日局15では，テトラサイクリン系化合物の化学名は，tetraceneを用いて命名した．

(例)

Methyl (1*R*, 2*R*, 4*S*)-4-{2, 6-dideoxy-4-*O*-[(2*R*, 6*S*)-6-methyl-5-oxo-3, 4, 5, 6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyl-(1→4)-2, 3, 6-trideoxy-3-dimethylamino-α-L-*lyxo*-hexopyranosyloxy}-2-ethyl-2, 5, 7-trihydroxy-6, 11-dioxo-1, 2, 3, 4-tetrahydrotetracene-1-carboxylate monohydrochloride（アクラルビシン塩酸塩）

(2*S*, 4*S*)-2-Acetyl-4-(3-amino-2, 3, 6-trideoxy-α-L-*lyxo*-hexopyranosyloxy)-2, 5, 12-trihydroxy-1, 2, 3, 4-tetrahydrotetracene-6, 11-dione monohydrochloride（イダルビシン塩酸塩）

架橋環式構造は，von Baeyer法を拡張して命名する．

(例)

(2*S*, 5*R*, 6*R*)-6-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)-acetylamino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate（アモキシシリン水和物）

Tricyclo[3.3.1.1^{3,7}]dec-1-ylamine monohydrochloride（アマンタジン塩酸塩）

スピロ環状構造は，接頭辞spiroを冠して命名する．

(例)

(2*S*, 6′*R*)-7-Chloro-2′, 4, 6-trimethoxy-6′-methylspiro[benzo[*b*]furan-2(3*H*), 1′-(cyclohex-2′-ene)]-3, 4′-dione（グリセオフルビン）

縮合多環構造を部分構造の縮合体として命名する場合には，縮合環の縮合位置番号あるいは位置記号は，[ ]で囲んで表記する．

(例)

1*H*-Pyrazolo[3, 4-d]pyrimidin-4-ol（アロプリノール）

8-Chloro-6-phenyl-4*H*-[1, 2, 4]triazolo[4, 3-a][1, 4]benzodiazepine（エスタゾラム）

縮合多環構造に異性体が存在しそれらを区別して命名する必要がある場合には，指示水素（位置番号を付けた*H*）あるいは付加水素（丸括弧に入れ位置番号を付けた*H*）を使って命名する．

(指示水素を用いた例)

6-(1-Methyl-4-nitro-1*H*-imidazol-5-ylthio)purine（アザチオプリン）

1*H*-Pyrazolo[3, 4-d]pyrimidin-4-ol（アロプリノール）

8-Chloro-6-phenyl-4*H*-[1, 2, 4]triazolo[4, 3-*a*][1, 4]benzodiazepine（エスタゾラム）

3-(10, 11-Dihydro-5*H*-dibenzo[*a*, *d*]cyclohepten-5-ylidene)-*N*, *N*-dimethylpropylamine monohydrochloride（アミトリプチリン塩酸塩）

7-Chloro-1-methyl-5-phenyl-1, 3-dihydro-2*H*-1, 4-benzodiazepin-2-one（ジアゼパム）

N, *N*, 2-Trimethyl-3-(10*H*-phenothiazin-10-yl)propylamine hemitartrate（アリメマジン酒石酸塩）

10-{2-[(2*RS*)-1-Methylpiperidin-2-yl]ethyl}-2-methylsulfanyl-10*H*-phenothiazine monohydrochloride（チオリダジン塩酸塩）

(付加水素を用いた例)

5-Ethyl-5-(3-methylbutyl)pyrimidine-2, 4, 6(1*H*, 3*H*, 5*H*)-trione（アモバルビタール）

5-[(2*RS*)-3-(1, 1-Dimethylethyl)amino-2-hydroxypropyloxy]-3, 4-dihydroquinolin-2(1*H*)-one monohydrochloride（カルテオロール塩酸塩）

(3*S*, 3a*S*, 5a*S*, 9b*S*)-3, 5a, 9-Trimethyl-3a, 5, 5a, 9b-tetrahydronaphtho[1, 2-*b*]furan-2, 8(3*H*, 4*H*)-dione（サントニン）

縮合多環構造の水素付加体を示す接頭語hydroは，分離不可能接頭語として扱う．

(例)

Methyl (1*R*, 2*R*, 4*S*)-4-{2, 6-dideoxy-4-*O*-[(2*R*, 6*S*)-6-methyl-5-oxo-3, 4, 5, 6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyl-(1→4)-2, 3, 6-trideoxy-3-dimethylamino-α-L-*lyxo*-hexopyranosyloxy}-2-ethyl-2, 5, 7-trihydroxy-6, 11-dioxo-1, 2, 3, 4-tetrahydrotetracene-1-carboxylate monohydrochloride（アクラルビシン塩酸塩）

Dihydroxo(5-oxo-4-ureido-4, 5-dihydro-1*H*-imidazol-2-yl)oxoaluminium（アルジオキサ）

環状ペプチドでは，cycloを用いて環状構造を命名する．

(例)

cyclo{-[(2*S*, 3*R*, 4*R*, 6*E*)-3-Hydroxy-4-methyl-2-methylaminooct-6-enoyl]-L-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-}（シクロスポリン）

(6) 同一要素集合型の命名

同一の置換基が重複する複雑な構造の化合物の場合には，置換式命名法ではなく同一要素集合型の命名法により命名する．

(例)

3, 5-Bis(acetylamino)-2, 4, 6-triiodobenzoic acid（アミドトリゾ酸）

2, 2′-Succinyldioxybis(*N*, *N*, *N*-trimethylethylaminium) dichloride dihydrate（スキサメトニウム塩化物水和物）
Disodium 3, 3′-dioxo-[$\Delta^{2,2'}$-biindoline]-5, 5′-disulfonate（インジゴカルミン）
2, 2′-(Ethylenediimino) bis[(2*S*)-butan-1-ol] dihydrochloride（エタンブトール塩酸塩）
2, 2′, 2′′, 2′′′-{[4, 8-Di(piperidin-1-yl) pyrimido[5, 4-*d*] pyrimidine-2, 6-diyl] dinitrilo} tetraethanol（ジピリダモール）
2, 2′-[(1, 2-Dioxoethane-1, 2-diyl) diimino] bis[*N*-(2-chlorobenzyl)-*N*, *N*-diethylethylaminium] dichloride（アンベノニウム塩化物）
N, *N*-Bis(2-chloroethyl)-3, 4, 5, 6-tetrahydro-2*H*-1, 3, 2-oxazaphosphorin-2-amine 2-oxide monohydrate（シクロホスファミド水和物）
N, *N*′-Ethylenebis(*N*-butylmorpholine-4-carboxamide)（ジモルホラミン）
3, 3′-[Hexamethylenebis(methyliminocarbonyloxy)] bis(1-methylpyridinium) dibromide（ジスチグミン臭化物）
Tris(aziridin-1-yl) phosphine sulfate（チオテパ）
(2-Hydroxycyclohexane-1, 1, 3, 3-tetrayl) tetramethyl tetranicotinate（ニコモール）

重合体は，単体名を括弧に入れ，その前に間隔を開けずに poly を表記する．
(例)
Poly[(2-oxopyrrolidin-1-yl) ethylene] iodine（ポビドンヨード）
Poly(oxymethylene)（パラホルムアルデヒド）

(7) 立体構造の命名

二重結合のシス，トランス異性体を区別する場合には，*E*，*Z* を用いて命名する．cis，trans は使用しない．原則として *E*，*Z* の前に位置番号を付ける．
(例)
(3*S*, 5*Z*, 7*E*, 22*E*)-9, 10-Secoergosta-5, 7, 10(19), 22-tetraen-3-ol（エルゴカルシフェロール）
(2*E*, 4*E*, 6*E*, 8*E*)-3, 7-Dimethyl-9-(2, 6, 6-trimethylcyclohex-1-en-1-yl) nona-2, 4, 6, 8-tetraen-1-yl acetate（レチノール酢酸エステル）
(5*Z*)-7-{(1*R*, 2*R*, 3*R*, 5*S*)-3, 5-Dihydroxy-2-[(1*E*, 3*S*)-3-hydroxyoct-1-en-1-yl] cyclopentyl}-hept-5-enoic acid（ジノプロスト）

不斉原子の立体配置が明確な場合には，*R*, *S* 表示を用いて絶対配置を表示し，原則として *R*, *S* の前に位置番号を付ける．
(例)
(1*R*)-1-(3, 4-Dihydroxyphenyl)-2-(methylamino) ethanol（アドレナリン）
(1*R*, 2*S*)-2-Methylamino-1-phenylpropan-1-ol monohydrochloride（エフェドリン塩酸塩）
(2*S*)-1-[(2*S*)-2-Methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid（カプトプリル）

ラセミ体の場合には，*RS* 表示を用いて命名する．原則として *RS* の前に位置番号を付ける．
(例)
(2*RS*)-2-[4-(2-Methylpropyl) phenyl] propanoic acid（イブプロフェン）
(2*RS*)-2-Carbamoyloxy-*N*, *N*, *N*-trimethylpropylaminium chloride（ベタネコール塩化物）
(1*RS*, 4*RS*)-1, 7, 7-Trimethylbicyclo[2.2.1] heptan-2-ol（*dl*-カンフル）
(1*RS*, 2*SR*)-2-Dimethylamino-1-phenylpropan-1-ol monohydrochloride（*dl*-メチルエフェドリン塩酸塩）
(2*RS*, 6*RS*, 11*RS*)-6, 11-Dimethyl-3-(3-methylbut-2-en-1-yl)-1, 2, 3, 4, 5, 6-hexahydro-2, 6-methano-3-benzoazocin-8-ol（ペンタゾシン）
(1*RS*, 2*SR*)-4-[2-(4-Benzylpiperidin-1-yl)-1-hydroxypropyl] phenol hemi-(2*R*, 3*R*)-tartrate（イフェンプロジル酒石酸塩）

疑似不斉原子は，*r*, *s* で表記する．
(例)
(1*R*, 3*r*, 5*S*)-8-Methyl-8-azabicyclo[3.2.1] oct-3-yl [(2*RS*)-3-hydroxy-2-phenyl] propanoate hemisulfate hemihydrate（アトロピン硫酸塩水和物）
(1*R*, 3*r*, 5*S*)-8-Methyl-8-azabicyclo[3.2.1] oct-3-yl [(2*RS*)-2-hydroxy-2-phenyl] acetate monohydrobromide（ホマトロピン臭化水素酸塩）
(1*R*, 3*r*, 5*S*)-3-[(2*RS*)-3-Hydroxy-2-phenylpropanoyloxy]-8-methyl-8-(1-methylethyl) 8 azoniabicyclo[3.2.1] octane bromide monohydrate（イプラトピウム臭化物水和物）
(1*S*, 2*S*, 4*R*, 5*R*, 7*s*)-9-Methyl-3-oxa-9-azatricyclo[$3.3.1.0^{2,4}$] non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate monohydrobromide trihydrate（スコポラミン臭化水素酸塩水和物）

単環化合物の置換基の相対的立体構造は，*cis*-，*trans*-を用いて命名する．
(例)
trans-4-(Aminomethyl) cyclohexanecarboxylic acid（トラネキサム酸）
3-{4-[*trans*-4-(Aminomethyl) cyclohexylcarbonyloxy]-phenyl} propanoic acid monohydrochloride（セトラキサート塩酸塩）

meso 体は，*meso*-を用いて命名する．

（例）

meso-Xylitol（キシリトール）

（8）イオン，塩及び塩類似化合物の命名

有機及び無機の陽イオンと陰イオンは，IUPAC 命名法に従って命名する．

無機の陽イオンは，イオン数を mono，di，tri などを用いて表記する．

（例）

Monosodium benzoate（安息香酸ナトリウム）

Disodium (2*R*,3*S*)-3-methyloxiran-2-ylphosphonate（ホスホマイシンナトリウム）

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate（クエン酸ナトリウム水和物）

無機の陰イオンは，数が1個の場合には mono を付けない．数が2個以上の場合には，di などを用いて表記する．

（例）

N-Ethyl-3-hydroxy-*N*,*N*-dimethylanilinium chloride（エドロホニウム塩化物）

2,2′-[(1,2-Dioxoethane-1,2-diyl)diimino]bis[*N*-(2-chlorobenzyl)-*N*,*N*-diethylethylaminium] dichloride（アンベノニウム塩化物）

四級アンモニウム塩は，aminium を用い，主基をアミノ基とする *N*-置換化合物の命名と同様に命名する．

（例）

(2*RS*)-2-Carbamoyloxy-*N*,*N*,*N*-trimethylpropylaminium chloride（ベタネコール塩化物）

2-Acetoxy-*N*,*N*,*N*-trimethylethylaminium chloride（注射用アセチルコリン塩化物）

塩基性化合物の無機酸塩あるいは有機酸塩の場合には，本体部分と酸部分の比率を接頭辞（整数値は，mono，di，tri など，分数値は，hemi，sesqui，hemipenta，hemihepta など）を用いて表記する．

（例）

(2*RS*)-1-(2-Allylphenoxy)-3-[(1-methylethyl)amino]propan-2-ol monohydrochloride（アルプレノロール塩酸塩）

2-Ethoxy-6,9-diaminoacridine monolactate monohydrate（アクリノール水和物）

2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine dimaleate（プロクロルペラジンマレイン酸塩）

2,6-Diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)-[2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl-(1→5)]-2-deoxy-D-streptamine trisulfate（フラジオマイシン B 硫酸塩）

N,*N*,2-Trimethyl-3-(10*H*-phenothiazin-10-yl)propylamine hemitartrate（アリメマジン酒石酸塩）

2-Amino-2,3,4,6-tetradeoxy-6-methylamino-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine hemipentasulfate（ミクロノマイシン硫酸塩）

酸性の水素を含むイオン構造は，無機陰イオンの場合には，hydrogen を陰イオンの名前の前に付けて表記する．有機陰イオンの場合には，hydrogen と陰イオンの間を空けて表示する．必要ならばその数を数詞で示す．

（例）

Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-dihydrogenphosphate（クリンダマイシンリン酸エステル）

11β,17,21-Trihydroxypregn-4-ene-3,20-dione 21-(hydrogen succinate)（ヒドロコルチゾンコハク酸エステル）

複雑な化合物の中に陽イオンあるいは陰イオン構造がある場合には，そのイオンの接頭辞を用いて命名する．

（陽イオンの例）

(1*R*,3*r*,5*S*)-8-(4-Butoxybenzyl)-3-[(2*S*)-hydroxy-2-phenylpropanoyloxy]-8-methyl-8-azoniabicyclo[3.2.1]octane bromide（ブトロピウム臭化物）

（陰イオンの例）

Disodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*R*)-2-phenyl-2-sulfonatoacetylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate（スルベニシリンナトリウム）

Monocalcium (4-amino-2-oxidobenzoate) hemiheptahydrate（パラアミノサリチル酸カルシウム水和物）

含窒素ヘテロ環に陽イオンが結合している場合には，そのヘテロ環をアニオン（…ate）として命名してもよい．

（例）

2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide dihydrate（サッカリンナトリウム水和物）

アルミニウム塩は，IUPAC 無機化合物命名法に従い配位化合物として命名する．

（例）

Dihydroxo(5-oxo-4-ureido-4,5-dihydro-1*H*-imidazol-2-yl)oxoaluminium（アルジオキサ）

Bis(2-acetoxybenzoato)hydroxoaluminium
(アスピリンアルミニウム)

金属を含む化合物で共有結合かイオン結合か不明な場合には塩として命名する.

(例)

Monogold monosodium monohydrogen(2*RS*)-2-sulfidobutane-1,4-dioate 及び
Monogold disodium (2*RS*)-2-sulfidobutane-1,4-dioate
(金チオリンゴ酸ナトリウム)

(9) 結晶水などの結晶溶媒が存在する場合の命名

結晶溶媒の数は,接頭辞(整数値は,mono, di, tri, penta, hepta など,分数値は,hemi, sesqui, hemihepta など)を用いて表記する.

(例)

1,3,7-Trimethyl-1*H*-purine-2,6-(3*H*,7*H*)-dione monohydrate (カフェイン水和物)
2,2′-Succinyldioxybis(*N*,*N*,*N*-trimethylethylaminium) dichloride dihydrate (スキサメトニウム塩化物水和物)
(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate
(モルヒネ塩酸塩水和物)
Monocalcium bis[(2*RS*)-2-hydroxypropanoate] pentahydrate (乳酸カルシウム水和物)
Monosodium (6*R*,7*S*)-7-{2-[(2*S*)-2-amino-2-carboxyethylsulfanyl]acetylamino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate heptahydrate
(セフミノクスナトリウム水和物)
8-Hydroxy-5-{(1*RS*,2*SR*)-1-hydroxy-2-[(1-methylethyl)amino]butyl}quinolin-2(1*H*)-one monohydrochloride hemihydrate
(プロカテロール塩酸塩水和物)
(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate (メチルドパ水和物)
Monosodium (5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-7-oxo-3-[(2*R*)-tetrahydrofuran-2-yl]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate hemipentahydrate
(ファロペネムナトリウム水和物)
Monosodium 3-[5-(4-nitrophenyl)furan-2-ylmethylene]amino-2,5-dioxo-1,3-imidazolidinate hemiheptahydrate (ダントロレンナトリウム水和物)

(10) 有機化合物の複合体の命名

有機化合物の複合体構造は,IUPAC の付加化合物の命名法に従い,それぞれの構成分子を命名し,それらを — で繋いで表示する.構成比は比率で表記する.

(例)

2-(Diphenylmethyloxy)-*N*,*N*-dimethylethylamine—8-chloro-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (1/1)
(ジメンヒドリナート)
7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl}heptanoic acid—α-cyclodextrin
(アルプロスタジル　アルファデクス)

(11) 生化学命名法を用いた命名

アミノ酸,糖,ペプチド,核酸などの誘導体は IUPAC 生化学命名法を用いて命名する.

1) アミノ酸及びアミノ酸誘導体の命名

アミノ酸は,IUPAC 命名法に従いアミノ酸の慣用名を用いて命名する.ただし,慣用名が医薬品各条の英名と同じ場合には,置換式命名法に従い体系的命名法により命名する.

(例)

(2*S*)-2,6-Diaminohexanoic acid monohydrochloride
(L-リジン塩酸塩)
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid
(L-メチオニン)

アミノ酸誘導体の命名には慣用名を用いる.慣用名を用いるとき,立体構造は D-, L-で表記する.

(例)

3-Hydroxy-L-tyrosine (レボドパ)

アミノ酸の塩は,慣用名の末尾…e を…ate に変えて命名する.

(例)

Monosodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosinate hydrate (レボチロキシンナトリウム水和物)

ポリペプチド,オリゴペプチドには化学名を付けない.

2) 糖類の命名

糖類は,IUPAC 生化学命名法に従い体系的命名法を用いて命名する.ただし,構造が非常に簡単なものでは,慣用名を使用してもよい.

(例)

D-Mannitol (D-マンニトール)

糖の基準炭素原子の立体配置は,接頭記号 D-, L-で表記する.また,糖のアノマー炭素原子の立体配置を示す場合には,アノマー接頭記号 α-, β-を基準炭素原子の立体配置を示す接頭記号 D-, L-と一緒に使用する.

(例)

L-*threo*-Hex-2-enono-1,4-lactone (アスコルビン酸)
β-D-Fructopyranose (果糖)

糖類の配置接頭辞は小文字のイタリックを用いて表記する．

（例）

L-*threo*-Hex-2-enono-1,4-lactone（アスコルビン酸）

オリゴ糖の糖鎖結合は，(○ → △)を用いて表記する．

（例）

3-Deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1 → 6)-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl-(1 → 4)]-2-deoxy-D-streptamine hemipentasulfate（シソマイシン硫酸塩）

Methyl (1*R*,2*R*,4*S*)-4-[2,6-dideoxy-4-*O*-[(2*R*,6*S*)-6-methyl-5-oxo-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyl-(1 → 4)-2,3,6-trideoxy-3-dimethylamino-α-L-*lyxo*-hexopyranosyloxy] -2-ethyl-2,5,7-trihydroxy-6,11-dioxo-1,2,3,4-tetrahydrotetracene-1-carboxylate monohydrochloride（アクラルビシン塩酸塩）

分岐状オリゴ糖は，側鎖(オリゴ糖又は単糖)にあたる一方のグリコシル置換基を[ ]に入れもう１つの主鎖側のグリコシル置換基と区別する．

（例）

3-Amino-3-deoxy-α-D-glucopyranosyl-(1 → 6)-[6-amino-6-deoxy-α-D-glucopyranosyl-(1 → 4)]-2-deoxy-D-streptamine sulfate（カナマイシン硫酸塩）

糖類似の５員環又は６員環では，糖の命名法を準用してもよい．

（例）

1,4：3,6-Dianhydro-D-glucitol（イソソルビド）

３）ステロイド誘導体の命名

ステロイド類は，IUPAC生化学命名法に従い基本炭素環部分に慣用名を用いて命名する．また，ステロイド骨格に付加した置換基の立体配置を表示するときは，α-，β-を使用する．

（例）

(3*S*,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol（エルゴカルシフェロール）

(3*S*,5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3-ol（コレカルシフェロール）

6-Chloro-3,20-dioxopregna-4,6-dien-17-yl acetate（クロルマジノン酢酸エステル）

3α,7β-Dihydroxy-5β-cholan-24-oic acid（ウルソデオキシコール酸）

Estra-1,3,5(10)-triene-3,16α,17β-triol（エストリオール）

9β,10α-Pregna-4,6-diene-3,20-dione（ジドロゲステロン）

４）核酸，核酸誘導体及び核酸類似体の命名

核酸誘導体の化学名は，原則として核酸の慣用名を用いて命名する．

（例）

5-Iodo-2′-deoxyuridine（イドクスウリジン）

6-(1-Methyl-4-nitro-1*H*-imidazol-5-ylthio)purine（アザチオプリン）

1,3,7-Trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione（無水カフェイン）

5-Fluoro-1-[(2*RS*)-tetrahydrofuran-2-yl]uracil（テガフール）

5-Fluorouracil（フルオロウラシル）

1-β-D-Arabinofuranosylcytosine（シタラビン）

（12）非体系的母核構造名を用いた命名

複雑な構造を有する天然物は，CASで使用している非体系的母核構造名を使用し命名する．ただし，慣用名が医薬品各条の正名（英名）と同じになる場合は置換式命名法に従い体系名で命名する．

（例）

(17*R*,21*R*)-Ajmalan-17,21-diol（アジマリン）

1-Bleomycinoic acid（ブレオマイシン酸）

Ethyl (8*S*,9*R*)-6′-methoxycinchonan-9-yl carbonate（キニーネエチル炭酸エステル）

*Co*α-[α-(5,6-Dimethylbenz-1*H*-imidazol-1-yl)]-*Co*β-cyanocobamide（シアノコバラミン）

*Co*α-[α-(5,6-Dimethylbenz-1*H*-imidazol-1-yl)]-*Co*β-hydroxocobamide monoacetate（ヒドロキソコバラミン酢酸塩）

(8*S*)-*N*-[(1*S*)-2-Hydroxy-1-methylethyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate（エルゴメトリンマレイン酸塩）

(5′*S*,10*R*)-5′-Benzyl-12′-hydroxy-2′-methyl-9,10-dihydroergotaman-3′,6′,18-trione monomethanesulfonate（ジヒドロエルゴタミンメシル酸塩）

(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate（モルヒネ塩酸塩水和物）

3-Amino-3-deoxy-α-D-glucopyranosyl-(1 → 6)-[6-amino-6-deoxy-α-D-glucopyranosyl-(1 → 4)]-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine disulfate（アミカシン硫酸塩）

7′,12′-Dihydroxy-6,6′-dimethoxy-2,2′,2′-trimethyltubocuraranium chloride monohydrochloride pentahydrate（ツボクラリン塩化物塩酸塩水和物）

Methyl (3*S*,16*S*,17*R*,18*R*,20*R*)-11,17-dimethoxy-18-(3,4,5-trimethoxybenzoyloxy)yohimban-16-carboxylate（レセルピン）

◇◇◇ 文　献 ◇◇◇

1）The graphic representation of chemical formulae in the publications of international nonproprietary names (INN) for pharmaceutical substances (WHO/Pharm/95.579 1996), http：//www.who.int/medicinedocs/collect/edmweb/pdf/h1807e/h1807e.pdf

2）平山健三：有機化学・生化学命名法（上，下），南江堂，1989.

3）A guide to IUPAC nomenclature of organic compounds：Recommendations 1993，IUPAC，Organic Chemistry Division.

4）IUPAC，http：//www.chem.qmul.ac.uk/iupac/search.html

〈参考〉

5）化合物命名法，日本化学会.

分子式と分子量

【技術情報】

1．分子式の表記法

（1）分子式の表記

有機化合物の場合は分子式を，また無機化合物の場合は組成式を表記する．ただし，分子組成が不明な場合，あるいは混合物の場合には，分子式（又は組成式）を省略する．

有機化合物の分子式（又は組成式）は，炭素（C），水素（H）の順に表記し，次いでそれ以外の構成元素をアルファベット順に並べる．

無機化合物の分子式（又は組成式）は，アルファベット順に並べて記載する．

［例］

$C_9H_7N_7O_2S$（アザチオプリン）

$Cl_2H_6N_2Pt$（シスプラチン）

（2）無機酸塩及び有機酸塩の分子式の表記

無機酸（塩酸，臭化水素酸，硝酸，硫酸，リン酸など），あるいは有機酸（乳酸，アジピン酸，クエン酸，酢酸，サリチル酸，酒石酸，ステアリン酸，フマル酸など）の塩の場合には，本体部分の分子式の後に酸部分の分子式を表記する．また，本体部分の分子式と酸部分の分子式の間には・を入れる．

［例］

$C_{42}H_{53}NO_{15}\cdot HCl$（アクラルビシン塩酸塩）

本体部分と酸部分の比率が1でない場合には，酸部分の分子式の前に整数あるいは分数で両者の比率を表記する．比率に分数を用いる場合には，$l\frac{n}{m}$ を用いる．

［例］

$C_{17}H_{35}N_5O_6\cdot 2\,H_2SO_4$（アストロマイシン硫酸塩）

$C_{16}H_{17}N_9O_5S_3\cdot \frac{1}{2}\,HCl$（セフメノキシム塩酸塩）

$C_{19}H_{37}N_5O_7\cdot 2\frac{1}{2}\,H_2SO_4$（シソマイシン硫酸塩）

本体部分と酸部分の比率が明らかでない場合には，酸部分の分子式の前に x を表記する．

［例］

$C_{18}H_{36}N_4O_{11}\cdot xH_2SO_4$（カナマイシン硫酸塩）

多塩基酸（酒石酸塩，硫酸塩など）の場合には，本体部分の分子式を（　）でくくり，その後に下付き数字で両者の比率を表記する．

［例］

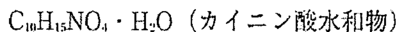
$(C_{11}H_{17}NO_3)_2\cdot H_2SO_4$（オルシプレナリン硫酸塩）

（3）結晶溶媒を含む化合物の分子式の表記

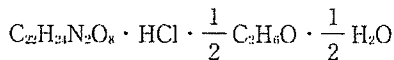
結晶溶媒（結晶水など）を含む場合には，本体の分子式の後に結晶溶媒の分子式を表記する．

本体部分と結晶溶媒の分子式の間に・を入れる．

［例］



$C_{10}H_{15}NO_4 \cdot H_2O$（カイニン酸水和物）



$C_{22}H_{24}N_2O_8 \cdot HCl \cdot \frac{1}{2}C_2H_6O \cdot \frac{1}{2}H_2O$

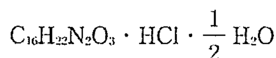
（ドキシサイクリン塩酸塩水和物）

結晶溶媒と本体との比率が1でない場合には，結晶溶媒の分子式の前に比率を数字あるいは分数で表記する．

［例］

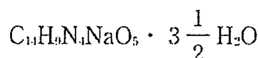
$C_{14}H_{30}Cl_2N_2O_4 \cdot 2H_2O$

（スキサメトニウム塩化物水和物）



$C_{16}H_{22}N_2O_3 \cdot HCl \cdot \frac{1}{2}H_2O$

（プロカテロール塩酸塩水和物）

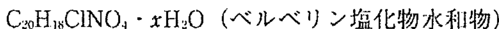


$C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$

（ダントロレンナトリウム水和物）

結晶溶媒の数が不明の場合には，結晶溶媒の分子式の前にxを表記する．

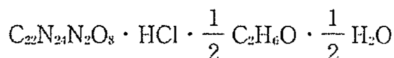
［例］



$C_{20}H_{18}ClNO_4 \cdot xH_2O$（ベルベリン塩化物水和物）

結晶水と水以外の結晶溶媒の両方を含む場合には，結晶水を最後に表記する．

［例］



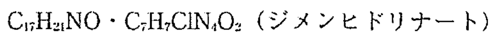
$C_{22}N_{24}N_2O_8 \cdot HCl \cdot \frac{1}{2}C_2H_6O \cdot \frac{1}{2}H_2O$

（ドキシサイクリン塩酸塩水和物）

（4）分子複合体の分子式の表記

有機化合物の複合体である場合には，それぞれの分子式を記載し，分子式間に・を入れて表記する．

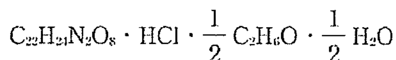
［例］



$C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$（ジメンヒドリナート）

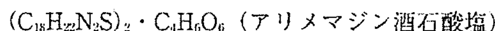
分子複合体の構成比が1でない場合には，比率を整数，分数，あるいはxで表記する．

［例］

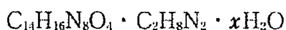


$C_{22}H_{24}N_2O_8 \cdot HCl \cdot \frac{1}{2}C_2H_6O \cdot \frac{1}{2}H_2O$

（ドキシサイクリン塩酸塩水和物）

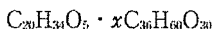


$(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$（アリメマジン酒石酸塩）



$C_{14}H_{16}N_8O_4 \cdot C_2H_8N_2 \cdot xH_2O$

（アミノフィリン水和物）



$C_{20}H_{34}O_5 \cdot xC_{36}H_{60}O_{30}$

（アルプロスタジル　アルファデクス）

2．分子量の表記法

（1）分子量の表記

有機化合物の場合は分子量を，また無機化合物の場合は式量を表記する．

分子量（又は式量）は，分子式（又は組成式）の後に表記する．

分子式（又は組成式）と分子量（又は式量）の間には：を入れる．

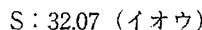
ただし，分子組成が不明な場合，あるいは混合物の場合には，分子量を省略する．

［例］

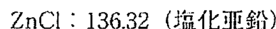
$C_{62}H_{86}N_{12}O_{16}$：1255.42（アクチノマイシンD）

$C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$：361.39

（アクリノール水和物）



S：32.07（イオウ）



ZnCl：136.32（塩化亜鉛）

（2）分子量の計算

分子量（あるいは式量）の計算には，最新の国際原子量表（日局15ではIUPAC 2004年国際原子量表）の原子量を用いる[1]．

原子量（あるいは式量）は，各元素の原子量をそのまま集計し，小数点以下第3位を四捨五入し小数点以下第2位まで記載する．

◇◇◇ 文　献 ◇◇◇

1）原子量表．化学と工業 57(4)，2004．

Regular Article

Glycomic/glycoproteomic analysis by liquid chromatography/mass spectrometry: Analysis of glycan structural alteration in cells

Noritaka Hashii, Nana Kawasaki, Satsuki Itoh, Mashashi Hyuga, Toru Kawanishi and Takao Hayakawa

Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Tokyo

The alteration of glycosyltransferase expression and the subsequent changes in oligosaccharide structures are reported in several diseases. The analysis of glycan structural alteration in glycoproteins is becoming increasingly important in the discovery of therapies and diagnostic markers. In this study, we propose a strategy for glycomic/glycoproteomic analysis based on oligosaccharide profiling by LC/MS followed by proteomic approaches, including 2-DE and 2-D lectin blot. As a model of aberrant cells, we used Chinese hamster ovary cells transfected with *N*-acetylglucosaminyltransferase III (GnT-III), which catalyzes the addition of a bisecting *N*-acetylglucosamine (GlcNAc) to β-mannose of the mannosyl core of *N*-linked oligosaccharides. LC/MS equipped with a graphitized carbon column (GCC) enabled us to elucidate the structural alteration induced by the GnT-III expression. Using 2-D lectin blot followed by LC/MS/MS, the protein carrying an extra *N*-acetylhexosamine in cells transfected with GnT-III was successfully identified as integrin α3. Thus, oligosaccharide profiling by GCC-LC/MS followed by proteomic methods can be a powerful tool for glycomic/glycoproteomic analysis.

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Keywords:
2DE / LC/MS / Lectin blotting / Oligosaccharides profiling

1 Introduction

It is common knowledge that approximately 50% of proteins in mammalian cells are glycosylated and that glycans play crucial roles in various biological events including cell recognition [1], adhesion [2] and cell-cell interaction [3]. The alteration of glycosyltransferase expression and subsequent changes in oligosaccharide structures are reported in several diseases, including inherited diseases [4], the progression of cancer [5] and autoimmune diseases [6–8]. The analysis of glycan structural alteration in glycoproteins is becoming increasingly important in the discovery of therapies and diagnostic markers.

Comprehensive analysis of proteins in a given cellular sample is the most effective means of elucidating the disease mechanism. Simultaneous separation and characterization of proteins by 2-DE and 2-D LC followed by MS have been utilized as the fundamental approaches to proteomic analysis; however, these approaches alone are ineffectual for the elucidation of the glycan structural alteration in glycoproteins. A strategy based on qualitative and quantitative glycomic analysis is necessary for the study of glycosylation-associated diseases.

LC/MS is widely used for glycosylation analysis in glycoproteins. Previously, we demonstrated that LC/MS equipped with a graphitized carbon column (GCC-LC/MS) is a useful means of oligosaccharide profiling and for the structural analysis of carbohydrates [9–12]. Using this method, oligosaccharides, including high mannose, hybrid and complex

Correspondence: Dr. Nana Kawasaki, 1–18–1, Kamiyoga, Setagaya-ku, Tokyo, 158–8501, Japan
E-mail: nana@nihs.go.jp
Fax: +81-3-3700-9084

Abbreviations: CHO, Chinese hamster ovary; **CHO-III cells**, CHO cells transfected with *N*-acetylglucosaminyltransferase III; **dHex**, deoxyhexose; **GCC**, graphitized carbon column; **GlcNAc**, *N*-acetylglucosamine; **GnT-III**, *N*-acetylglucosaminyltransferase III; **Hex**, hexose; **HexNAc**, *N*-acetylhexosamine; **NeuAc**, *N*-acetylneuraminic acid; **PNGase F**, peptide *N*-glycosidase F

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types with or without sialic acids, can be separated, and structural information can be obtained from their mass spectra and chromatographic behavior.

Here we propose a strategy for performing glycomic/glycoproteomic analysis based on a combination of GCC-LC/MS and proteomic approaches.

First, GCC-LC/MS is applied to the analysis of oligosaccharide structural alteration in aberrant cells. Chinese hamster ovary (CHO) cells, used as a model of aberrant cells, were transfected with *N*-acetylglucosaminyltransferase III (GnT-III), which catalyzes the addition of bisecting *N*-acetylglucosamine (GlcNAc) to the trimannosyl core of *N*-linked oligosaccharides [13] and is associated with cell adhesion [14] and the suppression of tumor cell metastasis [15–17]. Then, 2-D lectin blotting followed by LC/MS/MS was used to identify the protein in which glycosylation was altered by the expression of GnT-III.

2 Materials and methods

2.1 Cell lines and culture

The CHO cells were obtained from the Japanese Collection of Research Bioresources (Tokyo, Japan). The human GnT-III cDNA was cloned into the pCI-neo vector. The expression vector was transfected into CHO cells with LipofectAMINE plus reagent, according to the manufacturer's instructions. To screen the transformants, the transfectants were cultured with Ham's F12 medium supplemented with 10% fetal calf serum (FCS) and 1 mg/mL G418. After 2 weeks, the colonies were lifted with a micropipette. A high GnT-III-expressing clone was used in succeeding experiments.

The CHO cells and GnT-III-transfected CHO cells (CHO-III cells) were cultured in Ham's F12 medium supplemented with 10% FCS, 100 U/mL of penicillin and 100 μg/mL of streptomycin under a humidified atmosphere of 95% air and 5% CO_2. After harvesting CHO and CHO-III cells, they were rinsed with PBS containing protease inhibitors and 2 mM EDTA.

2.2 Preparation of insoluble and soluble fractions

The insoluble and soluble fractions were prepared from CHO and CHO-III cells using a Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit (Pierce Biotechnology, P.O., USA). The detergent in these fractions was removed with Detergent-OUT (Geno Technology, M.O., USA) three times. For desalting and degreasing, seven volumes of acetone were added to the sample solution, and the mixture was stirred and sonicated. The mixture was then incubated at −20°C for 1 h and centrifuged at 4°C for 15 min, 15 000 × g. The supernatants were discarded, and the pellets dried. The protein concentrations were determined using a BCA protein assay kit (Pierce).

2.3 Preparation of *N*-linked oligosaccharide alditols

The protein (500 μg) from each fraction was dissolved in 810 μL of 0.5 M Tris-HCl containing 8 M guanidine-HCl and 5 mM EDTA (pH 8.6), and then 6.0 μL of 2-mercaptoethanol were added in the solution. After incubation at room temperature for 2 h, freshly prepared 0.6 M sodium monoiodoacetamide (135 μL) was added to the solution. After incubation at room temperature for 2 h in the dark, the solution was desalted with PD10 column (Amersham Biosciences, NJ, USA), and the elute was lyophilized. The carboxylmethylated proteins were dissolved in 500 μL of 100 mM PBS (pH 7.2), and 20 U of peptide *N*-glycosidase F (PNGase F) (Roche Diagnostics, Mannheim, Germany) were added to the solution. After incubation at 37°C for 4 days, 1.74 mL of cold ethanol was added to the solution, the mixture was incubated at −20°C for 3 h, and proteins were removed by centrifugation at 4°C for 10 min (15 000 × g). The supernatants containing oligosaccharides were evaporated, and then lyophilized. The oligosaccharides were incubated with 500 μL of 0.5 M $NaBH_4$ at room temperature for 16 h, and then neutralized with 10% (v/v) acetic acid to pH 6.5 and desalted with Envi-Carb (Supelco, Bellefonte, USA).

2.4 GCC-LC/MS

LC was carried out using a MAGIC 2002 system (Michrom BioResources, Auburn, CA, USA). The GCC used was a Hypercarb column (150 × 0.2 mm, ThermoFinnigan, San Jose, CA, USA). The eluents were 5 mM ammonium acetate, pH 8.5, containing 2% ACN (pump A) and 5 mM ammonium acetate, pH 8.5, containing 80% ACN (pump B). The borohydride-reduced oligosaccharides were eluted at a flow rate of 2 μL/min with a gradient of 10–45% of pump B in 90 min. Mass spectra were recorded on a TSQ 7000 triple-stage quadruple mass spectrometer (ThermoFinnigan) equipped with a nanoelectorospray ion source (AMR, Inc., Tokyo, Japan). The mass spectrometer was operated in positive ion mode. Ions in the range of m/z 900–2400 were acquired with a scan duration of 3 s. The ESI voltage was set at 2.0 kV, and the capillary temperature was 175°C. The electron multiplier was set at 1.0 kV. Collisions for MS/MS were carried out with collision energy of 25%, scan duration of 4 s., and mass range of m/z 100–2000.

2.5 1-D SDS-PAGE and lectin blotting

Proteins were separated by 1-D SDS-PAGE (12.5% T, 3% C) as described by Laemmli [18] and stained with SYPRO Orange (Bio-Rad, Richmond, CA, USA) at room temperature for 30 min in transfer buffer (25 mM Tris-HCl, 20 mM glycine and 20% methanol). The gel images were scanned on a Tyhoon 9400 (Amersham Biosciences) at an excitation wavelength of 540/25 nm and an emission wavelength of 590/30 nm. After saving the gel image, the proteins were blotted to a PVDF membrane (Immun-Blot PVDF membrane,

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0.2 μm, Bio-Rad) at 3.0 mA/cm^2, 20 V for 30 min in transfer buffer containing 0.1% SDS using a semi-dry blotter (Trans-blot SD sel, Bio-Rad). The efficiency and position of the transfer were confirmed using SYPRO Orange transferred together with proteins. Nonspecific sites on the membrane were blocked at 4°C for 16 h in 0.5% casein-PBS. After the membranes were washed with 0.05% Tween-PBS (T-PBS) three times, they were treated with 0.1 U/mL of sialidase (Nacalai Tesque, Kyoto, Japan) at 37°C for 16 h in 0.5 M acetate buffer (pH 5.0). The membranes were then re-blocked with 0.5% casein-PBS at 37°C for 15 min, washed with T-PBS three times, and incubated with biotinylated phytohemagglutinin-E4 (PHA-E4, 2 μg/mL) at 4°C for 2 h in PBS (pH 7.4). The membranes were then washed with T-PBS and incubated with 1:1000 diluted avidin-alkaline phosphatase (AP) complex solution at 4°C for 1 h in PBS.

2.6 Concentration of target proteins in the gel

The band detected by lectin blotting on 1-D gel was excised and then mashed in 20 mM Tris-HCl (pH 8.0) containing 2% SDS. The proteins in the gel particles were extracted by intermittent sonication at 4°C for 30 min, followed by shaking at room temperature for 16 h. After extraction, the gel particles were removed by centrifugation (15 000 × g). The proteins in the supernatant were precipitated with sevenfold acetone at −20°C for 3 h, and then the precipitates were washed with acetone three times to remove salts and detergent.

2.7 2-DE

For first dimension IEF of the sample, Immobiline DryStrip gel (13 cm, pH4–7 NL, Amersham Biosciences) was used. The samples were dissolved in IEF solution containing 7 M urea, 2 M thiourea, 18 mM DTT, 0.5% IPG buffer, 2% CHAPS, and bromophenol blue. Dried IPG strips were rehydrated overnight in the sample solution. IEF was then performed using the following steps: 500 V for 1 h, 100 V for 1 h, and 8000 V for 2 h, *i.e.* a total of 17.5 kVh.

IPG strips were treated with 10 mL of 50 mM Tris-HCl (pH 8.8) containing 2% SDS, 6 M urea, 30% glycerol and 65 mM DTT for 15 min, and then treated with 10 mL of 50 mM Tris-Cl (pH 8.8) containing 2% SDS, 6 M urea, 30% glycerol and 135 mM iodoacetamide for 15 min in order to reduce the disulfide bonds of cysteinyl residues. SDS-polyacrylamide gels (7.5%T, 3%C, size 140 × 140 × 1 mm) and running buffer containing 25 mM Tris-HCl, 192 mM glycine and 0.1% SDS were used for the 2-DE. The gels were run at 25 mA/gel after setting the IPG strip on the gel. Fluorescent staining and scanning of gel, followed by lectin blotting, were performed as mentioned above. In 2-D lectin blotting, the proteins were blotted to a PVDF membrane at 3.0 mA/cm^2, 20 V for 90 min.

2.8 In-gel digestion and protein identification by LC/MS/MS

Interesting spots were excised from the 2-DE gel for in-gel trypsin digestion. The gel particles were destained with 20 mM ammonium bicarbonate containing 50% methanol in microcentrifuge tubes, and dehydrated in 100% ACN. Enzymatic digestion was performed overnight at 37°C with 5 μL of 20 μg/mL trypsin (Promega, Madison, WI, USA) in 20 mM ammonium bicarbonate (pH 8.5). Digested peptides were extracted with 1% TFA in 50% ACN, and samples were dried with a Speed-Vac and redissolved in 0.1% TFA for LC/MS.

LC was carried out using a Paradigm MS4 (Michrom BioResources) equipped with Magic C18 column (50 × 0.2 mm, Michrom BioResources). The eluents were 0.1% formic acid containing 2% ACN (pump A), and 0.1% formic acid containing 90% ACN (pump B). The peptides were eluted at a flow rate of 2 μL/min with a gradient of 5–70% of pump B in 30 min. Mass spectra were recorded on an API QSTAR Pulsar i (Applied Biosystems, Foster City, CA, USA) in the positive ion mode. The proteins were identified by searching the Swiss-Prot database using MASCOT (Matrix Science, UK). The mass range and MS/MS range were *m/z* 400–2000 and *m/z* 100–2000, respectively, and the ESI voltage was set at 2.5 kV.

3 Results

3.1 Analysis of glycans in the insoluble fractions

N-linked oligosaccharides were released from soluble and insoluble fractions by PNGase F and reduced with $NaBH_4$ to prevent the separation of anomers by GCC. Figure 1A shows the *N*-linked oligosaccharide profile of the insoluble fraction from CHO cells (5 × 10^7). Diverse oligosaccharide ions were detected by full scan in the positive ion mode of MS. Oligosaccharides were numbered with the labels on peaks where they were detected, and the multiple oligosaccharides in single peak were classified by the digits behind alphabets, such as peaks A1 and A2. Their monosaccharide compositions were deduced from the *m/z* values as shown in Table 1. *N*-linked oligosaccharides from CHO cells have a high proportion of high mannose-type and bi-, tri- and tetra-antennary complex type oligosaccharides [19, 20]. High mannose-type oligosaccharides, $[Man]_{5-9}[GlcNAc]_2$ were detected at 9–23 min (peaks A–E and K). Major components (peaks N2, Q2, R1, S1, T1, U1 and V2) were deduced as fucosylated and non-fucosylated biantennary forms with mono- and di-sialic acids from previous articles and their monosaccharide compositions. Various oligosaccharides, including mono- (peak N1 and Q1), tri- (peak P1, U2, V1), tetra-antennary (peak V3), and hybrid-type (peak F1 and I1) oligosaccharides were detected as minor components together with low molecular weight oligosaccharides such as the trimannosyl core (peaks G1 and O1).

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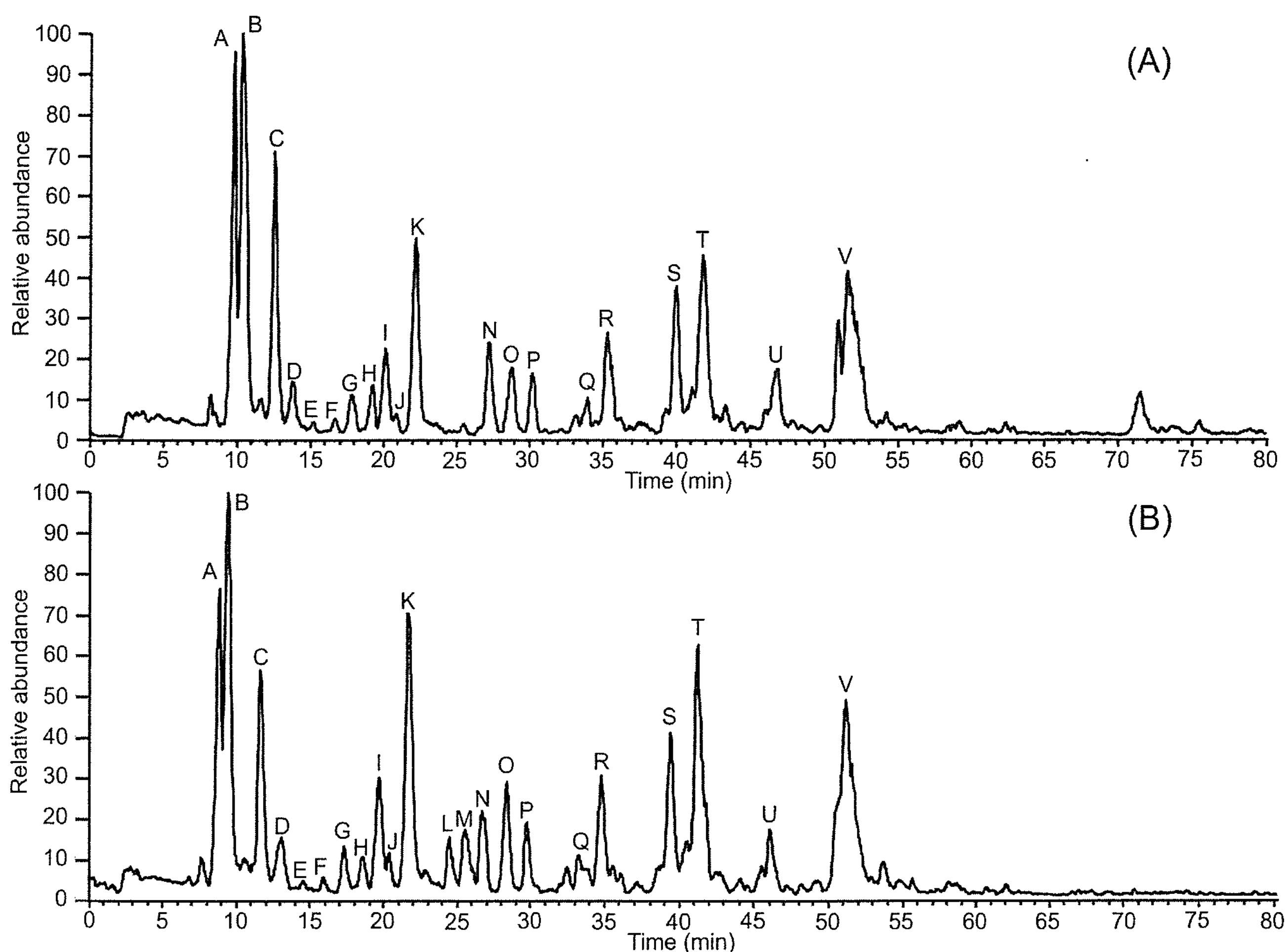

Figure 1. Total ion chromatograms of GCC-LC/MS of borohydride-reduced *N*-linked oligosaccharides released from insoluble fraction of CHO (A) and CHO-III (B) in positive ion mode. Pump A: 5 mM ammonium acetate, pH 8.5, containing 2% ACN. Pump B: 5 mM ammonium acetate, pH 8.5, containing 80% ACN. The borohydride-reduced oligosaccharides were eluted at a flow rate of 2 μL/min with a gradient of 10–45% of pump B in 90 min.

Figure 1B shows the oligosaccharide profile of the CHO-III-insoluble fraction. The distribution of oligosaccharides in CHO-III was different from that in the CHO cell insoluble fraction. Some additional peaks (peaks L1 and M1) were detected in the CHO-III-insoluble fraction, and their doubly charged ions at *m/z* 1143.2 and 1143.0 were consistent with the theoretical *m/z* values of fucosylated biantennary-bearing NeuAc with one additional HexNAc. Figure 2 shows the MS/MS spectrum of peak M1. Detection of $B_{1\gamma}/Y_6^{2+}$ at *m/z* 894.1 and an intense ion of $[HexNAc]^+$, at *m/z* 204 suggest that the oligosaccharide (peak M1) carries one GlcNAc at either of the non-reducing ends. Peak M1 is possibly assigned to bisected biantennary form. In addition, peaks D1, I2 and S2, which were not found in the profile of CHO, were detected in that of CHO-III (Fig. 1B). They can also be deduced as bisected biantennary forms from their MS/MS spectra. Other than these oligosaccharides bearing GlcNAc at either of the non-reducing ends in CHO-III cells, there was no significant difference in glycosylation between CHO and CHO-III cells. These results suggest that only limited oligosaccharides are altered by the expression of GnT-III.

3.2 Analysis of glycans in the soluble fractions

Figure 3A and B shows the *N*-linked oligosaccharide profiles of the soluble fractions of CHO and CHO-III, respectively. The oligosaccharide components of soluble fractions are very different from those of insoluble fractions (Table 1). High mannose-type oligosaccharides, $[Man]_{5-9}[GlcNAc]_2$, were detected as major components (peaks A–C and K), and complex-type and hybrid-type oligosaccharides were detected as minor oligosaccharides in the soluble fraction. Oligosaccharides bearing extra GlcNAc (D1, L1 and M1) were also detected in the soluble fraction of CHO-III.

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Table 1. Observed *m/z* values and carbohydrate compositions of peaks A-V in total ion chromatogram 3 of CHO-insoluble (Fig. 1A), CHO-III-insoluble (Fig. 1B), CHO-soluble (Fig. 3A) and CHOIII-soluble (Fig. 3B) fractions

Carbohydrate composition[a]	Theoretical mass[b]	Peak No.		Insoluble fraction				Soluble fraction			
				CHO		CHO-III		CHO		CHO-III	
				Charge state	Observed *m/z*	Charge state	Observed *m/z*	Charge state	Observed *m/z*	Charge state	Observed *m/z*
$[Hex]_7[HexNAc]_2$	1561.4	A	A1	H^+	1562.2	H^+	1562.0	Na^+	1584.4	Na^+	1584.2
$[Hex]_8[HexNAc]_2$	1723.5		A2	Na^+	1746.3	Na^+	1746.3	Na^+	1746.5	Na^+	1746.1
$[Hex]_9[HexNAc]_2$	1885.7	B	B1	Na^+	1908.4	Na^+	1908.5	Na^+	1908.4	Na^+	1908.9
$[Hex]_6[HexNAc]_2$	1399.3	C	C1	H^+	1400.1	H^+	1400.0	H^+	1399.7	H^+	1399.9
$[Hex]_7[HexNAc]_2$	1561.4		C2	Na^+	1584.2	Na^+	1584.0	Na^+	1584.8	Na^+	1584.0
$[dHex]_1[Hex]_5[HexNAc]_5$	1992.9	D	D1		N.D.[c]	$2H^+$	**997.4**		N.D.	$2H^+$	**997.5**
$[Hex]_6[HexNAc]_2$	1399.3		D2	Na^+	1422.0	Na^+	1421.9		N.D.		N.D.
$[Hex]_7[HexNAc]_2$	1561.4		D3	Na^+	1584.2	Na^+	1584.1		N.D.		N.D.
$[Hex]_8[HexNAc]_2$	1723.5		D4	Na^+	1746.2	Na^+	1746.4		N.D.		N.D.
$[Hex]_4[HexNAc]_2$	1075.0	E	E1	Na^+	1097.9	Na^+	1097.6		N.D.		N.D.
$[Hex]_6[HexNAc]_2$	1399.3		E2	H^+	1400.1	H^+	1400.0		N.D.		N.D.
$[Hex]_6[HexNAc]_3$	1602.5	F	F1	H^+	1604.0	H^+	1603.1		N.D.		N.D.
$[Hex]_3[HexNAc]_2$	912.8	G	G1	H^+	913.7	H^+	913.7	Na^+	935.7	Na^+	935.6
$[Hex]_5[HexNAc]_4$	1643.5	H	H1	H^+	1644.5	H^+	1644.2	Na^+	1666.3	Na^+	1666.4
$[Hex]_6[HexNAc]_4$	1827.6	I	I1	$2Na^+$	914.7	$2Na^+$	914.7		N.D.		N.D.
$[Hex]_5[HexNAc]_5[NeuAc]_1$	2137.9		I2		N.D.	$2H^+$	**1069.8**		N.D.		N.D.
$[dHex]_1[Hex]_3[HexNAc]_4$	1465.4	J	J1	H^+	1466.1	H^+	1466.1	Na^+	1488.2	Na^+	1487.9
$[Hex]_5[HexNAc]_2$	1237.1	K	K1	H^+	1238.0	H^+	1238.0	H^+	1237.9	H^+	1237.9
$[dHex]_1[Hex]_5[HexNAc]_5[NeuAc]_1$	2284.1	L	L1		N.D.	$2H^+$	**1143.2**		N.D.	$2H^+$	**1142.9**
$[dHex]_1[Hex]_5[HexNAc]_5[NeuAc]_1$	2284.1	M	M1		N.D.	$2H^+$	**1143.0**		N.D.	$2H^+$	**1143.3**
$[dHex]_1[Hex]_4[HexNAc]_3$	1424.3	N	N1	H^+	1425.4	H^+	1425.3	Na^+	1447.1	Na^+	1447.1
$[dHex]_1[Hex]_5[HexNAc]_4$	1789.7		N2	H^+	1790.1	H^+	1790.3	Na^+	1812.3	Na^+	1812.1
$[dHex]_1[Hex]_3[HexNAc]_2$	1059.0	O	O1	H^+	1059.7	H^+	1059.7	H^+	1059.8	H^+	1059.7
$[dHex]_1[Hex]_6[HexNAc]_5$	2155.0	P	P1	$2H^+$	1078.5	$2H^+$	1078.5		N.D.		N.D.
$[dHex]_1[Hex]_3[HexNAc]_3$	1262.2	Q	Q1	H^+	1263.0	H^+	1263.0		N.D.		N.D.
$[Hex]_5[HexNAc]_4[NeuAc]_1$	1934.7		Q2	$2H^+$	968.4	$2H^+$	968.4		N.D.		N.D.
$[Hex]_5[HexNAc]_4[NeuAc]_1$	1934.7	R	R1	$2H^+$	968.4	$2H^+$	968.4	$2H^+$	968.7	$2H^+$	968.2
$[dHex]_1[Hex]_5[HexNAc]_4[NeuAc]_1$	2080.9	S	S1	$2H^+$	1041.4	$2H^+$	1041.4	$2H^+$	1041.4	$2H^+$	1041.3
$[dHex]_1[Hex]_5[HexNAc]_5[NeuAc]_2$	2574.0		S2		N.D.	$2H^+$	**1288.5**		N.D.		N.D.
$[dHex]_1[Hex]_5[HexNAc]_4[NeuAc]_1$	2080.9	T	T1	$2H^+$	1041.4	$2H^+$	1041.5	$2H^+$	1041.4	$2H^+$	1041.3
$[Hex]_5[HexNAc]_4[NeuAc]_2$	2226.0	U	U1	$2H^+$	1114.0	$2H^+$	1113.9	$2H^+$	1113.9	$2H^+$	1113.9
$[dHex]_1[Hex]_6[HexNAc]_5[NeuAc]_1$	2446.2		U2	$2H^+$	1224.2	$2H^+$	1224.3	$2Na^+$	1124.9		N.D.
$[dHex]_1[Hex]_6[HexNAc]_5[NeuAc]_2$	2737.5	V	V1	$2H^+$	1370.0	$2H^+$	1370.0		N.D.		N.D.
$[dHex]_1[Hex]_5[HexNAc]_4[NeuAc]_2$	2372.1		V2	$2H^+$	1187.1	$2H^+$	1187.1	$2H^+$	1187.2	$2H^+$	1187.2
$[dHex]_1[Hex]_7[HexNAc]_6[NeuAc]_1$	2811.6		V3	$2H^+$	1406.8	$2H^+$	1406.6		N.D.		N.D.

The characteristic *m/z* values observed in total ion chromatograms of CHO-III are depicted in bold type.

a) [dHex], deoxyhexose; [Hex], hexose; [HexNAc], *N*-acetylhexosamine; [NeuAc], *N*-acetylneuraminic acid.

b) Monoisotopic mass values.

c) Not detected.

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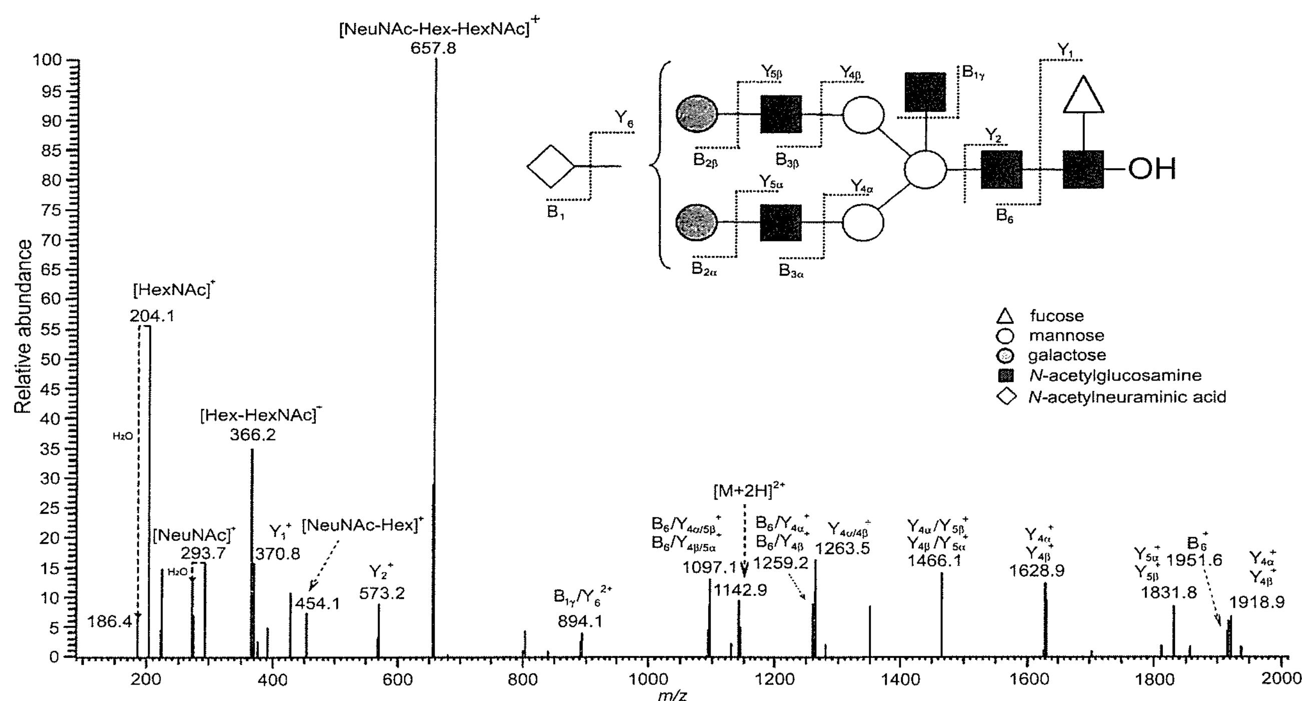

Figure 2. MS/MS spectrum of fucosylated biantennary *N*-linked oligosaccharide (peak M1) detected in the insoluble fraction from CHO-III.

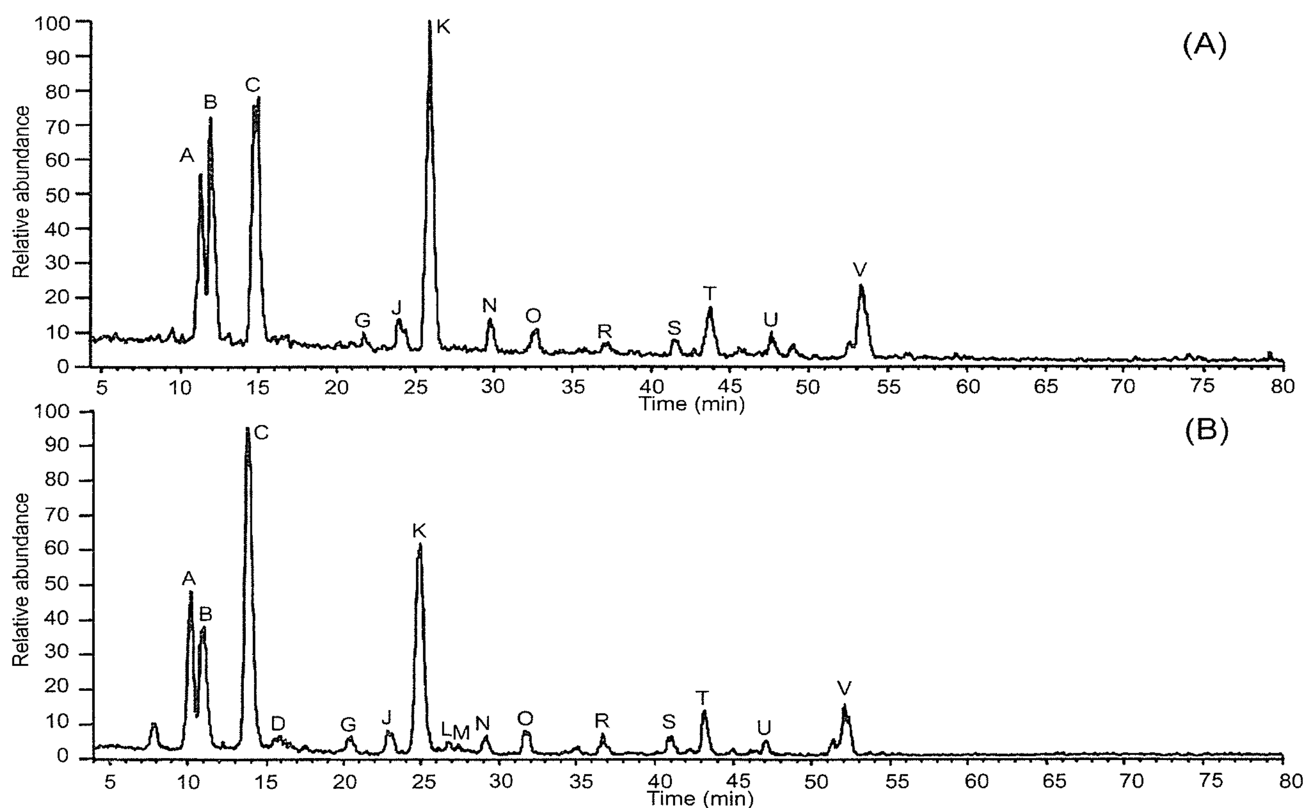

Figure 3. Total ion chromatograms of GCC-LC/MS of borohydride-reduced *N*-linked oligosaccharides released from the soluble fractions of CHO (A) and CHO-III (B) in positive ion mode. Pump A: 5 mM ammonium acetate, pH 8.5, containing 2% ACN. Pump B: 5 mM ammonium acetate, pH 8.5, containing 80% ACN. The borohydride-reduced oligosaccharides were eluted at a flow rate of 2 μL/min with a gradient of 10–45% of pump B in 90 min.

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3.3 Identification of protein bearing bisected oligosaccharides

To identify proteins with altered glycans by the expression of GnT-III, we performed 2-DE followed by lectin blotting using PHA-E4 lectin, which recognizes bisecting GlcNAc in complex-type oligosaccharides. Although some bisected glycoproteins (70–120 kDa) could be visualized by 2-D lectin blotting, their expressions were too low to be detected on 2-DE gel. Lectin affinity chromatography, which is generally used for the concentration of glycoproteins, cannot be used for the insoluble fraction due to the presence of detergent in the solvent medium. Therefore, we first performed 1-D SDS-PAGE followed by lectin blotting, and then proteins in the range of 70–120 kDa were extracted from the gel (Fig. 4). 2-DE followed by lectin blotting was then performed, and interesting spots were successfully detected on 2-DE gel. Figure 5A and B shows the 2-DE gel images and the 2-D lectin blot of extracted proteins, respectively. The remarkable train spots (120 Da) of glycoprotein were picked up and in-gel digested with trypsin. The digest was subjected to LC/MS/MS, and the integrin α3 precursor was identified as the GnT-III target protein.

4 Discussion

The development of a simple and rapid method to explore glycan structural alteration in a complex mixture is required to elucidate the mechanisms of diseases involving glycan alteration. In this study, we demonstrated that GCC-LC/MS, which is used for glycosylation analysis in glycoproteins, is applicable for the exploration of changes in glycosylation between samples. Using GCC-LC/MS, high mannose, hybrid, and complex types as well as neutral and acidic oligosaccharides could be separated and characterized by a single analysis. GCC-LC/MS clearly shows differences in glycosylation between soluble and insoluble fractions. High mannose-type oligosaccharides were detected as major components in the soluble fraction. The soluble fraction contains endoplasmic reticulum and Golgi apparatus, where *N*-linked oligosaccharides are constructed. The predominance of high-mannose-type oligosaccharides in the soluble fraction may be the cause of immature oligosaccharides in the process of biosynthesis. In contrast, complex and hybrid types with or without sialic acids were detected in the insoluble fraction, suggesting that membrane proteins carry mature oligosaccharides.

In addition, GCC-LC/MS revealed differences in glycosylation between control cells and aberrant model cells. Biantennary forms bearing extra GlcNAc were obviously increased in cells transfected with the GnT-III gene, indicating that our methodology allows us to explore changes in the glycosyltransferase expression followed by glycan alteration. Although MS is frequently used for the analysis of glycosylation, identification of oligosaccharide isomers by MS alone

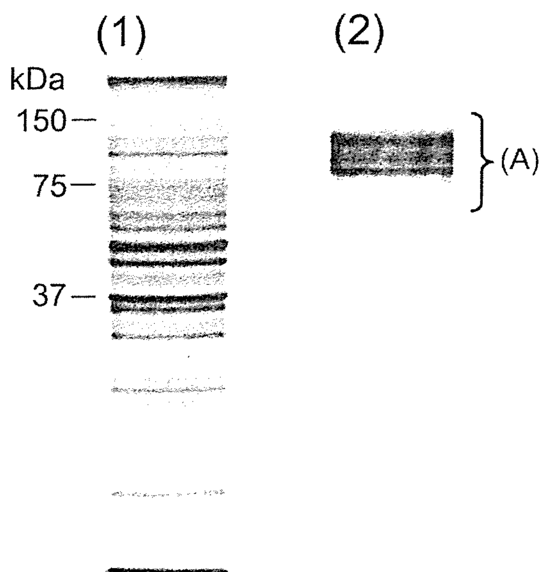

Figure 4. (1) 1-D SDS-PAGE and (2) lectin blot images of the CHO-III insoluble fraction. Proteins were separated on a 12.5% SDS-PAGE gel and stained with SYPRO Orange.

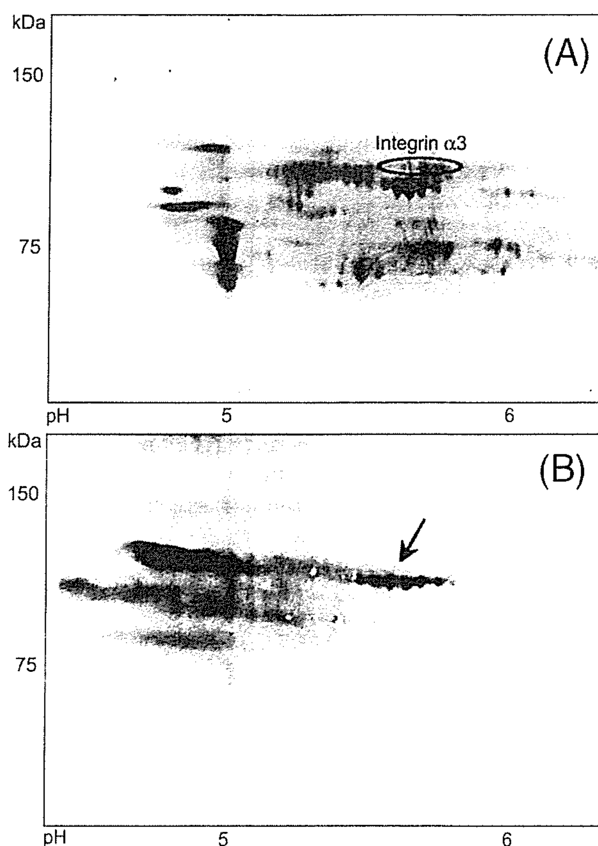

Figure 5. Enlarged partial (A) 2-DE and (B) lectin blot images from band A in Fig. 4.

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still remains challenges. Positional isomers could be differentiated by multiple-stage tandem mass spectrometry (MS^n); however, MS^n itself failed to identify oligosaccharides if the sample contained positional isomers. The use of GCC-LC/MS enables us to differentiate the structural isomers and perform differential analysis in glycosylation between normal and aberrant cells.

GnT-III is reported to involve the suppression of tumor cell metastasis and is assumed to be a marker of cancerous alteration in hepatic carcinoma [21, 22]. To identify the protein in which glycosylation was modified by GnT-III expression, we carried out 2-DE followed by lectin blotting, and Integrin α3 was identified as a target protein of GnT-III. Integrin α3, a type I membrane protein, is known to be a receptor of adhesion molecules, such as laminin 5 and 10/11 [23–25]. Our finding, in which integrin α3 is a target molecule of GnT-III, might be a clue to clarify the suppression mechanism of metastasis by GnT-III.

5 Concluding remarks

We propose a strategy for glycomic/glycoproteomic analysis using GCC-LC/MS in Fig. 6. First, GCC-LC/MS is used for oligosaccharide profiling to identify disease-related oligosaccharides. Based on the carbohydrate structure, appropriate lectins or antibodies could be selected for western blotting. Proteins carrying disease-related oligosaccharides could then be identified by 2-D lectin blotting followed by MS/MS analysis. Using several groups, 2-D lectin blotting has been proposed for the characterization of glycoproteins on gel [26, 27]. The use of mass spectrometric oligosaccharide profiling, which can directly characterize glycan structures, is worthwhile to obtain structural information about disease-related carbohydrate and is helpful in the subsequent choice of appropriate lectins and antibodies. Our method is expected to be a powerful tool for glycomic/glycoproteomic analysis.

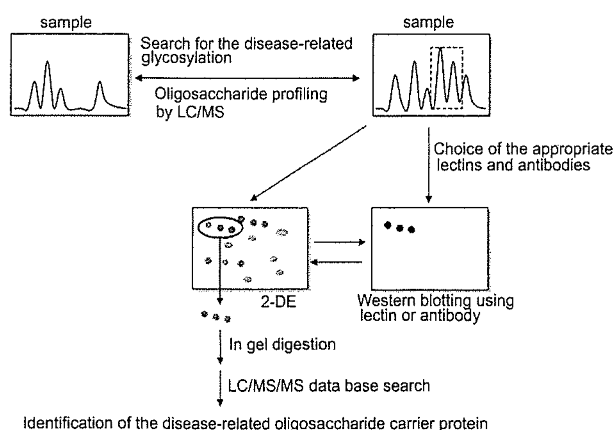

Figure 6. Strategy for glycomics/glycoproteomics using GCC-LC/MS, 2-DE and 2-D Western blotting using lectin or antibody.

6 References

[1] Varki, A., *Glycobiology* 1993, *3*, 97–130.

[2] Isaji, T., Gu, J., Nishiuchi, R., Zhao, Y. *et al.*, *J. Biol. Chem.* 2004, *279*, 19747–19754.

[3] Stanley, P., *Biochim. Biophys. Acta* 2002, *1573*, 363–368.

[4] Schachter, H., *Cell Mol. Life Sci.* 2001, *58*, 1085–1104.

[5] Dennis, J. W., Granovsky, M., Warren, C.E., *Biochim. Biophys. Acta* 1999, *1473*, 21–34.

[6] Delves, P. J., *Autoimmunity* 1998, *27*, 239–253.

[7] Gleeson, P. A., *Biochim. Biophys. Acta* 1994, *1197*, 237–255.

[8] Chui, D., Sellakumar, G., Green, R., Sutton-Smith, M. *et al.*, *Proc. Natl. Acad. Sci. USA* 2001, *98*, 1142–1147.

[9] Itoh, S., Kawasaki, N., Ohta, M., Hyuga, M. *et al.*, *J. Chromatogr. A* 2002, *968*, 89–100.

[10] Kawasaki, N., Haishima, Y., Ohta, M., Itoh, S. *et al.*, *Glycobiology* 2001, *11*, 1043–1049.

[11] Kawasaki, N., Ohta, M., Itoh, S., Hyuga, M. *et al.*, *Biologicals* 2002, *30*, 113–123.

[12] Davies, M., Smith, K. D., Harbin, A. M., Hounsell, E. F., *J. Chromatogr.* 1992, *609*, 125–131.

[13] Narasimhan, S., *J. Biol. Chem.* 1982, *257*, 10235–10242.

[14] Yoshimura, M., Ihara, Y., Matsuzawa, Y., Taniguchi, N., *J. Biol. Chem.* 1996, *271*, 13811–13815.

[15] Taniguchi, N., Miyoshi, E., Ko, J. H., Ikeda, Y., Ihara, Y., *Biochim. Biophys. Acta* 1999, *1455*, 287–300.

[16] Yoshimura, M., Nishikawa, A., Ihara, Y., Taniguchi, S., Taniguchi, N., *Proc. Natl. Acad. Sci. USA.* 1995, *92*, 8754–8758.

[17] Bhaumik, M., Harris, T., Sundaram, S., Johnson, L. *et al.*, *Cancer Res.* 1998, *58*, 2881–2887.

[18] Laemmli, U. K., *Nature* 1970, *227*, 680–685.

[19] Stanley, S., Sundaram, S., Tang, J., Shi, S., *Glycobiology* 2005, *15*, 43–53.

[20] Lee, J., Sundaram, S., Shaper, N. L., Raju, S., Stanley, P., *J. Biol .Chem.* 2001, *276*, 13924–13934.

[21] Yamashita, K., Koide, N., Endo, T., Iwaki, Y., Kobata, A., *J. Biol. Chem.* 1989, *264*, 2415–2423.

[22] Yamashita, K., Hitoi, A., Taniguchi, N., Yokosawa, N. *et al.*, *Cancer Res.* 1983, *43*, 5059–5063.

[23] Carter, W. G., Ryan, M. C., Gahr, P. J., *Cell* 1991, *65*, 599–610.

[24] Kikkawa, Y., Umeda, M., Miyazaki, K., *J. Biochem (Tokyo)* 1994, *116*, 862–869.

[25] Kikkawa, Y., Sanzen, N., Sekiguchi, K., *J. Biol. Chem.* 1998, *273*, 15854–15859.

[26] Kim, Y. S., Hwang, S. Y., Oh, S., Shon, H. *et al.*, *Proteomics* 2004, *4*, 3353–3358.

[27] Rahman, M. A., Karsani, S. A., Othman, I., Rahman, P. S. A., Hashim, O. H., *Biochem. Biophys. Res. Commun.* 2002, *295*, 1007–1013.

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Regulatory perspectives from Japan — Comparability of biopharmaceuticals ☆

Toru Kawanishi*

Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

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Abstract

In Japan there is no official guideline about comparability assessment of biotechnological products at present. However, there is some notifications which should be referred to, when the manufacturer changes the manufacturing process. Here, regulatory perspectives from Japan on the comparability assessment are presented. When establishing the comparability of biotechnological products derived from different manufacturing processes and the validity of modified manufacturing process, rational step-by-step approaches based on both product and process aspects would be useful. At first, relevant physicochemical and biological properties of products including purity, impurity profiles and stability should be compared before and after the manufacturing change, depending on the type and nature of the desired products. It is also necessary to examine the capacities of the new manufacturing process for ensuring the consistent production of the active protein product as well as the anticipated elimination of potential impurities and contaminants. Further relevant assessment of preclinical and clinical comparability of product may be necessary in some cases.
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1. Introduction

Biotechnological products were developed and produced based on many innovative technologies, which are always advancing by themselves. The products are, therefore, often subject to change in the manufacturing process for improvement of the product quality and production economy, increase in production yield, and so on. It is not reasonable that the manufacturers are required to submit the same full data to obtain the authorization of the manufacturing change as to obtain the new drug authorization. USA-FDA and EU-CPMA have already set each guideline for comparability assessment of biotechnological/biological products. We had also started the discussion about the comparability guideline in Japan. However, we stopped developing it, because comparability assessment of biotechnological products was nominated as a candidate of the new topic in the ICH-Quality. Drafting of the harmonized guideline has just started in the ICH-EWG. Here, I would like to give the regulatory perspectives about the comparability assessment of biopharmaceuticals from Japan.

2. Present official notifications relating with comparability assessment of biotechnological products before and after manufacturing changes in Japan

In Japan, we do not have any official guideline for the comparability assessment of biotechnological/biological products whose manufacturing processes are changed, yet. However, there is a notification, which should be referred to, when the manufacturer changes the manufacturing process of biotechnology-derived drugs which have already been approved. That is the Notification No. 243 from the Pharmaceutical Affairs Bureau, MHW of 1984. However, nearly 20 years have already passed since the Notification was made and some parts

☆ The perspectives are before the discussion in the ICH-EWG.

* Tel./fax: +81 3 3700 9064.
E-mail address: kawanish@nihs.go.jp

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of the requirement are assumed to be too strict. At present we usually treat each case as summarized below.

The following recombinant drugs would be treated as "not new drugs", which are categorized as "1-(8) other drugs" in the Pharmaceutical Affairs Bureau Notification No. 698: the first is the product which contains identical active ingredient although the culture method is different from the approved drug; the second is that which contains identical active ingredient although the purification process is different from the approved drug; and the third is the other drug in which difference is not specified. The followings are also usually treated as "not new drugs" but decided on a case-by-case basis: the product which contains identical active ingredient but its structure gene is identified by different process; and the product which contains identical active ingredient but host cell/vector system is different from the approved drug. In the case of the category 1-(8) other drugs as "not new drug", the data on specification and test methods, stability, and bioequivalence are required to be submitted for the registration as the pharmaceuticals, and a list of literature references concerning toxicity, pharmacological action, absorption, distribution, metabolism and excretion, and clinical trials for active ingredients concerned, as well as an outline of the list contents and the results of evaluation test are also required. In addition, in the case of the biotechnology-derived drugs, the following data are also needed on a case-by-case basis:

data on the manufacturing process, physicochemical analysis, specifications and test methods, stability;
data on single dose administration toxicity in one species of animals;
data on bioequivalency study;
data on clinical study for safety, etc.

The present notifications relating with the comparability of the products between before and after the changes in the manufacturing process in Japan are very simple, as summarized above. However, we have discussed much how to assess comparability of biotechnological products to draft the guideline, within Japanese experts. The following is the perspectives obtained from the discussion.

3. Regulatory perspectives from Japan: "how should we assess comparability of biotechnological products before and after the manufacturing change?"

To date, various topics related to the characterization and quality assessment as well as the manufacturing process for biotechnological products have been the subject of ICH harmonized guidelines and have proven very useful, in allowing manufacturers to develop a global approach to these issues. However, there is no specific international guideline on comparability of biotechnological products subject to changes in the manufacturing process. The subject we are facing is how to develop and establish rational concepts and approaches for establishing comparability of protein products derived from different biopharmaceutical manufacturing processes.

3.1. When is comparability assessment needed?

A comparability assessment is needed when a manufacturer wants to claim that the product of new manufacturing process Y is comparable to the already existing product of manufacturing process X with respect to quality, safety and efficacy (Fig. 1). The new process Y would be employed by either the same manufacturer, innovator or by different subsequent-entry manufacturer(s). The existing product from process X may be either an already licensed one or one under development for new drug application for approval. In case where there is an already licensed drug, subsequent-entry product(s) from different manufacturer(s) will be dealt with as a so-called generic product(s). On the other hand, the application from the innovator will be handled as a partial variation from already licensed conditions for the drug with respect to the manufacturing process. In the case of manufacturing variation of the product under development, the issue becomes the verification of such change within a single manufacturer at various stages of product development from early stage research to pre-approval. Here, the followings should be mentioned: it has been already decided that the generic products are excluded from the scope of the ICH-Q5E comparability guideline, but in Japan we still think that the comparability of the generic products could be evaluated following the same scientific approach.

3.2. General principles of comparability assessment

When establishing the comparability of biotechnological products derived from different manufacturing processes and the validity of modified manufacturing process, rational step-by-step approaches based on both product and process aspects would be useful. In this approach, the following parameters should be considered as key points:

(1) physicochemical and biological characterizations;
(2) impurities profile and the presence of potential contaminants;
(3) batch analysis;
(4) product stability;
(5) manufacturing process evaluation/validation studies; and in wider perspective
(6) preclinical and clinical studies.

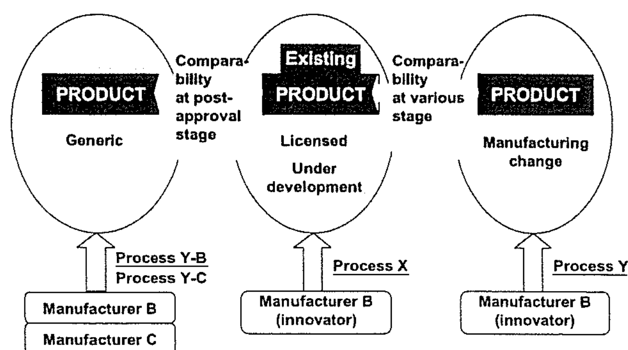

Fig. 1. Various cases of comparability assessment.

3.3. Strategies for comparability assessment

From the viewpoint of product aspects, the essential and critical first step is to establish whether the new candidate product in question is comparable to the existing product in terms of molecular and quality attributes. This is because whatever changes (minor or major) in the manufacturing process are made, if the new candidate product in question is not comparable to the existing product in terms of molecular and quality attributes, the new one will rather be regarded as a novel molecular entity for new drug application, but not as a qualified candidate for further comparability studies. The candidate product should be, therefore, the subject of extensive identification and characterization, as well as quality assessments including tests on impurities profile and the presence of potential contaminants. If these attributes of the candidate product and process are found to be comparable to those of the previous ones, further assessment of preclinical and clinical comparability would be performed, where necessary and appropriate.

3.4. Comparability from product aspects

Before going into some details about the need for further assessment of preclinical and clinical comparability, however, one should ask the following key question: "what is the identity or comparability of the biosynthetic protein product which possesses the inherent degree of structural heterogeneity?" In other words, what kind of criteria should be applied for establishing the identity or comparability of the candidate product(s) compared to the previous product with respect to molecular and quality attributes?

To answer this question, we should remind new concepts in the ICH-Q6B document. In the document we have introduced the concept, which has defined the desired product and variants, so that an inherent degree of structural and quality heterogeneity can be dealt within a relevant way. Desired product is defined as: (1) the protein which has the expected structure, or (2) the protein which is expected from the DNA sequence and anticipated post-translational modification (including glyco-forms), and from the intended downstream modification to produce an active biological molecule. When molecular variants of the desired product are formed during manufacture and/or storage and have properties comparable to the desired product, they are considered to be product-related substances and incorporated into active ingredient. When molecular variants of the desired product do not have properties comparable to those of the desired product, they are considered to be product-related impurities. In the concept, active ingredient may be composed of the desired product and multiple product-related substances; the desired product can be a mixture of several molecular entities derived from anticipated post-translational modification. Impurities may be either process-related or product-related (Fig. 2).

Various cases are considered for minimum qualification for further comparability assessments depending on each following specific type of desired product (A–D):

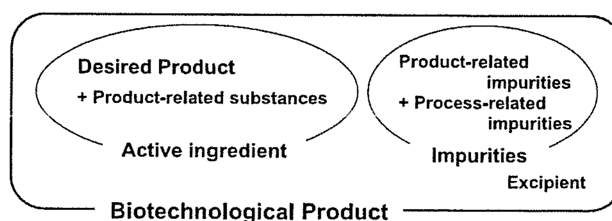

Fig. 2. New concept about biotechnological product in the ICH-Q6B.

(A) the protein which has the expected structure (e.g., monoclonal antibodies);
(B) the protein which is expected from the DNA sequence (simple protein);
(C) the protein which is expected from the DNA sequence and anticipated post-translational modification; and
(D) the protein which is expected from the intended downstream modification to produce an active biological molecule.

In the case of the "Desired product" being defined as the protein which has the expected structure, like monoclonal antibodies, minimum qualification for a candidate product for further comparability assessments should be that the product is derived from the same initial cell clone as a previous one and has comparable molecular and quality attributes compared to a previous one with respect to: (1) structural features, (2) physicochemical, (3) immunological properties, and (4) impurities profile. Variation of carbohydrate heterogeneity due to changes in culture conditions should be considered on a case-by-case basis.

In the case of the "Desired product" being defined as the protein which is expected from the DNA sequence, like recombinant insulin, minimum qualification for a candidate (product) for further comparability assessments should be that the product is the same as an already existing one with respect to protein structure, physicochemical and biological properties, as well as comparable impurities profiles.

In cases where the in vivo biological activity is closely related to the intended clinical effectiveness, further preclinical and clinical assessments with respect to efficacy may be omitted.

In the case of the "Desired product" being defined as the protein which is expected from the DNA sequence structure and anticipated post-translational modification, typically like glycoproteins, minimum qualification for a candidate product for further comparability assessments should be that the product is derived from the same initial cell clone as a previous product and has the same protein structure, comparable physicochemical properties, comparable carbohydrate patterns compared to a previous product with respect to the types of sialic acids and their contents, and antennary profile. Here, comparable biological properties, especially ensuring higher-order structure, in vivo activity and representing the clinical effectiveness, if any, is a critical factor for the qualification.

In the case of the protein which is expected from the intended downstream modification to produce an active

biological molecule, qualification for further comparability assessment of this type of products should be considered as a case-by-case issue, taking into account of types of modification and process change. Where necessary and appropriate, manufacturers should refer to the above cases A–C.

In this way, each specific type of candidate product can be qualified to be comparable to the pre-existing product with respect to molecular and quality attributes including impurity profile. The quality and extent of data obtained from studies on the molecular and quality attributes of the candidate would become one of the crucial elements for determining the necessity and extent of further comparability assessments, as well as for establishing the entire comparability to the pre-existing product.

3.5. Comparability from process aspect

As another aspect of quality comparability assessments, it is necessary to examine the capacities of a new manufacturing process for ensuring the consistent production of the active protein product as well as the anticipated elimination of potential impurities and contaminants. The capacities of the new process should not be less potent than those of the old process.

Changes in the manufacturing process used to make a particular product can be made in a variety of stages or steps of the process. Examples of such changes include: (1) method for generating cell substrate; (2) cell culture methods; (3) isolation and purification procedure; and (4) final product formulation. For changes in a certain stage of manufacturing process including cell substrate matters, relevant and complementary use of the ICH-guidelines (Q5A, Q5B, Q5EC, 5D, Q6B, and S6) would be encouraged.

Whatever changes in the manufacturing process are made, the effects of the changes, both direct and indirect, on the consistent production of the product should be considered and the modified process should be re-evaluated or re-validated as needed. The appropriate process re-evaluation or re-validation programs and criteria will vary depending on the nature and extent of the change. According to the results of process re-evaluation/re-validation studies on the new process, sometimes applicants may need to modify in-process controls including in-process testing and specifications of critical intermediates or final product (Fig. 3). The applicant should provide justification of such modification, if any.

3.6. Suitability of analytical method

As another dimension to comparability study, it is necessary to consider suitability of available analytical methods. Manufacturers should provide assurance that an appropriate set of analytical methods has been selected in order to assess the comparability of the product and to what extent the analytical methods used are suitable for comparability studies. The validation of the analytical methods used should be appropriate.

New analytical technology and modifications to existing technology are continually being developed and should be utilized when appropriate.

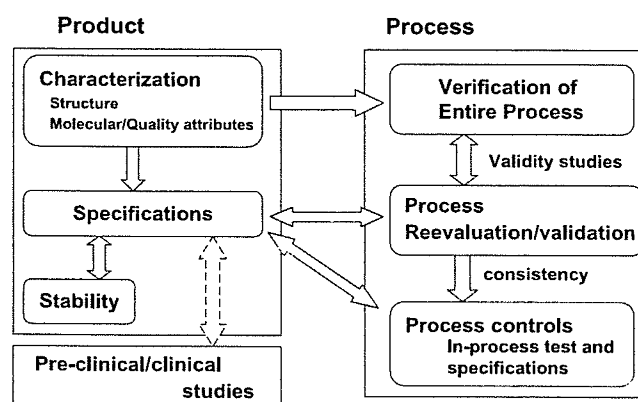

Fig. 3. Elements for ensuring product quality and consistency.

3.7. Preclinical and clinical studies

Further relevant assessment of preclinical and clinical comparability of product may be necessary, when it cannot be determined if the pre-existing product and the candidate product are comparable or not from the quality studies. The extent and nature of preclinical and clinical studies should be determined on a case-by-case basis in consideration of various factors. These include the followings:

- the nature of the product;
- intended clinical use;
- the extent of comparability of the candidate product to the existing counterpart with respect to molecular and quality attributes including impurity profile;
- the nature and extent of changes in manufacturing process;
- the results of the evaluation/validation studies on the new process including the results of relevant in-process tests;
- the capabilities and limitations of tests used for any comparability study;
- availability of existing preclinical and clinical data;
- the extent of existing information and experiences pertaining to the product in question; and
- stage of the product development.