

図 27 ミクロLC/MS、及びミクロLC/MS/MSによって得られたトロンボモジュリンのペプチド/糖ペプチドマップ (A)、及び糖ペプチドマップ (B)

表 7

図 27 中のピークのアミノ酸残基、糖鎖構造、分子量理論値、及び実測値

Peak No.	Peptide No.	Amino acid residues	Carbohydrates	Theoretical mass	Observed mass		
					Positive (low)	Positive (high)	Negative (high)
1	K17	298-304		809.9	809.5	-	-
2	K10	104-106		431.5	431.2	-	-
3	K2	39-45		813.0	812.5	-	-
4	K8	84-88		554.7	554.4	-	-
5	(K23)	(467-498)					
6	K15	247-260		1476.5	1476.3	7786.0	7785.3
7	K9	89-103	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	4084.0	4083.5	4084.2	4084.2
			[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	3792.7	3791.9	3793.2	3792.6
			[Fuc][Hex] ₅ [HexNAc] ₄	3501.4	3502.0	3501.7	
8	K14	220-246		2886.0	2885.8	2886.5	2886.8
9	K18	305-321		2093.1	2092.6	2093.4	2093.4
10	(K16)	(261-297)	Hex	4516.5	4515.7	4517.8	4518.6
11	K20	354-385	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	6204.3	6204.4	6206.2	6204.6
			[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	5913.0	5913.8	5913.8	5914.6
			[Fuc][Hex] ₅ [HexNAc] ₄	5621.7	5621.9	5622.4	
12	K19	322-353	-OH	3916.0	3915.7	3917.0	3917.0
13	K6	68-82		1651.9	1651.3		1651.8
	K1	1-38	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	6487.6	6487.4	6489.3	6489.3
				6196.3	6197.5	6199.3	6199.8
				5905.0	5905.5		5907.2
14	K13	183-219		3704.2	3704.4	3704.7	3705.4
15	K21	386-456	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	10597.7		10593.3	10594.4
16	K12	144-182		4113.6	4113.6	4114.7	4115.0
17	K11	107-143		4042.4	4042.8	4043.8	4043.0
18	K3	46-65		1900.1	1899.8	1900.9	1900.0

Fuc, fucose; Hex, hexose; HexNAc, N-acetylhexosamine; NeuAc, N-acetylneuramic acid

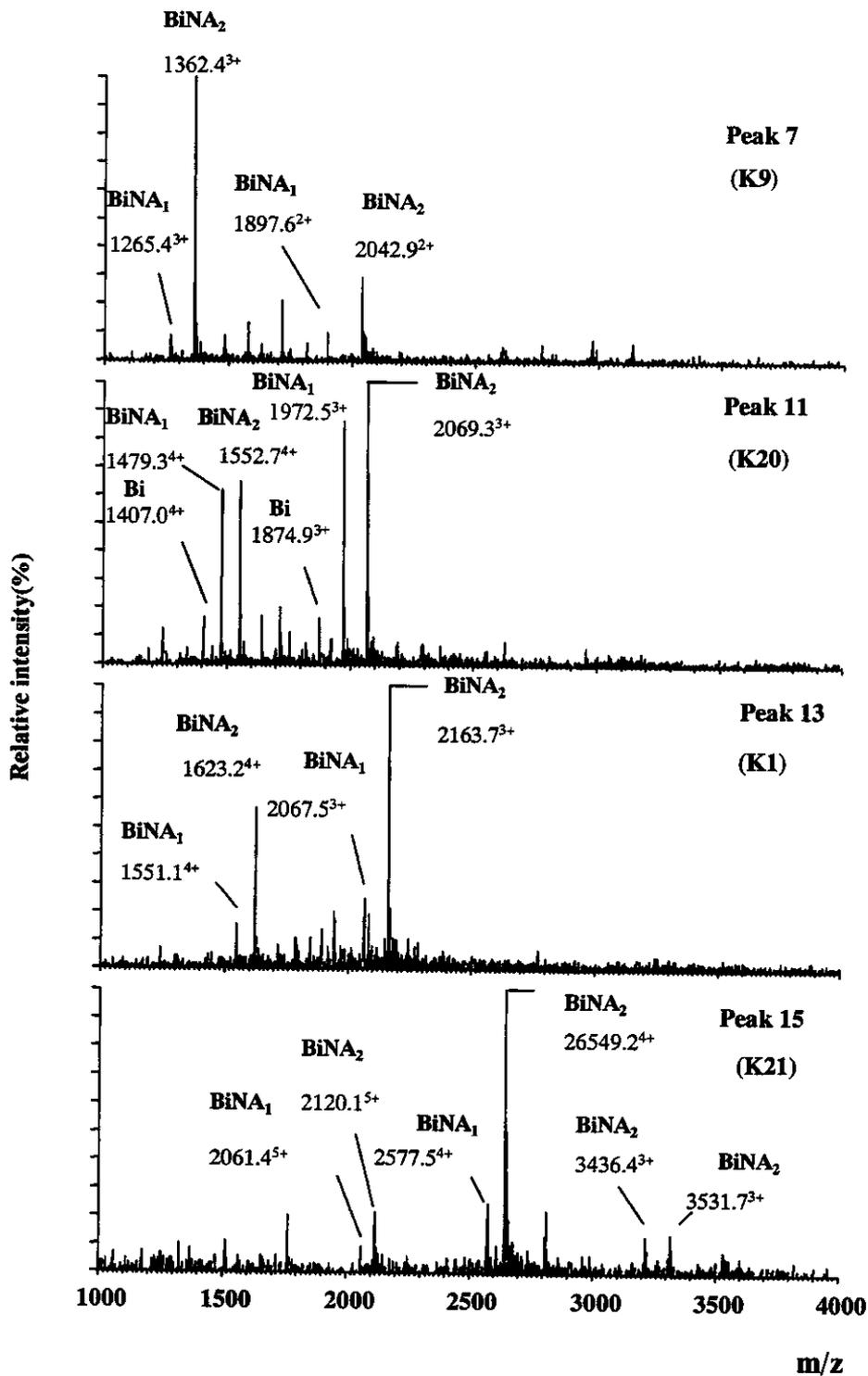
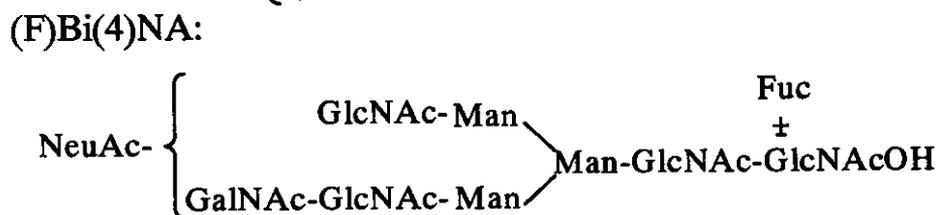
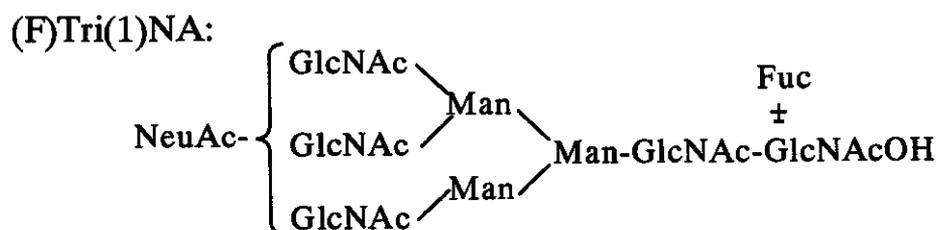
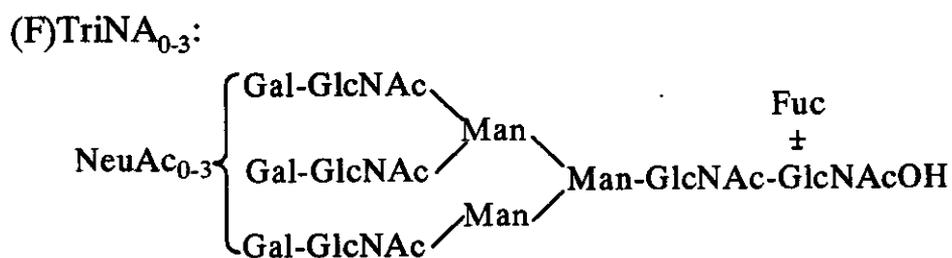
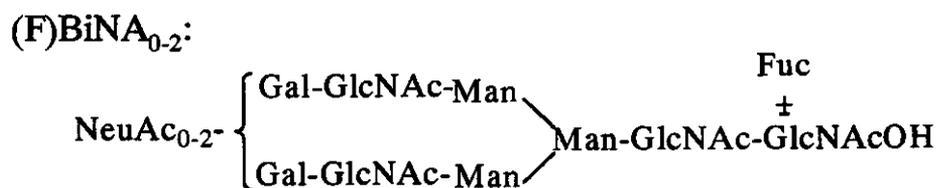
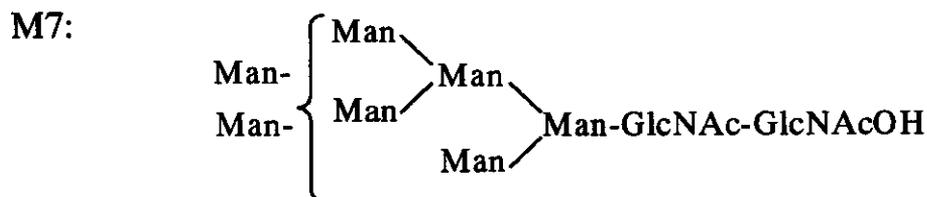
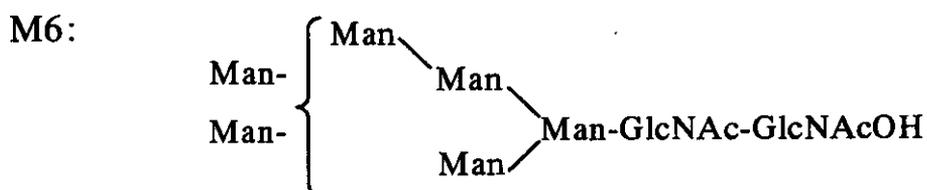
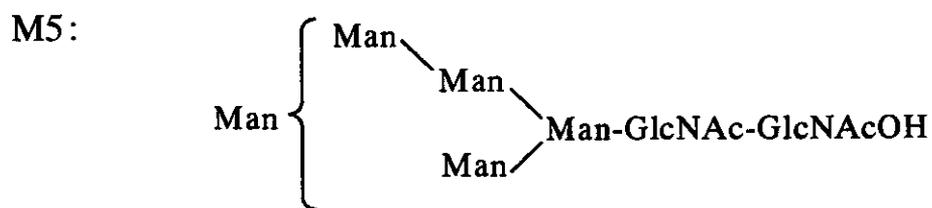


図 28 トロンボモジュリン糖ペプチドのマススペクトル
Bi, biantennary; NA, NeuAc

表8 糖鎖の基本構造



GNCWLRQAKN GRCQVLYKTE LSKEECCSTG RLSTSWTEED
K1
VN /DNTLFKWM IFNGGAPNCI PCKETCENV/D CGPGKKCRMN
K2 K3
KKNKPRCVCA P /DCSNITWKG PVCGL/DGKTY RNECALLKAR
K4 K5
CKEQPELEVQYQGRCKKTCR /DVFCPGSSTC VV /DQTNNAYC
K6 K7
VTCNRICPEPASSEQYLCGN /DGVTYSSACH LRKATCLLGR
K8
SIGLAYEGKC IKAKSCE/DIQ CTGGKKCLWD FKVGRGRCSL
K9 K10 K11
C/DELCP/DSKS /DEPVCAS/DNA TYASECAMKE AACSSGVLL//E
K12 K13 K14 K15 ①
VKHSGSCN
K15②

図 29 フォリスタチンの一次構造、及びAsp-Nによる開裂位置
 下線は糖鎖結合可能性部位を示す。//は部分的開裂位置を示す

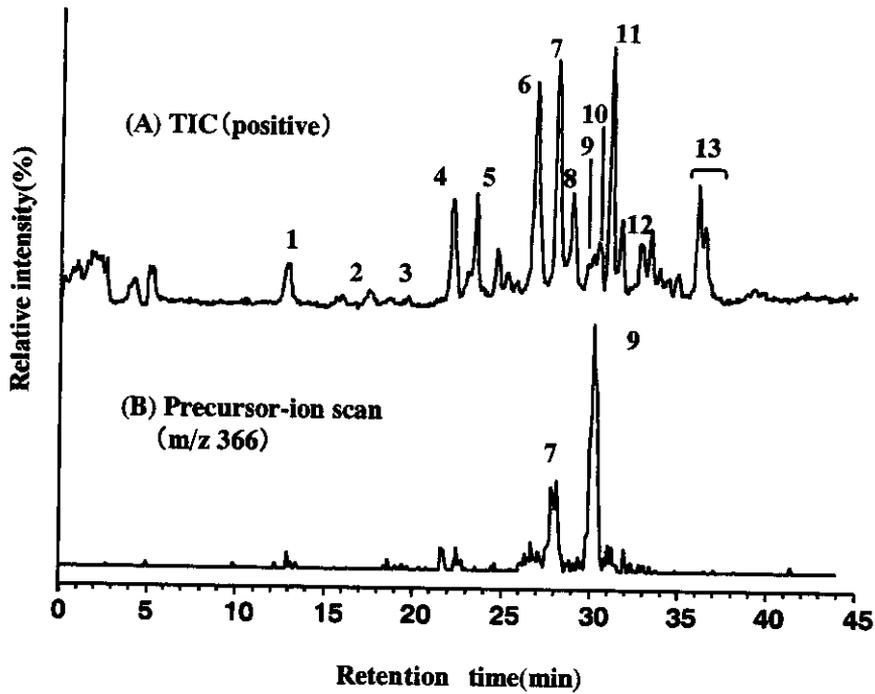


図 30 ミクロLC/MS、及びミクロLC/MS/MSによって得られたフォルリスタチンのペプチド/糖ペプチドマップ(A)、及び糖ペプチドマップ(B)

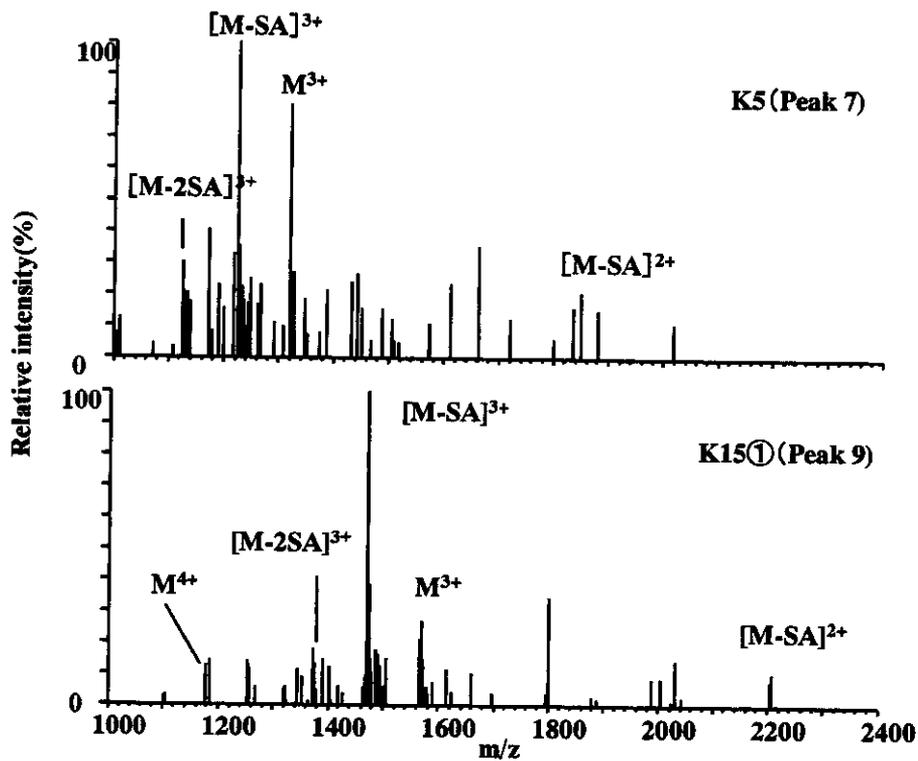


図 31 フォルリスタチン糖ペプチドのマスペクトル

表 9

図 30 中のピークのアミノ酸残基、糖鎖構造、分子量理論値、及び実測値

Peak No.	Peptide No.	Amino acid residues	Carbohydrates	Theoretical mass		Observed mass	
				Positive (low)	Negative (high)	Positive (high)	Negative (high)
1	K4	70-91		2665.0	2666.0	2665.9	2666.6
2	K14	251-257		777.6	777.8	-	-
3	K15②	280-288		1018.4	1018.0	1017.3	
4	K11	230-241		1455.8	1456.6	1456.7	1457.4
5	K6	106-140		4378.9	4378.8	4379.0	4379.6
6	K8	153-180		3325.4	3326.4	3326.4	3325.4
	K10	218-229		1466.8	1467.6	1467.7	1466.8
7	K1	1- 39		4729.4	4728.1	4728.7	4728.6
	K5	92-105	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	3959.8	3960.9	3961.8	3962.4
			[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	3668.3	3669.6	3669.9	3673.6
			[Fuc][Hex] ₅ [HexNAc] ₄	3379.6	3378.3	3379.4	
8	K7	141-152		1328.5	1329.4	1328.9	1329.0
9	K15①	258-249	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	4699.3	4702.8	4703.0	4703.8
			[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	4410.0	4411.4	4411.6	4411.2
			[Fuc][Hex] ₅ [HexNAc] ₄	4116.0	4120.2	4120.1	4121.0
10	K5	92-105		1608.1	1608.7	1608.2	1608.3
11	K9	181-217		4164.6	4165.6	4166.3	4165.8
12	K15①	258-279		2349.6	2350.6	2349.7	2351.2
13	K3	43-69		3218.9	3219.5	3220.2	3219.6

Fuc, fucose; Hex, hexose; HexNAc, *N*-acetylhexosamine; NeuAc, *N*-acetylneuramic acid

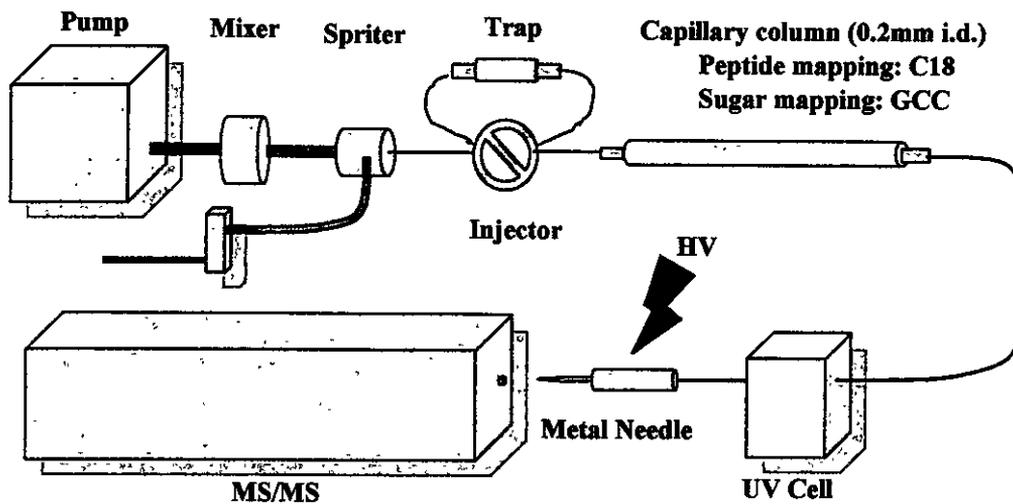


図 32 CapLC/MSの概略

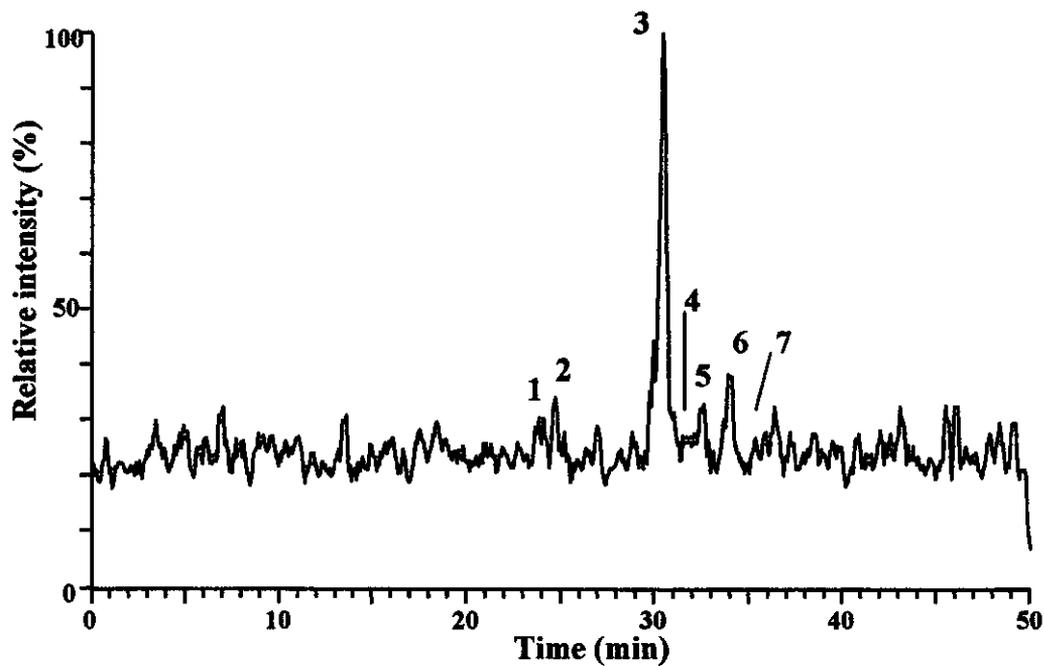


図 33 CapGCC-LC/MSによって得られたrhTMのN結合糖鎖の糖鎖プロファイル

表 10 図33中のピークの糖鎖構造、分子量理論値、及び実測値

Peak No.	Carbohydrate composition ^a	Sugar type ^b	Theoretical mass ^c	Observed <i>m/z</i>	
				M ⁺	M ²⁺
1	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	FucBiNA	2080.9	2081.1	1041.5
2	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	FucBiNA	2080.9	2080.1	1041.6
3	[Fuc][Hex] ₄ [HexNAc] ₃ [NeuAc]	FucBi(1)NA	1715.6	1716.7	
	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	FucBiNA ₂	2372.2	2373.3	1187.4
	[Fuc][Hex] ₆ [HexNAc] ₅ [NeuAc] ₂	FucTriNA ₂ , FucBiLacNA ₂	2737.5		1369.9
4	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	FucBiNA ₂	2372.2	2373.0	1186.9
	[Fuc][Hex] ₆ [HexNAc] ₅ [NeuAc] ₂	FucTriNA ₂ , FucBiLacNA ₂	2737.5		1369.6
5	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	FucBiNA ₂	2372.2	2373.0	1186.9
	[Fuc][Hex] ₆ [HexNAc] ₅ [NeuAc] ₂	FucTriNA ₂ , FucBiLacNA ₂	2737.5		1369.7
6	[Fuc][Hex] ₄ [HexNAc] ₃ [NeuAc]	FucBi(1)NA	1715.6	1716.5	
	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	FucBiNA ₂	2372.2	2372.9	1187.8
	[Fuc][Hex] ₆ [HexNAc] ₅ [NeuAc] ₃	FucTriNA ₃	3028.8		1515.9
7	[Fuc][Hex] ₆ [HexNAc] ₅ [NeuAc] ₃	FucTriNA ₃	3028.8		1516.4
	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	FucBiNA ₂	2372.2	2372.2	1187.5

^a Fuc, fucose; Hex, hexose; HexNAc, *N*-acetylhexsamine; NeuAc, *N*-acetylneuramic acid.

^b Bi, biantennary; Tri, Triantennary; Tetra, tetraantennary; Lac, *N*-acetyllactosamine; NA, NeuAc; FucBi(1),

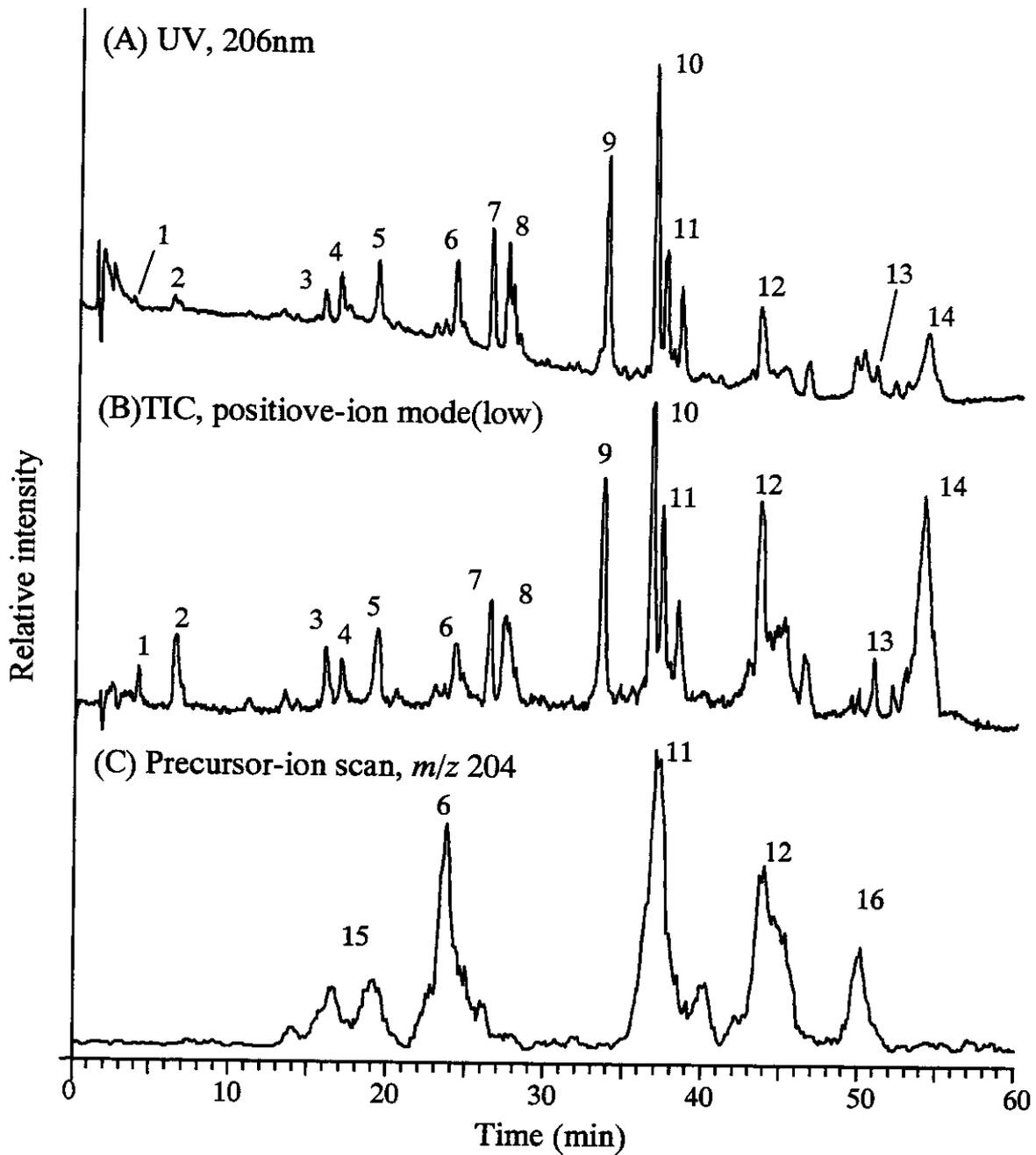
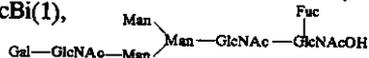


図 34 CapLC/MS及びCapLC/MS/MSによって得られたrhTMのペプチド/糖ペプチドマッピング (A)、(B)、及び糖ペプチドマップ(C).

表 11 図 34 中のピークのアミノ酸残基、糖鎖構造の帰属と理論値、及び実測値

Peak in Fig.1	Peptide No.	Amino acid residues	Carbohydrate structure ^a	Theoretical mass ^b	Observed <i>m/z</i> value				
					M ⁺	M ²⁺	M ³⁺	M ⁴⁺	M ⁵⁺
1	K17	298-304		809.9	810.6				
2	K10	104-106		431.5	432.3				
	K22	457-466		1090.1	1090.5	546.0			
3	K8	84-88		554.7	555.6				
4	K2	39-45		813.0	813.6	407.4			
5	K15	247-260		1476.5	1476.9	739.2			
6	K9 ^c	89-103	FucBiNA ₂	4084.0		2043.4	1362.3		
			FucBiNA	3792.7		1897.8	1265.2		
			FucBi	3501.4		1751.5	1168.7		
			FucBiLacNA ₃ , FucTriNA ₃	4740.5			1582.1		
			FucBiLacNA ₂ , FucTriNA ₂	4449.3			1484.6		
			FucBiLacNA, FucTriNA	4158.0			1387.7		
			FucBi(1)NA	3427.4		1714.6			
7	K18	305-321		2093.1	2093.7	1047.3			
8	K14	220-246		2886.0		1444.1	963.1		
9	K16 ^c	261-297	Hex	4516.5		2259.3	1506.3		
10	K19 ^d	322-353	OH	3916.0		1958.8	1306.2		
	K6	68-82		1651.9	1652.2	826.9			
11	K20 ^c	354-385	FucBiNA ₂	6204.3			2069.7	1552.7	1242.0
			FucBiNA	5913.0			1972.2	1479.3	
			FucBiLacNA ₂ , FucTriNA ₂	6569.6				1643.7	1315.0
			FucBiLacNA, FucTriNA	6278.3				1570.9	
			FucBi(1)	5256.4				1315.0	
			Bi	5475.6			1826.7		
12	K13	183-219		3704.2			1853.7	1235.9	
	K1 ^c	1-38	FucBiNA ₂	6487.6			2164.4	1623.0	
			FucBiNA	6196.3			2067.0	1550.4	
			FucBiLacNA ₂ , FucTriNA ₂	6852.9			2286.0		
			FucBi(1)NA	5831.0			1945.1		
13	K3	46-65		1900.1	1901.7	951.4			
14	K11	107-143		4042.4		2023.3	1348.9		
	K12	144-182		4113.6		2057.6	1371.5		

^a Fuc, fucose; Hex, glucose; Bi, biantennary; Tri, Triantennary; Tetra, Tetraantennary; NA, *N*-acetylneuramic acid; Lac, *N*-acetyllactosamine; FucBi(1),



^b Average mass value.

^c Glycopeptide.

^d Hydroxylated peptide.

10	20	30	40	50
SYQVICRDEK	TQMIYQQHQS	WLRPVLRSNR	VEYCWCNSGR	AQCHSVPVKS
60	70	80	90	100
CSEPRCFNGG	TCQQALYFSD	FVCQCPEGFA	GKCCEIDTRA	TCYEDQGISY
	*			
110	120	130	140	150
RGTWSTAESG	AECTNWNSSA	LAQKPYSGRR	PDAIRLGLGN	HNYCRNPDRD
160	170	180	190	200
SKPWCYVFKA	GKYSSEFCST	PACSEGNSDC	YFGNGSAYRG	THSLTESGAS
210	220	230	240	250
CLPWNSMILI	GKVYTAQNPS	AQALGLGKHN	YCRNPDGDAK	PWCHVLKNRR
260	270	280	290	300
LTWEYCDVPS	CSTCGLRQYS	QPQFRIKGGI	FADIASHPWQ	AAIFAKHRRS
310	320	330	340	350
PGERFLCGGI	LISSCWILSA	AHCFQERFPP	HHLTVILGRT	YRVVPGEEEQ
360	370	380	390	400
KFEVEKYIVH	KEFDDDTYDN	DIALLOLKSD	SSRCAQESSV	VRTVCLPPAD
410	420	430	440	450
LQLPDWTECE	LSGYGKHEAL	SPFYSERLKE	AHVRLYPSSR	CTSQHLLNRT
460	470	480	490	500
VTDNMLCAGD	TRSGGPQANL	HDACQGDSSG	PLVCLNDGRM	TLVGIISWGL
510	520	527		
GCGQKDVPGV	YTKVTNYLDW	IRDNMRP		

図 35 t-PA の 1 次構造

トリプシンによる開裂位置を太字で示す。*印はフコース結合位置と示す。下線はN結合糖鎖の結合可能位置を示す。

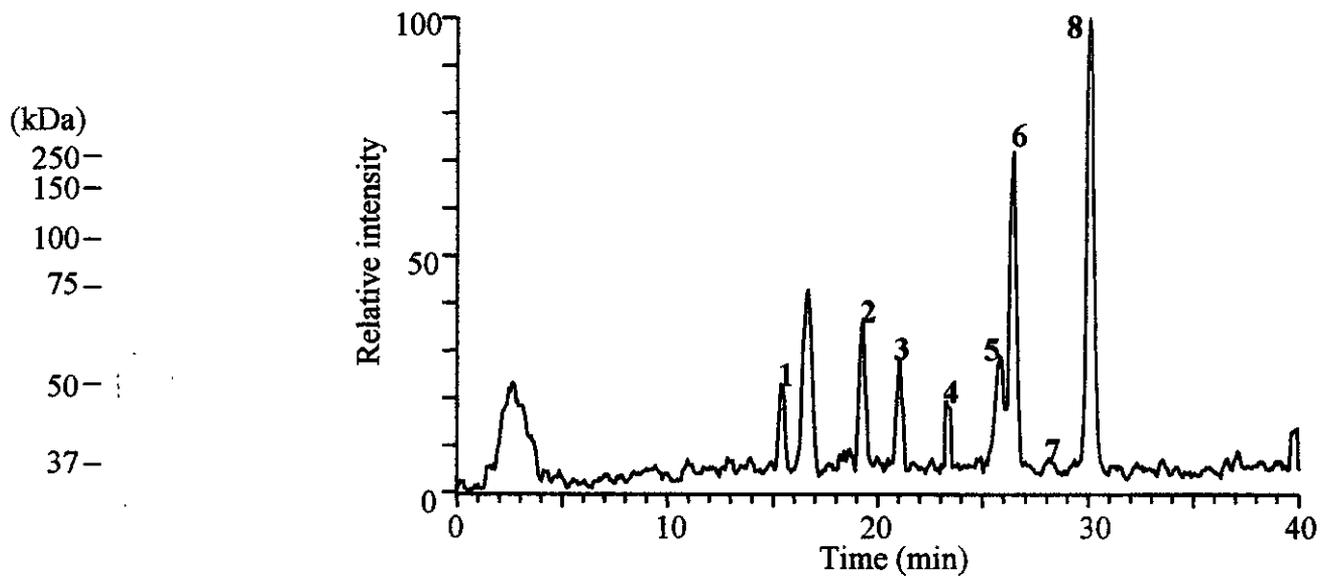


図 36 t-PAのSDS-PAGE

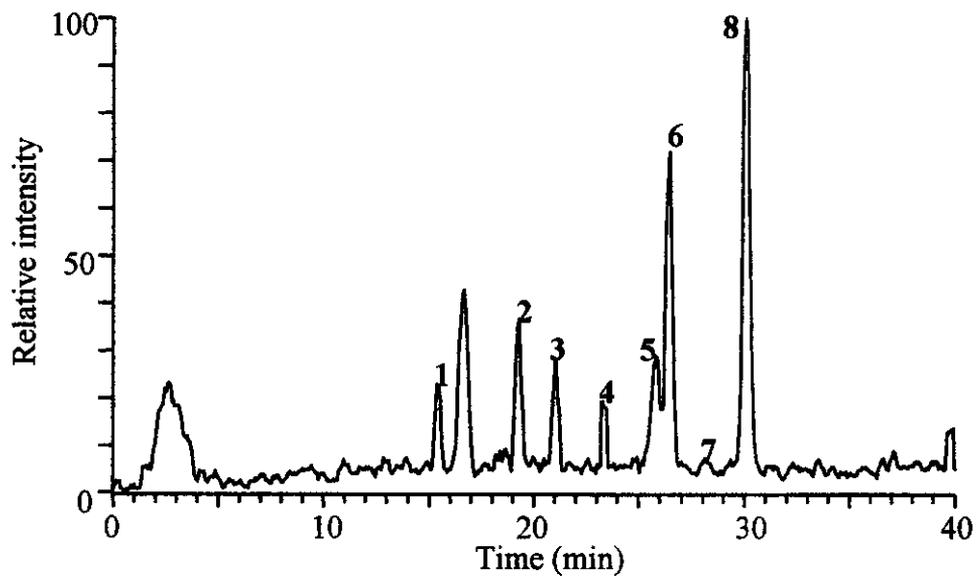


図 37 t-PAの糖鎖プロファイル

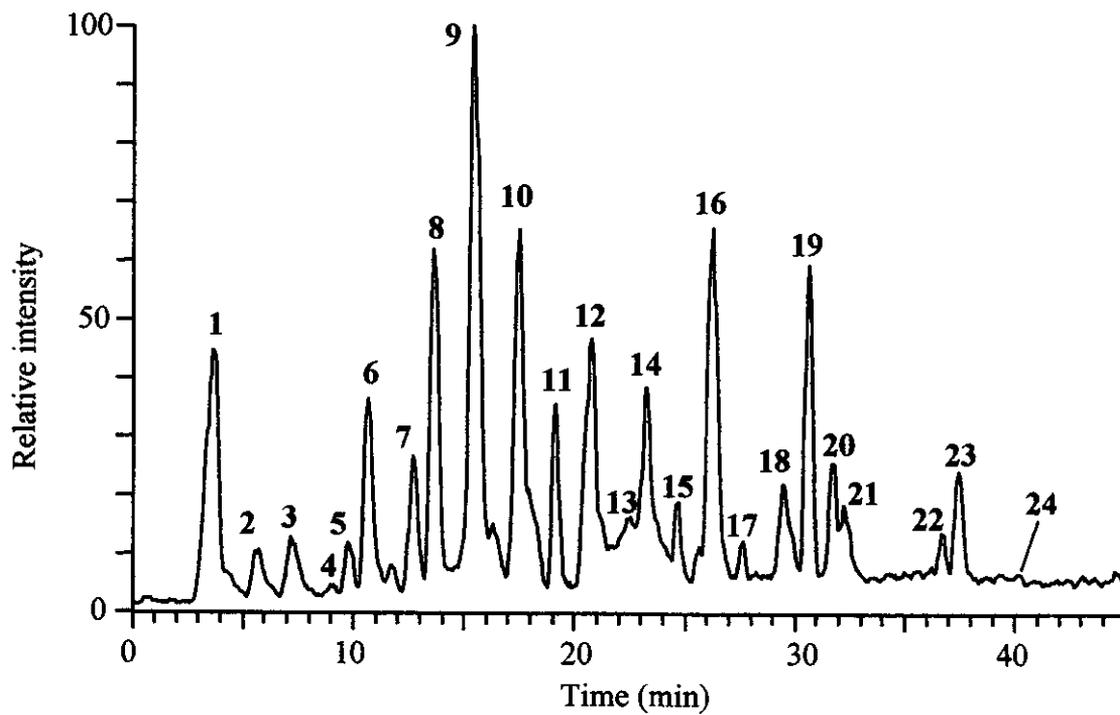


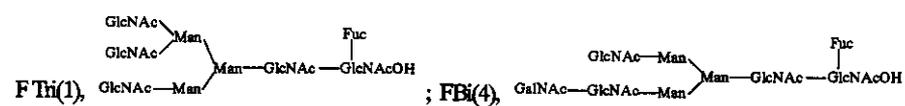
図 38 t-PAのペプチド/糖ペプチドマップ

表 12 図 37 中の各ピークの糖組成、理論分子量、及び実測値

Peak No.	Carbohydrate composition ^a	Sugar type ^b	Theoretical mass ^c	Observed <i>m/z</i>	
				<i>M</i> ⁺	<i>M</i> ²⁺
1	[Hex] ₆ [HexNAc] ₂	M6	1399.3	1400.2	
	[Hex] ₇ [HexNAc] ₂	M7	1561.4	1562.1	
2	[Hex] ₅ [HexNAc] ₂	M5	1237.1	1238.2	
3	[Hex] ₅ [HexNAc] ₂	M5	1237.1	1238.1	
	[Fuc][Hex] ₅ [HexNAc] ₄	FBi	1789.7		895.9
4	[Fuc][Hex] ₃ [HexNAc] ₅ [NeuAc]	FTri(1)NA, FBi(4)NA	1959.8		980.2
5	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	FBiNA	2080.9		1041.1
6	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc]	FBiNA	2080.9		1041.4
7	[Fuc][Hex] ₆ [HexNAc] ₅ [NeuAc] ₂	FTriNA ₂	2737.5		1370.4
8	[Fuc][Hex] ₅ [HexNAc] ₄ [NeuAc] ₂	FBiNA ₂	2372.2		1187.3

^aHex, hexose; Fuc, fucose; HexNAc, *N*-acetylhexosamine; NeuAc, *N*-acetylneuramic acid.

^bF, fucose; NA, *N*-acetylneuramic acid; Bi, biantennary; Tri, triantennary.



^c Average mass value.

表 13 図 38 中の各ピークのアミノ酸残基、理論分子量、及び実測値

Peak No.	Amino acid residues	Theoretical mass ^a	Observed <i>m/z</i>				
			M ⁺	M ²⁺	M ³⁺	M ⁴⁺	M ⁵⁺
1	130-135	726.8	727.5				
	384-392	1036.1	1036.6	519.0			
	428-434	852.0	852.7	426.9			
	435-440	721.8	722.5				
2	41-55	1743.9		872.7	582.2	436.9	
3	506-513	878.0	878.5	439.8			
4	50-55	735.7	736.0				
5	506-513	878.0	878.4	439.8			
6	1-7	926.0	926.5	463.8			
	1-10	1298.4	1298.9	650.0	433.7		
7	146-162	2069.3		1036.6	518.9		
8	90-101	1463.5	1464.0	732.6			
	136-145	1204.3	1204.8	603.0	402.4		
	450-462	1454.6	1454.7	728.0	485.8		
9	343-356	1646.8	1647.8	824.2	549.9		
	28-40	1689.7	1690.1	845.6	564.1		
10	213-228	1617.8	1619.5	809.7	540.3		
	268-275	1053.1	1054.6	527.9			
	229-247	2369.6		1186.1	790.7	593.4	
11	234-247	1637.8	1638.9	819.7	546.9		
	102-129 ^b (M5)	4235.2		2119.1	1412.4		
12	102-129 ^b (M6)	4397.4		2199.9	1466.7		
	102-129 ^b (M7)	4559.5			1520.9		
	463-489	2754.8		1378.8	919.2		
13	163-189	3077.0		1540.5	1026.9		
14	11-27	2184.5		1092.9	729.1	547.1	
	146-159	1813.0	1814.2	907.5	605.3		
	250-267	2263.4		1132.4	755.2		
15	136-159	2999.3		1500.2	1000.7	750.7	601.7
	251-267	2107.2	2109.2	1054.2			
	357-378	2668.9		1335.2	890.5	668.2	
16	357-378	2668.9		1336.1	890.5	668.3	
	514-522	1179.3	1179.8	590.6			
17	362-378	2028.2	2028.6	1015.0	677.1		
18	56-82 ^c	3299.4		1651.0	1101.2		
	190-212	2460.8		1231.8	821.2		
	514-527	1793.0	1794.6	897.3	598.7		
19	276-296	2241.6	2242.1	1122.0	748.2	561.4	
20	278-296	2000.3	2000.5	1000.9	667.7		
	393-416	2752.0		1376.6	918.1		
21	393-427	4069.4		2035.9	1358.0	1018.3	
22	229-249	2639.9		1320.5	880.0		
	490-513	2581.0		1291.5	861.2	646.2	
23	490-505	1721.0	1722.6	861.2	574.6		
24	305-327	2729.0		1366.1	910.6		

^a Average mass value. ^b Glycopeptide. M5; Man₅GlcNAc₂, M6; Man₆GlcNAc₂, M7; Man₇GlcNAc₂. ^c Peptide with fucose.

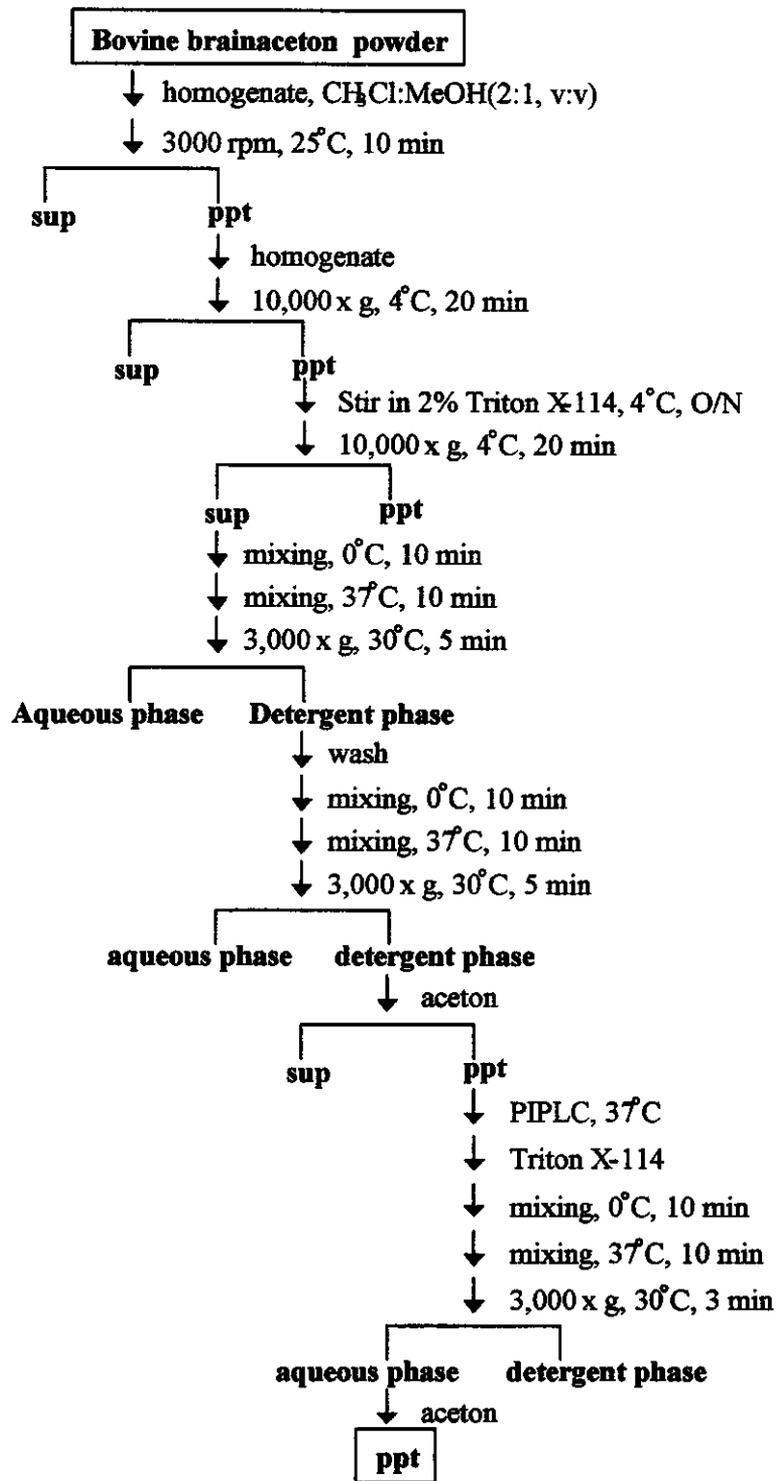


図 39 ウシ脳可溶性膜タンパク質画分の調製方法の概略.

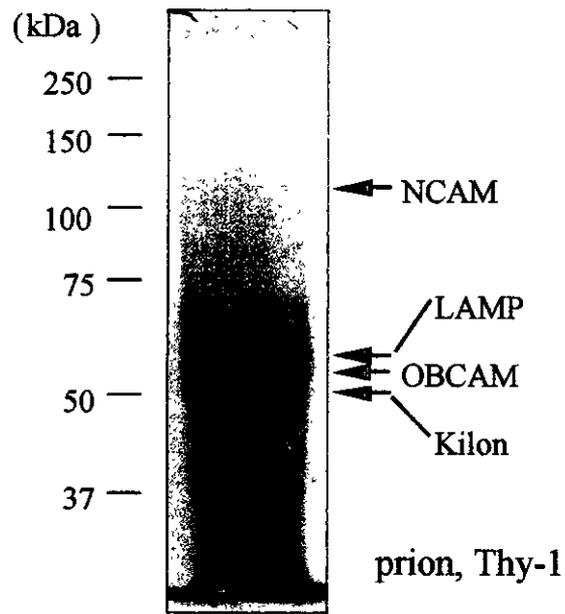


図 40 ウシ脳可溶性膜タンパク質の SDS-PAGE

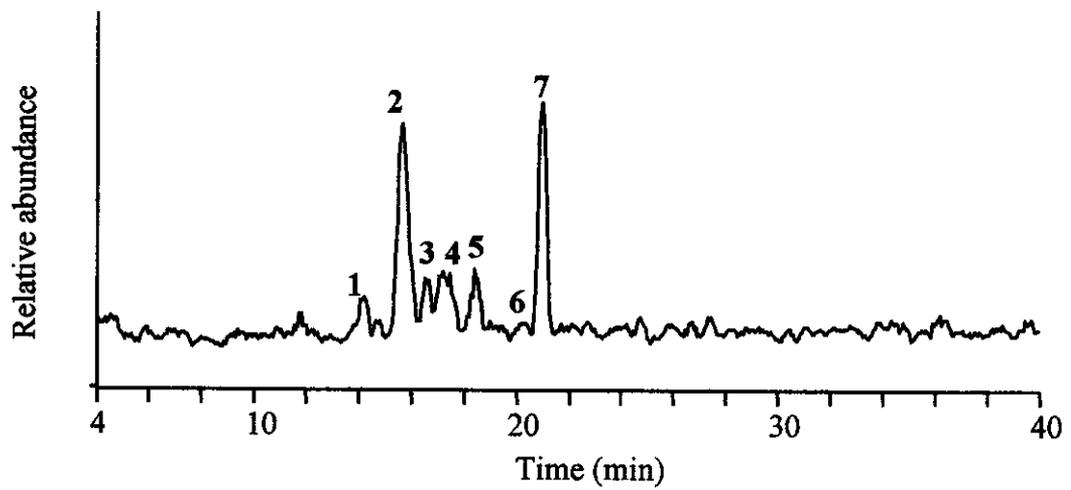


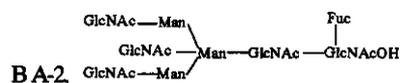
図 41 Kilonの糖鎖プロファイル

表 14 図 41 中の各ピークの糖組成、糖鎖推定構造、理論分子量、及び実測値

Peak No.	Carbohydrate composition ^a	Proposed sugar type ^b	Theoretical mass ^c	Observed mass ^c
1	[dHex] ₂ [Hex] ₅ [HexNAc] ₄	[FucCore]+[dHex] ₁ [Hex] ₂ [HexNAc] ₂	1935.8	1935.8
	[dHex] ₁ [Hex] ₄ [HexNAc] ₄	[FucCore]+[Hex][HexNAc] ₂	1627.5	1628.0
2	[dHex] ₃ [Hex] ₄ [HexNAc] ₄ [SO ₃] ₁	[FucCore]+[dHex] ₂ [Hex] ₁ [HexNAc] ₂ [SO ₃] ₁	1999.9	1999.0
	[dHex] ₁ [Hex] ₄ [HexNAc] ₅	[FucCore]+[Hex] ₁ [HexNAc] ₃	1976.9	1997.4
	[dHex] ₁ [Hex] ₃ [HexNAc] ₅	[FucCore]+[HexNAc] ₃ (BA-2)	1668.6	1669.0
	[dHex] ₁ [Hex] ₅ [HexNAc] ₄	[FucCore]+[Hex] ₂ [HexNAc] ₂	1789.7	1790.2
3	[Hex] ₃ [HexNAc] ₆ [SO ₃] ₂	[Core]+[Hex] ₃ [HexNAc] ₄ [SO ₃] ₂	1885.8	1885.8
	[Hex] ₉ [HexNAc] ₂	[Core]+[Hex] ₆ (M9)	1885.7	1885.8
4	[Hex] ₆ [HexNAc] ₂	[Core]+[Hex] ₃ (M6)	1399.3	1399.5
	[Hex] ₇ [HexNAc] ₂	[Core]+[Hex] ₄ (M7)	1561.4	1521.6
	[dHex] ₁ [Hex] ₆ [HexNAc] ₃	[FucCore]+[Hex] ₃ [HexNAc] ₁	1748.6	1749.6
	[dHex] ₁ [Hex] ₅ [HexNAc] ₄	[FucCore]+[Hex] ₂ [HexNAc] ₂	1789.7	1790.2
6	[dHex] ₃ [Hex] ₅ [HexNAc] ₆	[FucCore]+[dHex] ₂ [Hex] ₂ [HexNAc] ₄	2082.0	2082.8
	[dHex] ₁ [Hex] ₅ [HexNAc] ₄ [NeuAc] ₁	[FucCore]+[Hex] ₂ [HexNAc] ₂ [NeuAc] ₁ (FucBiNA)	2080.9	2082.8
7	[Hex] ₅ [HexNAc] ₂	[Core]+[Hex] ₂ (M5)	1237.1	1237.8

^aHex, hexose; dHex, deoxyhexose; HexNAc, *N*-acetylhexosamine; NeuAc, *N*-acetylneuramic acid; SO₃, sulfate.

^bFuc, fucose; Core, trimannosyl core; M5, Man₅GlcNAc₂; M6, Man₆GlcNAc₂; M7, Man₇GlcNAc₂; M9, Man₉GlcNAc₂.



^c Average mass value.

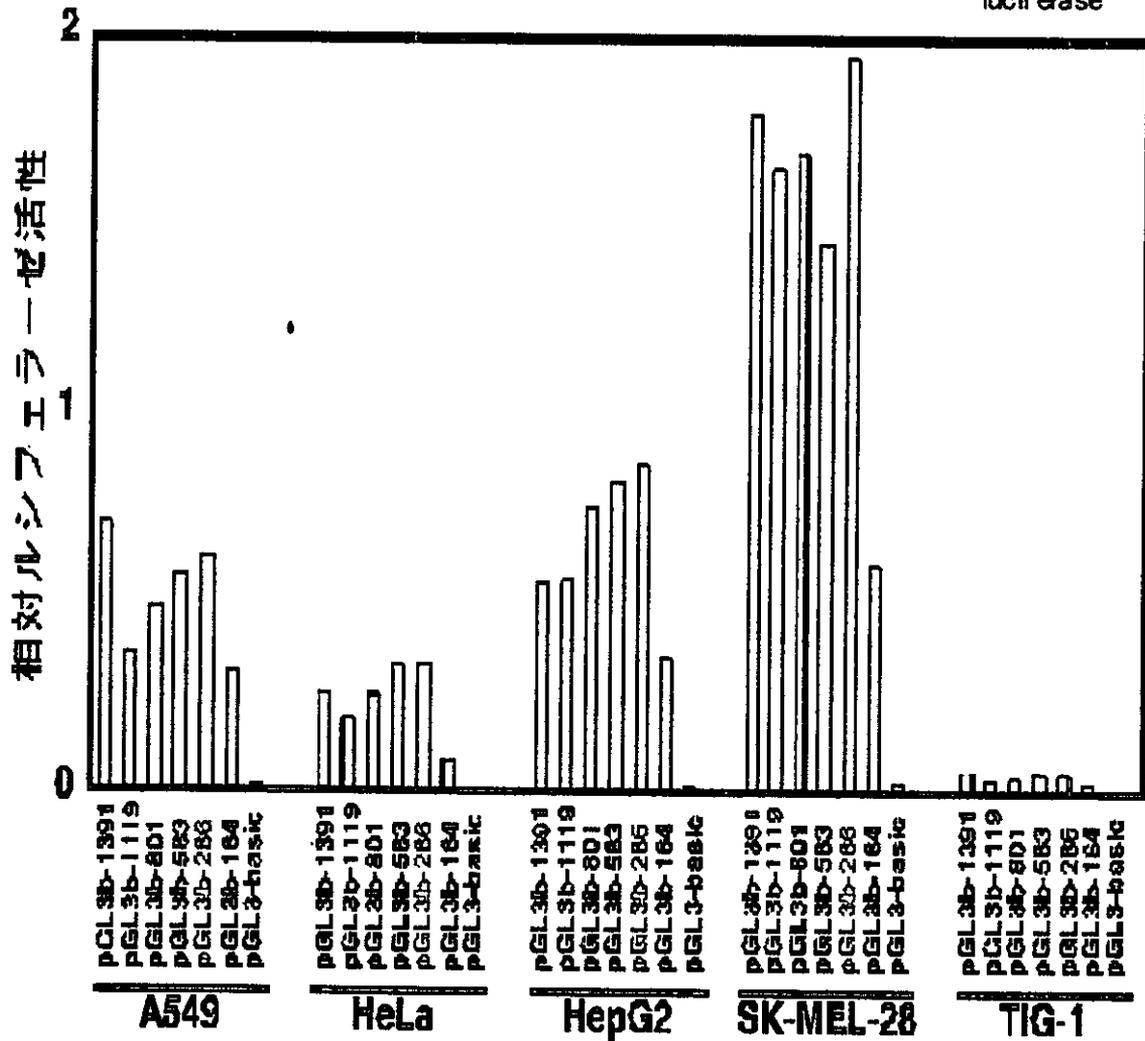
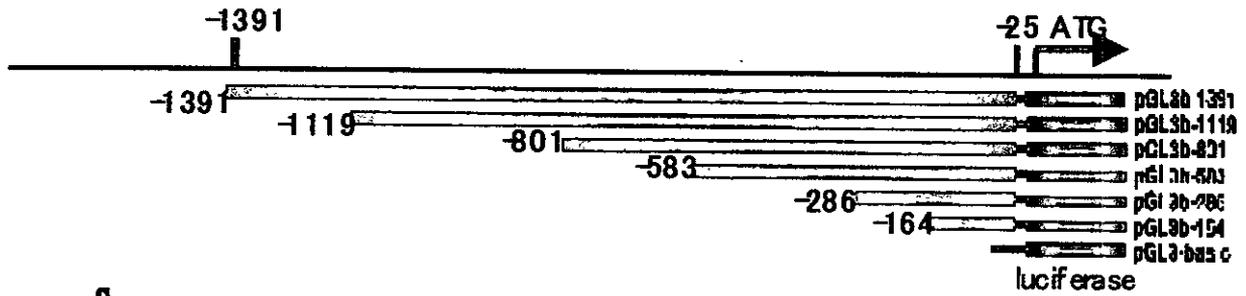


図42 hTERT プロモーターのガン細胞特異的活性化

図43 c-mycによるhTERTのコアプロモーターの転写活性について

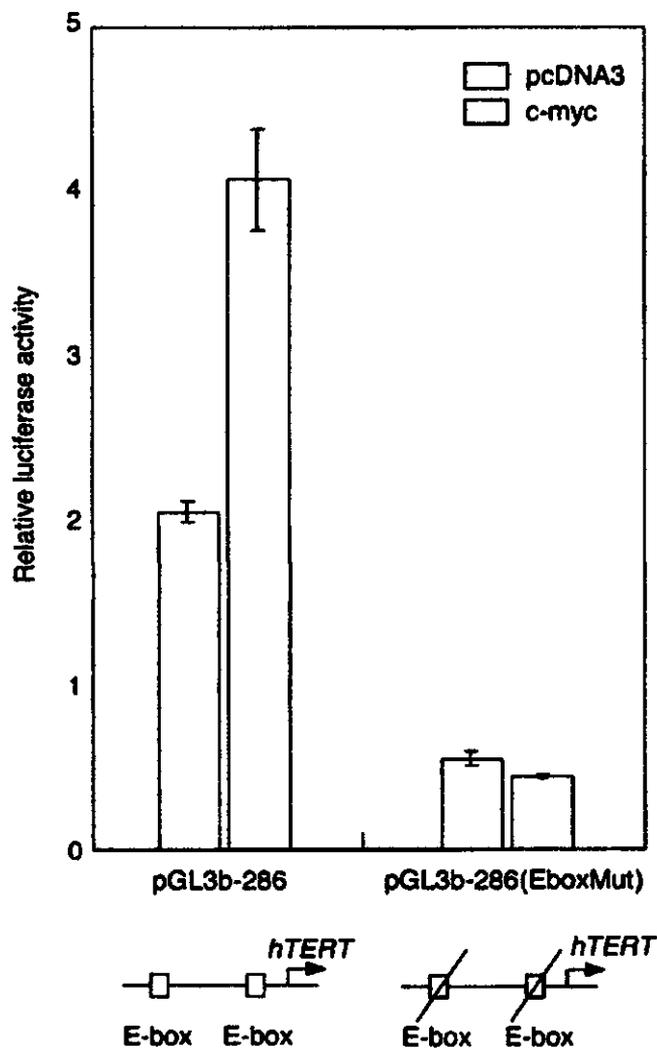


Figure Transcriptional activation of the core promoter region of *hTERT* by *c-myc*. A human *c-myc* cDNA expression plasmid (pcDNA3/HA-*c-myc*) or vector alone (pcDNA3) was co-transfected with the firefly luciferase construct (pGL3b-286 or pGL3b-286 (EboxMut)) and the pRL-TK into the A549 cells. The means from the three independent experiments are shown. Bars indicate standard deviations.