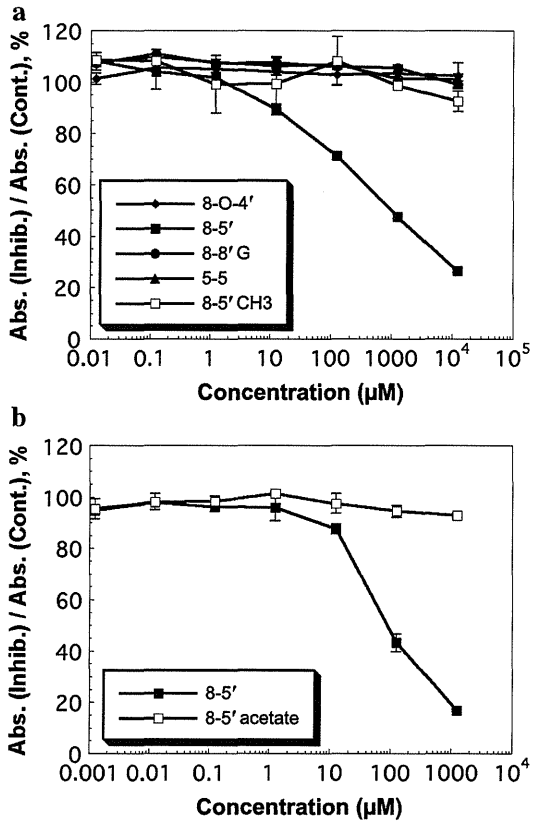

Fig. 3 Specificity of KM1 antibody as revealed by competitive ELISA. Dehydrodiconiferyl alcohol-*p*AHA–BSA conjugate was coated on microtiter plate wells. Different lignin model compounds (Fig. 1) were added to compete with the coated antigen for KM1 antibody recognition. Antibodies bound to coated antigen were detected with a secondary antibody labeled with peroxidase using *o*-phenylendiamine as substrate. Dehydrodiconiferyl alcohol was the only lignin model compound able to inhibit the binding of KM1 antibody. The difference was at least 1000-fold. Values are expressed as absorbance ratio ± SD ($n = 3$). **a** Comparison between model compounds with different linkages. **b** Comparison between dehydrodiconiferyl alcohol and dehydrodiconiferyl alcohol acetate

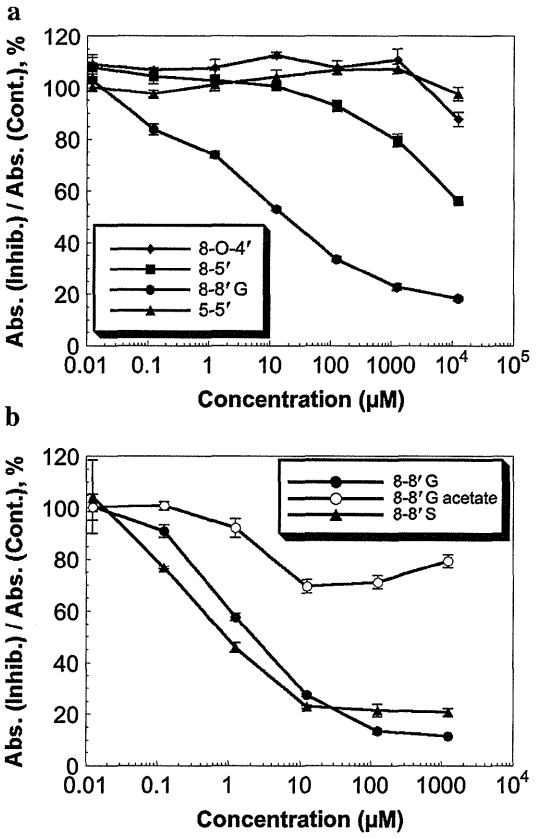

Fig. 4 Specificity of KM2 antibody as revealed by competitive ELISA. Pinoresinol-*p*AHA–BSA conjugate was coated on microtiter plate wells. Different lignin model compounds (Fig. 1) were added to compete with the coated antigen for KM2 antibody recognition. Antibodies bound to coated antigen were detected with a secondary antibody labeled with peroxidase using *o*-phenylendiamine as substrate. Pinoresinol and syringaresinol were the lignin model compounds able to inhibit the binding of KM2 antibody. The difference was at least 1000-fold. Values are expressed as absorbance ratio ± SD ($n = 3$). **a** Comparison between model compounds with different linkages. **b** Comparison between pinoresinol, pinoresinol acetate and syringaresinol

IgG2b) for further characterization. In addition, ascites fluid was produced for KM1 antibody and also used for immunolabeling at a dilution of 1:100.

Competitive ELISA tests

The specificities of the antibodies were determined by competitive ELISA tests using eight model lignin compounds (inhibitors) with various kinds of linkages, as shown in Fig. 1. The inhibitors were soluble in methanol; thus, the experimental tests included methanol. The control (0 % inhibition) had the same concentration of methanol but no inhibitors. The absorbance at 492 nm measured the extent of antibody binding to dehydrodiconiferyl alcohol- or pinoresinol-*p*AHA–BSA conjugate. Competition by the model lignin compounds for antibody recognition (i.e., inhibition) could be detected as a decrease in the absorbance. The absorbance ratio was calculated as the value from the experimental test over the value from the control. Figures 3 (KM1 antibody) and 4 (KM2 antibody) show the absorbance ratios plotted against the common logarithm of inhibitor concentrations for the various model compounds. The curves shift to lower concentrations when the antibody reacts well with the model compound used.

From Fig. 3a, it is evident that inhibition of the KM1 antibody by dehydrodiconiferyl alcohol with 8-5′ linkage was much greater (>1000-fold) than the inhibition by guaiacylglycerol-beta-guaiacyl ether with 8-*O*-4′ linkage, pinoresinol with 8-8′ linkage, or dehydrodidihydroeugenol with 5-5′ linkage. Thus, the KM1 antibody reacted specifically with dehydrodiconiferyl alcohol with 8-5′ linkage. Interestingly, no clear inhibition was found by dehydrodiisoeugenol,

which also contains an 8-5′ linkage but with a different side chain. This indicates that the KM1 antibody recognized structural differences in the side chains. No clear inhibition was found by dehydrodiconiferyl alcohol acetate (Fig. 3b), indicating that the presence of a free phenolic group and/or a hydroxyl group in the side chain was important for recognition by the KM1 antibody.

Similarly, Fig. 4a shows that inhibition of the KM2 antibody by pinoresinol with 8-8′ linkage was at least three orders of magnitude greater than the inhibition by guaiacylglycerol-beta-guiaiacyl ether with 8-*O*-4′ linkage, dehydrodiconiferyl alcohol with 8-5′ linkage, or dehydrodidihydroeugenol with 5-5′ linkage. Thus, the KM2 antibody reacted specifically with pinoresinol with 8-8′ linkage. The inhibition by pinoresinol acetate was also less than that by pinoresinol (Fig. 4b), showing that the presence of at least one free phenolic group was important for the reactivity of the KM2 antibody. Interestingly, inhibition by syringaresinol was almost similar to that by pinoresinol (Fig. 4b), showing that the KM2 antibody should react with 8-8′ linked structure with both guaiacyl and syringyl units.

Immunogold silver staining for light microscopy

Among different dilutions (1:10, 1:5, and no dilution) of hybridoma supernatant, the best signal to noise ratio was obtained from the supernatant with no dilution. Light micrographs of transverse sections treated with KM1 and KM2 antibodies are shown in Figs. 5 and 6, respectively. Although there was some background labeling especially in cell lumina, there was no labeling on the cell walls in control sections without primary antibody (Fig. 5a, c). In phloem, strong labeling of KM1 antibody was found in secondary walls of phloem fibers, but there was no labeling

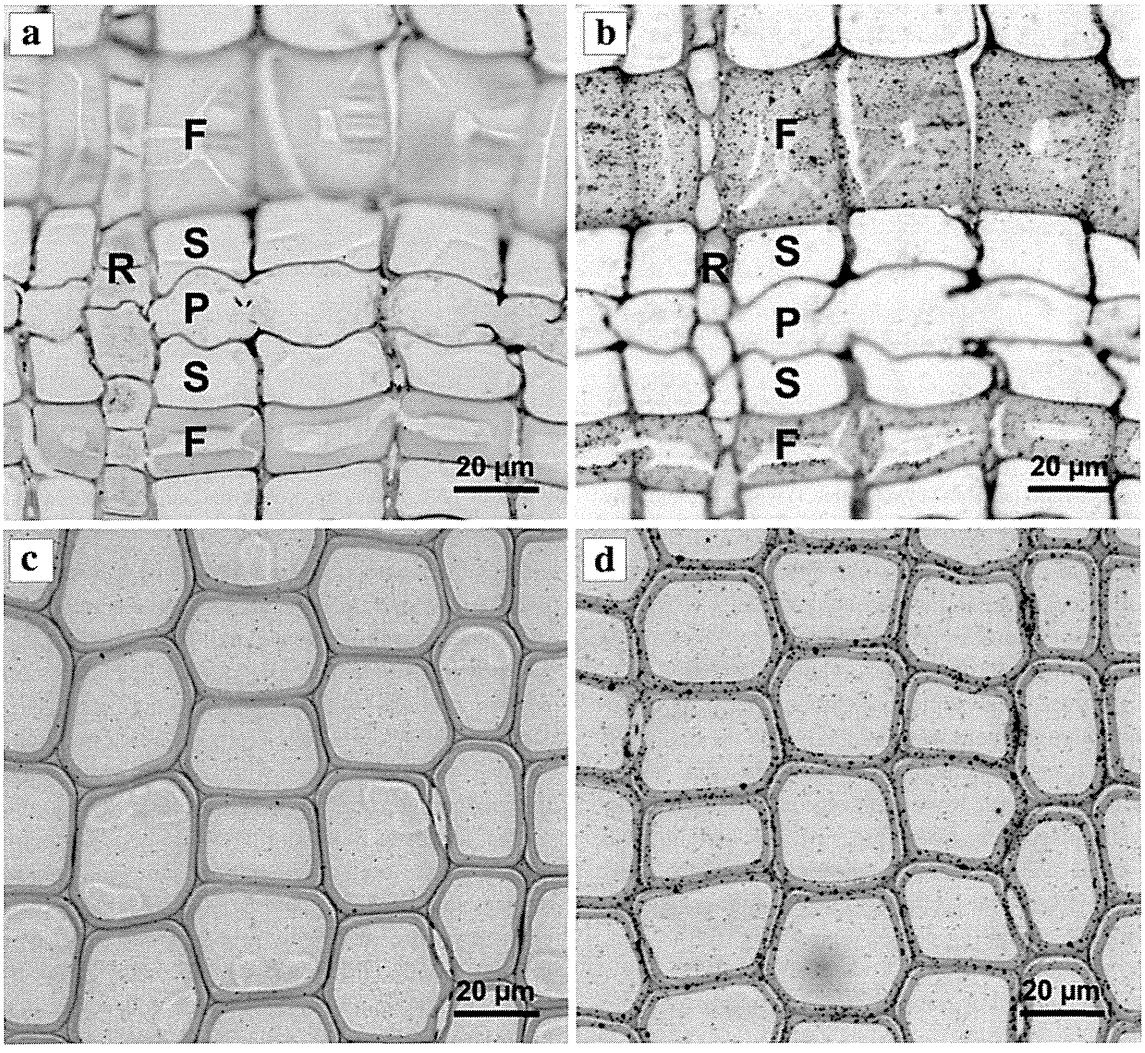

Fig. 5 Immunogold silver staining of a transverse section of *Chamaecyparis obtusa* (Japanese cypress, tree 1) with KM1 antibody. **a**, **c** Control (omission of the primary antibody). **b**, **d** Sections treated with KM1 antibody (1 % ascites fluid) in 5 % skim milk in PBS. **a**, **b** Secondary phloem. **c**, **d** Developed xylem. *F* phloem fiber, *P* phloem parenchyma cell, *R* phloem ray parenchyma cell, *S* sieve cell

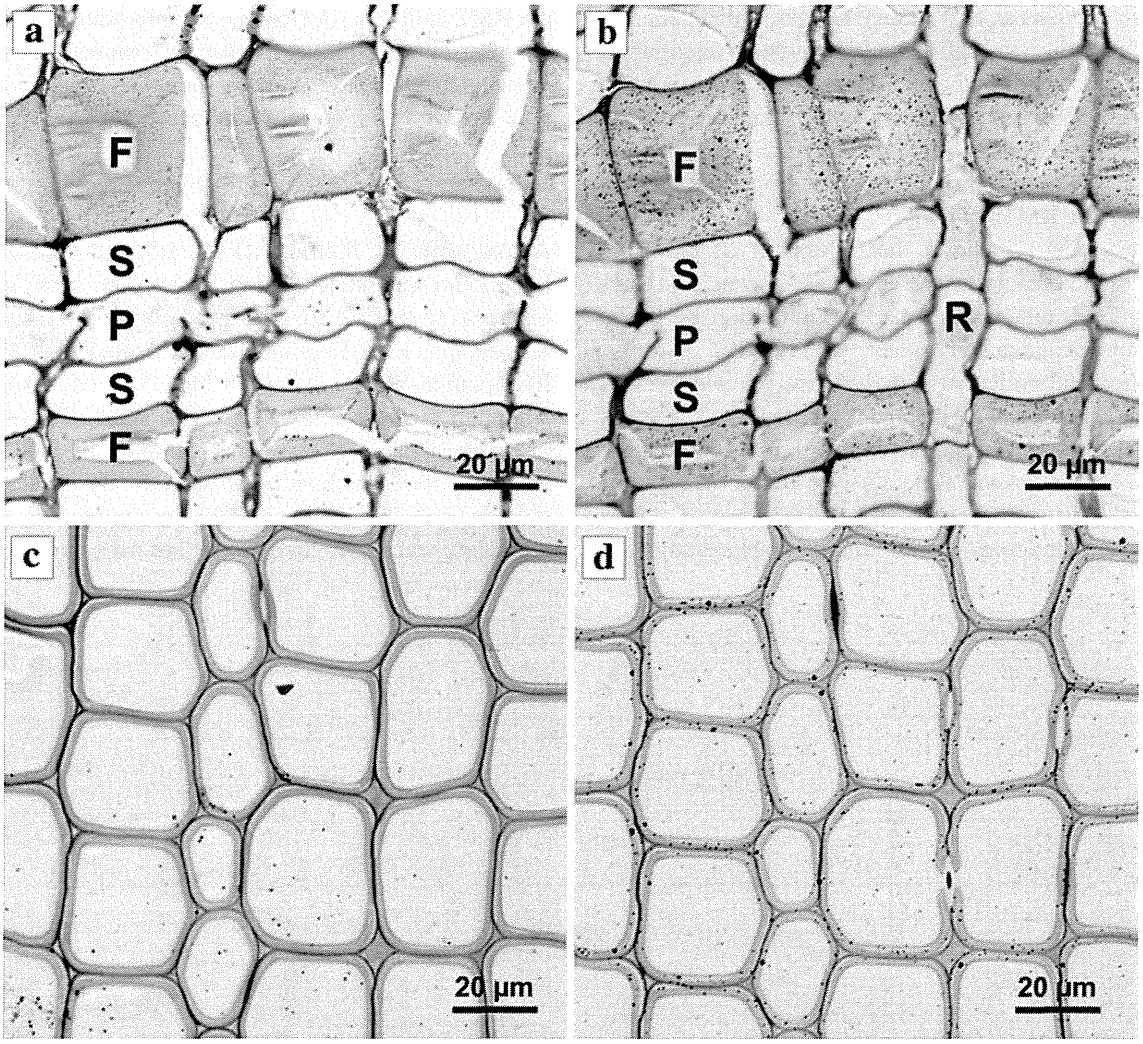

Fig. 6 Immunogold silver staining of a transverse section of *Chamaecyparis obtusa* (Japanese cypress, tree 1) with KM2 antibody. **a**, **c** Control (omission of the primary antibody). **b**, **d** Sections treated with hybridoma supernatant. **a**, **b** Phloem. **c**, **d** Developed xylem. *F* phloem fiber, *P* phloem parenchyma cell, *R* phloem ray parenchyma cell, *S* sieve cell

in other phloem cells, namely, sieve cells, phloem parenchyma cells, and phloem ray parenchyma cells (Fig. 5b). The labeling was found in both compound middle lamella and secondary walls in developed xylem (Fig. 5d).

Although there was some background labeling especially in cell lumina, there was no labeling on the cell walls in control sections without primary antibody (Fig. 6a, c). Moderate labeling with KM2 antibody was found in secondary walls of phloem fiber (Fig. 6b). No labeling was found in other phloem cells. In developed xylem, weak labeling was found in secondary walls and compound middle lamellae excluding corner region (Fig. 6d).

Immunogold labeling for transmission electron microscopy

As for immunogold silver staining, the best signal-to-noise ratio was obtained from the supernatant with no dilution among different dilutions (1:10, 1:5, and no dilution) of hybridoma supernatant. Transmission electron micrographs of transverse sections treated with KM1 and KM2 antibodies are shown in Fig. 7. There was no labeling on the cell walls in control sections without primary antibody (Fig. 7c, d). Strong labeling with KM1 antibody was found in the S_1 and S_2 layers of phloem fibers, with virtually no labeling found in the compound middle lamellae (Fig. 7a). In developed xylem, however, labeling of KM1 antibody was found uniformly in the compound middle lamellae, including cell corners and the S_1, S_2, and S_3 layers of tracheids (Fig. 7e). Labeling was occasionally found in the warty layers. Weak labeling by the KM2 antibody was found in the S_1 and S_2 layers of phloem fibers, with no labeling found in the compound middle lamellae (Fig. 7b). In developed xylem, weak labeling by the KM2 antibody was found in the S_1 and S_2 layers of tracheids, and virtually no labeling was found in the compound middle lamellae or warty layers (Fig. 7f).

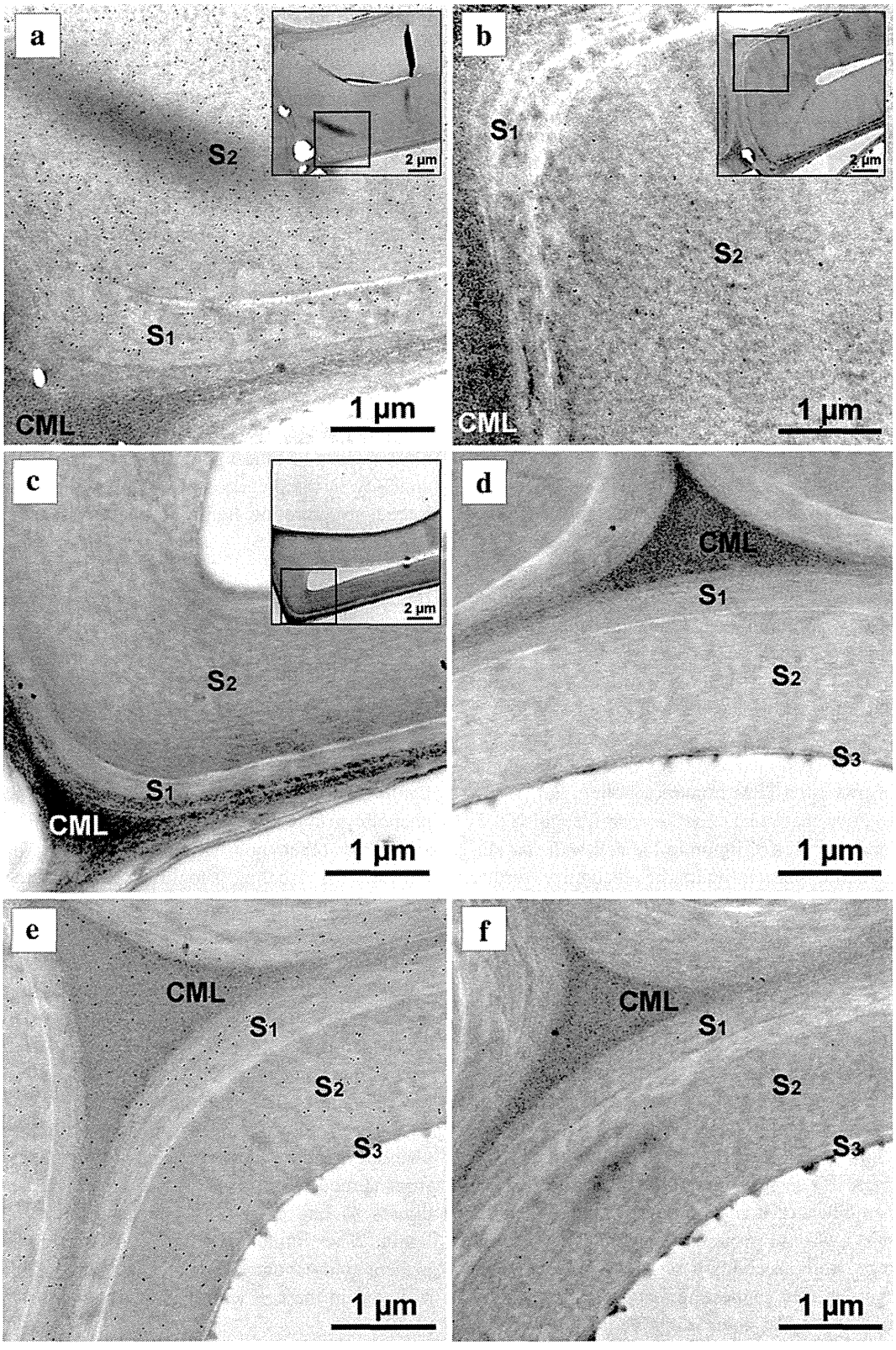

Fig. 7 Transmission electron micrographs of transverse sections of phloem fiber (**a, b, c**) and developed tracheids (**d, e, f**) from *Chamaecyparis obtusa* (Japanese cypress, tree 2). **a, e** Treated with KM1 antibody. **b, f** Treated with KM2 antibody. **c, d** Control (omission of the primary antibody). **a, b, c** Enlargement of the *square region* shown in each *inset* (low-magnification image)

Discussion

Specificity of monoclonal antibodies

The results of the competitive ELISA tests clearly showed that the KM1 and KM2 antibodies specifically recognized 8-5′ and 8-8′ linked dimers, respectively. In addition, KM2 antibody also recognizes 8-8′ structure with both guaiacyl and syringyl units. Morreel et al. (2004) detected dehydrodiconiferyl alcohol and pinoresinol in methanol extracts of differentiating xylem from poplar. Therefore, these antibodies should make useful probes for in situ detection of the presence of the dimers and/or oligolignols and/or their glucosides in differentiating xylem, provided samples can be prepared so as to minimize loss of the dimers.

Competitive ELISA with acetate derivatives of dehydrodiconiferyl alcohol or pinoresinol showed that the presence of at least one hydroxyl group (the phenolic or the aliphatic) is needed for the recognition with the KM1 antibody and the presence of at least one phenolic hydroxyl group is necessary for the recognition of the KM2 antibody.

Although the presence of phenolic and/or aliphatic hydroxyl groups was important for their reactivities, these antibodies should also make effective probes for the detection of 8-5′ and 8-8′ linked structures of polymerized lignin in the cell wall if the specimens were prepared so that free dimers are removed. Since the possibility of the presence of free dimers in the specimen is quite low in our sample preparation of tree 2 for TEM immunolabeling, our immunolabeling suggests that our antibodies can recognize 8-5′ and 8-8′ linked structures of lignin in the cell wall. To elucidate exact structure that our antibodies recognize, further studies should be necessary using trimers with monolignols attached to specific sites of the dimers used in this study.

Immunolocalization of the 8-5′ linked structure in Japanese cypress

Strong immunolabeling with KM1 antibody was found in the secondary walls of phloem fibers, whereas almost no labeling was found in compound middle lamella. Although there is very limited information about the lignin structure in phloem fibers, this result nevertheless indicates that the lignin in phloem fibers contains 8-5′ linkages with phenolic and/or aliphatic hydroxyl groups. As compared with tracheid secondary walls, secondary walls of phloem fibers showed stronger labeling suggesting that lignin structure of secondary walls of phloem fibers might be different (richer in 8-5′ structure) from that of secondary walls of tracheids in xylem. In secondary phloem, phloem fibers only have lignified secondary walls (Miyakawa et al. 1973). Therefore, the manner of lignification of phloem fibers might be different from that of tracheids in xylem as demonstrated previously by Ruel et al. (2009) in fibers and vessels from xylem. Since no labeling was found in cambial zone and other non-lignified tissues in the secondary phloem, the labeling should be specific to lignin substructures, as opposed to other cell wall components, like cellulose, hemicellulose, and pectin.

Labeling with the KM1 antibody was also found in the compound middle lamellae and secondary walls of developed tracheid cells. This result shows that 8-5′ linked structures of lignin were present in mature tracheid cell walls. Lapierre et al. (1991) analyzed dimers from spruce lignin after thioacidolysis and reported that the major carbon–carbon bonds were observed to be represented by the 8-5′, 5-5′ and 8-1′ linkages established between two guaiacyl units. Jouanin et al. (2000) also reported that the frequency of 8-5′ dimers was relatively high among that of main dimers of pine lignin. The immunolabeling of KM1 antibody in the present study showed the presence of 8-5′ linked structure of lignin in the cell wall in Japanese cypress.

Immunolocalization of the 8-8′ linked structure in Japanese cypress

Moderate labeling with KM2 antibody was found in the secondary walls of phloem fibers, whereas almost no labeling was found in compound middle lamella. This result indicates that the walls of phloem fibers contain the lignin substructure with 8-8′ linkage and at least one free phenolic group. Since no labeling was found in cambial zone and other non-lignified tissues in the secondary phloem, the labeling should be specific to lignin substructures as well as KM1 antibody.

Weak labeling with KM2 antibody was found in the compound middle lamellae and secondary walls of developed tracheid cells. KM2 labeling in TEM (Fig. 7) was weaker than that in light microscopy (Fig. 6). This inconsistency might be due to non-specific silver deposition in silver enhancement. Although the labeling was weak, this result suggests that developed tracheid cell walls contain lignin substructures with 8-8′ linkage with at least one free phenolic groups. Lapierre et al. (1991) analyzed dimers from spruce lignin after thioacidolysis and detected 8-8′ dimers as one of minor condensed structures of spruce lignin. The immunolabeling of KM2 antibody in the present study showed the presence of 8-8′ linked structure of lignin in the cell wall in Japanese cypress.

Conclusion

The monoclonal antibodies KM1 and KM2 specifically recognized dehydrodiconiferyl alcohol with 8-5′ linkage

and pinoresinol with 8-8′ linkage, respectively. Interestingly, KM2 antibody also reacted with syringaresinol with 8-8′ linkage. These antibodies should make effective tools for detecting lignin dimers in differentiating xylem if samples are prepared so as to minimize loss of the dimers.

In the condition that these dimers are absent, the antibodies should also prove to be useful probes for the immunolocalization of 8-5′ and 8-8′ linked structure of lignin. Our immunoassay suggested that the presence of free phenolic or aliphatic hydroxyl groups was an important factor in their reactivity. The immunolabeling of KM1 and KM2 antibodies showed the presence of 8-5′ and 8-8′ linked structure of lignin in the cell wall in the secondary phloem and developed xylem of Japanese cypress. We suggested that lignin structure of secondary walls of phloem fibers might be different (richer in 8-5′ structure) from that of secondary walls of tracheids in xylem.

Work is currently in progress using these antibodies to make detailed observations of developing tracheids in normal and compression wood by TEM immunolabeling.

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References

Clausen MH, Ralet M-C, Willats WGT, McCartney L, Marcus SE, Thibault J-F, Knox JP (2004) A monoclonal antibody to feruloylated-(1–4)-beta-D-galactan. Planta 219:1036–1041

Davin LB, Jourdes M, Patten AM, Kim K-W, Vassão DG, Lewis NG (2008) Dissection of lignin macromolecular configuration and assembly: comparison to related biochemical processes in allyl/propenyl phenol and lignan biosynthesis. Nat Prod Rep 25:1015–1090

Erlanger BF (1980) The preparation of antigenic hapten-carrier conjugates: a survey. Methods Enzymol 70:85–104

Gani M, Mcguinness BJ, Davies AP (1998) Monoclonal antibodies against tea polyphenols: a novel immunoassay to detect polyphenols in biological fluids. Food Agr Immunol 10:13–22

Joseleau J-P, Ruel K (1997) Study of lignification by non-invasive techniques in growing maize internodes—an investigation by Fourier transform infrared, cross-polarisation-magic angle spinning ^{13}C-nuclear magnetic resonance spectroscopy and immunocytochemical transmission electron microscopy. Plant Physiol 114:1123–1133

Joseleau J-P, Faix O, Kuroda K, Ruel K (2004a) A polyclonal antibody directed against syringylpropane epitopes of native lignins. C R Biologies 327:809–815

Joseleau J-P, Imai T, Kuroda K, Ruel K (2004b) Detection in situ and characterization of lignin in the G-layer of tension wood fibres of *Populus deltoides*. Planta 219:338–345

Jouanin L, Goujon T, de Nadaï V, Martin M-T, Mila I, Vallet C, Pollet B, Yoshinaga A, Chabbert B, Petit-Conil M, Lapierre C (2000) Lignification in transgenic poplars with extremely reduced caffeic acid *O*-methyltransferase activity. Plant Physiol 123:1363–1373

Kim YS, Koh HB (1996) Origin of milled wood lignin: an immunocytochemical approaches in combination with transmission electron microscopy. Mokchae Konghak 24:68–74

Kim YS, Koh HB (1997) Immuno electron microscopic study on the origin of milled wood lignin. Holzforschung 51:411–413

Kukkola EM, Koutaniemi S, Gustafsson M, Karhunen P, Ruel K, Lundell TK, Saranpää P, Brunow G, Teeri TH, Fagerstedt KV (2003) Localization of dibenzodioxocin substructures in lignifying Norway spruce xylem by transmission electron microscopy-immunogold labeling. Planta 217:229–237

Kukkola EM, Koutaniemi S, Pöllänen E, Gustafsson M, Karhunen P, Lundell TK, Säranpää P, kilpeläinen I, Teeri TH, Fagerstedt KV (2004) The benzodioxocin lignin substructure is abundant in the inner part of the secondary wall in Norway spruce and silver birch xylem. Planta 218:497–500

Lapierre C, Pollet B, Monties B, Rolando C (1991) Thioacidolysis of spruce lignin: gC-MS analysis of the main dimers recovered after Raney nickel desulphuration. Holzforschung 45:61–68

McCartney L, Marcus SE, Knox JP (2005) Monoclonal antibodies to plant cell wall xylans and arabinoxylans. J Histochem Cytochem 53:543–546

Miyakawa M, Fujita M, Saiki H, Harada H (1973) The cell wall structure of the secondary phloem elements in *Cryptomeria japonica* D. Don. Bulletin Kyoto Univ. Forests 45:181–191

Morreel K, Ralph J, Kim H, Lu F, Goeminne G, Ralph S, Messens E, Boerjan W (2004) Profiling of oligolignols reveals monolignol coupling conditions in lignifying poplar xylem. Plant Physiol 136:3537–3549

Nagasaki T, Yasuda S, Imai T (2001) Preparation of antibody against agatharesinol, a norlignan, using a hapten-carrier conjugate. Phytochemistry 58:833–840

Pew JC (1963) Evidence of a Biphenyl Group in Lignin. J Org Chem 28:1048–1054

Puhlmann J, Bucheli E, Swain MJ, Dunning N, Albersheim P, Darvill AG, Hahn MG (1994) Generation of monoclonal antibodies against plant cell-wall polysaccharides. Plant Physiol 104:699–710

Quideau S, Ralph J (1994) A biomimetic route to lignin model compounds via silver oxide oxidation. Holzforschung 48:12–22

Ralet M-C, Tranquet O, Poulain D, Moïse A, Guillon F (2010) Monoclonal antibodies to rhamnogalacturonan I backbone. Planta 231:1373–1383

Ruel K, Faix O, Joseleau J-P (1994) New immunogold probes for studying the distribution of the different lignin types during plant cell wall biogenesis. J Trace Microprobe Tech 12:247–265

Ruel K, Burlat V, Joseleau J-P (1999) Relationship between ultrastructural topochemistry of lignin and wood properties. IAWA J 20:203–211

Ruel K, Chabannes M, Boudet A-M, Legrand M, Joseleau J-P (2001) Reassessment of quantitative changes in lignification of transgenic tobacco plants and their impact on cell wall assembly. Phytochemistry 57:875–882

Ruel K, Berrio-Sierra J, Derikvand MM, Pollet B, Thévenin J, Lapierre C, Jouanin L, Joseleau J-P (2009) Impact of CCR1 silencing on the assembly of lignified secondary walls in *Arabidopsis thaliana*. New Phytol 184:99–113

Tranquet O, Saulnier L, Utille J-P, Ralph J, Guillon F (2009) Monoclonal antibodies to *p*-coumarate. Phytochemistry 70:1366–1373

Willats WGT, Knox JP, Mikkelsen JD (2006) Pectin: new insights into an old polymer are starting to gel. Trends Food Sci Technol 17:97–104

解説・主張〈Research Trends〉 2

セルロース系コポリマー・コオリゴマーの精密合成と機能発現

Precise synthesis of cellulosic copolymers/cooligomers with novel functions

This article briefly reviews our recent works regarding the synthesis of cellulosic copolymer/cooligomer of cellulose acetate or methylcellulose. For instance, our synthetic strategy regarding CTA enabled us to align gold nanoparticles by CTA derivatives and to control interparticle distances depending on the length of CTA molecules. Moreover, we have synthesized diblock methylcellulose derivatives with regioselective functional patterns. These derivatives permitted us to find that the diblock structure consisting of cellobiosyl block and approx. ten 2,3,6-tri-*O*-methyl-glucopyranosyl units was of crucial importance for thermoreversible gelation of methylcellulose.

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1．はじめに

我々はセルロース誘導体の構造－物性相関を深く理解する目的で、合成化学的手法を用いて新しい化学構造を有するセルロース／セロオリゴ糖誘導体の調製を行ってきた．本記事ではセルロースエステル、エーテルとして代表的なセルロースアセテート、メチルセルロースを中心に、関連する新規セルロース系コポリマー・コオリゴマーの合成と機能について最近の我々の研究成果を概説する．

2．セルロースアセテート関連化合物

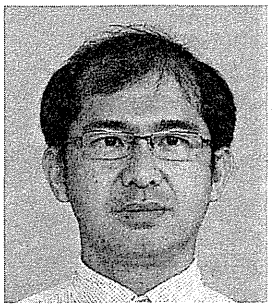
セルローストリアセテート（CTA）は、特に偏光板保護用フィルムとして[1]、または血液透析膜[2]や海水淡水化のための逆浸透膜[3]として広く利用されている極めて重要なセルロース誘導体の一つである．最近では、セルロースアセテートから更なる高付加価値製品を生み出すべく、様々な新規セルロース系ポリマーが開発されつつある．

2－1．一般的なセルロースアセテート系グラフトポリマー

市販のセルロースアセテートは遊離の水酸基を有するが、その水酸基を反応開始点として、ラクチド[4]やラクトン[5]の重合によりグラフト側鎖をセルロース主鎖に導入する方法（"Grafting from"法）が報告されており、主に生分解性プラスティックを指向した研究が展開されている．

2－2．新しいセルロースアセテート系グラフトポリマー

我々は新しい化学構造を有するセルロース誘導体の開発を目指し、非セルロース系ポリマーを主鎖にセルロースアセテートを側鎖に有する新規セルロースアセテート系化合物の調製を行った．セルロースアセテートを有するメタクリル酸エステルモノマーを用いた"Grafting through"法ではセルロースアセテート側鎖の導入量が少なかったが[6]、Huisgen 1,3-dipolar cycloaddition によりpoly（2-propyn-1-yl methacrylate）主鎖上にアジ



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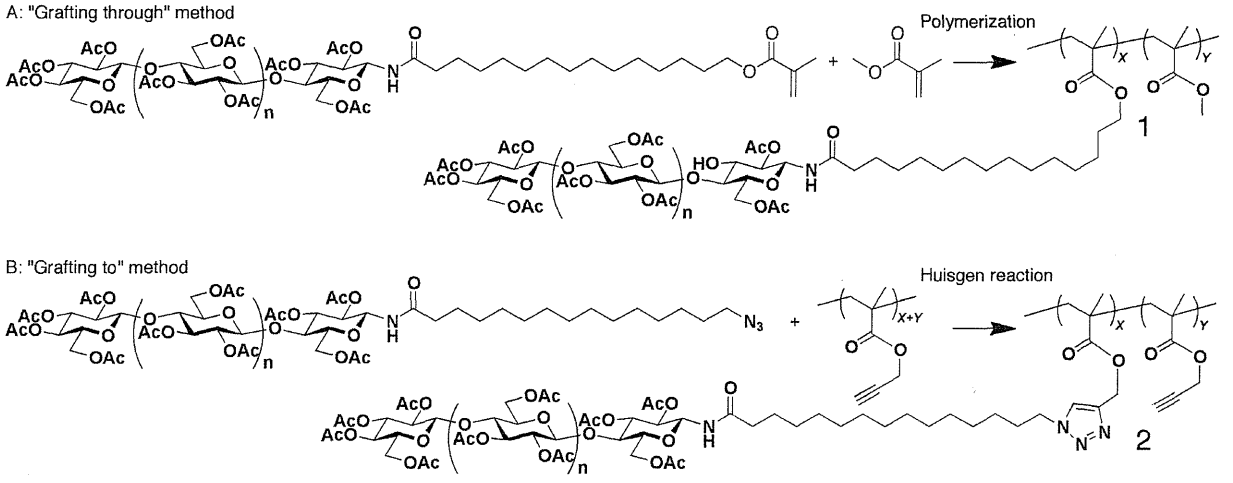


Fig. 1 Synthetic scheme for cellulosic graft copolymers **1** and **2** *via* "Grafting through" (a) and "Grafting to" (b) methods, respectively.

ド基を分子末端に有するセルロースアセテートを側鎖として密に導入することに成功した[7]．このClick反応による"Grafting to"法はセルロースおよびセルロース誘導体を側鎖とする新規セルロース系グラフトコポリマー調製に有効であることが判った．セルロース誘導体を側鎖とするグラフトコポリマーは特殊な物性を有する可能性があり、その利用法は現在検討中である．

2－3．セルロースアセテート系ジブロックコポリマー

ジブロックコポリマーからなる薄膜はミクロ相分離構造を有することが知られており、光学材料、電子材料、磁性材料として様々な機能が期待されている．セルロース系ブロックポリマーの合成は古くから報告されている[8-13]が、当時の限られた機器分析手法を用いたポリマーの化学構造解析では詳細は不明と言える．そこで我々は構造の明確なセルロース系ブロックポリマーの合成を計画し最新の分析手法により化学構造を調べた．

はじめに我々はヘミアセタール性水酸基をアジド基、アミノ基へと変換し、アミド結合によりオリゴアミド15鎖を段階的に導入する合成例を報告した[14, 15]．次いで、光機能性官能基であるピレンをアミド結合によりセルロース分子鎖の末端に導入する研究例を報告した[16]．更に、セルロースアセテート分子末端のアミノ基にα-リポ酸を反応させ得られた分子末端にジスルフィド基を有するセルロースアセテート系ポリマーは、金ナノ粒子表面に共有結合し、金粒子間距離はセルロースアセテートの重合度により制御可能であることを見

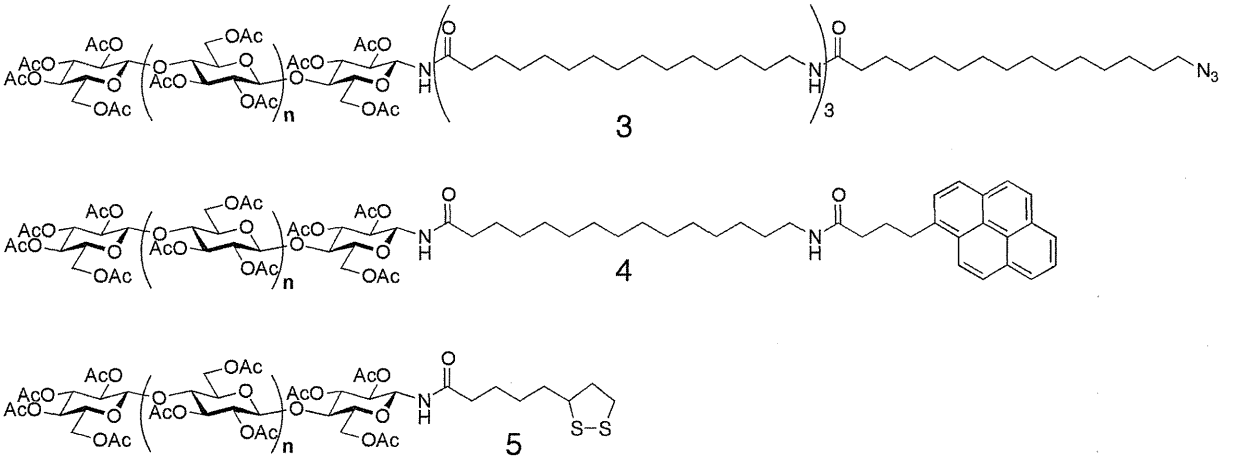


Fig. 2 Chemical structure of CTA-*block*-oligoamide-15 (**3**), CTA carrying a single pyrene group at the reducing-end (**4**), and *N*-lipoyl-tri-*O*-acetyl-β-cellulosylamine (**5**).

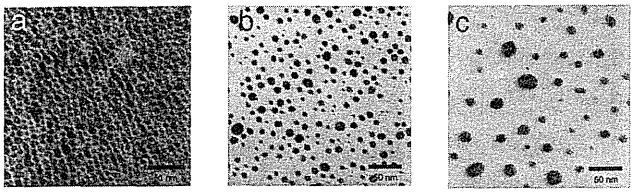


Fig. 3　TEM images of radially oriented CTA chains on gold nanoparticles. DPs of CTA are 2 (a), 13 (b), and 41 (c). CTA chains were invisible. Distance between gold nanoparticles depended on the length of CTA chains.

出した[17]．

2－4．自己組織化によるナノ構造体の生成

また最近、我々は分子末端にピレン基を有するセルロースアセテート誘導体のアセチル基を除去することにより、分子が自己組織化しナノ粒子構造体が生成することを見いだした[18]．興味深いことにDP=30の化合物の場合、ナノ粒子の平均直径40nmは伸びきり分子鎖の長さ21.3nmの約２倍であり、疎水部を粒子中央にセルロース部を外側に向けたセルロース分子１層からなる粒子が生成したと考えている．そのX線回折の結果、弱いながらCell-IIの結晶型が現れた．放射状にセルロース分子鎖の方向がそろった場合でも、一般に逆平行鎖と考えられているCell-IIの結晶型が現れた事実は興味深い．

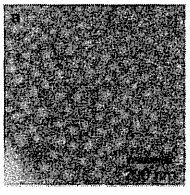


Fig. 4　TEM image of self-assembled nanoparticles carrying a single pyrene group at the reducing-end. Compound **4** (DP of cellulose is 30) was deacetylated by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in methanol/1,4-dioxane (1:4, v/v). See experimental details in our literature. It was suggested that the nanoparticles were monolayered, consisting of cellulose shell with radial orientation and hydrophobic core of long-chain alkyl group. Pyrene group should be located inside of the hydrophobic domain.

3. メチルセルロース関連化合物

メチルセルロースは重要なセルロースエーテル誘導体の一つであり、医薬品、食品、土木用途などで広く利用されている．市販のメチルセルロースは不均一反応で調製されており、メチル基は不均一に導入されていると考えられている．その不均一性には、(1) 無水グルコース単位内の2, 3, 6位水酸基への官能基導入位置の違い、(2) セルロース分子鎖に沿った置換基導入位置の違い、(3) 分子鎖間での置換度の違いという可能性が存在する．さらに、セルロースの重合度は分布を持っている．このように、置換度や重合度などの実験データは統計的に表すこととなるが、その事実こそがメチルセルロースの物性（ゲル化、界面活性など）に極めて大きな影響を与えている．そこで、市販のメチルセルロースのモデルとして、我々は位置特異的置換セルロース誘導体[19-22]の他に、構造の明確なジブロックコポリマー・コオリゴマーを調製し、化学構造が物性に与える影響を検討した．

3－1．メチルセルロース系ジブロックコオリゴマーおよびトリブロックコオリゴマーの合成と溶解性

合成ブロックとして、グルコース誘導体やセロビオース誘導体を調製し、グリコシル化法により単分散なジブロックおよびトリブロックコオリゴマーを調製した[23,24]．興味深い事に、AB-ジブロックオリゴマー **6-9**、ABA-トリブロックオリゴマー **10**は両親媒性を示したが、BAB-トリブロックオリゴマー **11**は水にのみ溶解した．同じトリメチル化グルコースを４残基、非修飾グルコースを２残基有するセロヘキサマーでも水への溶解性が異なることは予想外であり、この結果は一本の分子鎖に沿ったメチル置換位置の制御（シークエンス制御）は物性制御に極めて大きな影響を与えることを示している．一方、メチル化セロテトラオースの非還元性末端側グルコース残基の4位水酸基にグルコースオルトエステル誘導体を反応させた場合は、ジブロックコオリゴマーの混合物が得られた[25]．この化合物は水中で自己組織化しナノ粒子構造を形成することがわかった[26]．このようにオリゴ糖レベルの分子鎖長であっても、化合物のブロック構造はその物性や機能に大きな影響を与え得ることが判った．

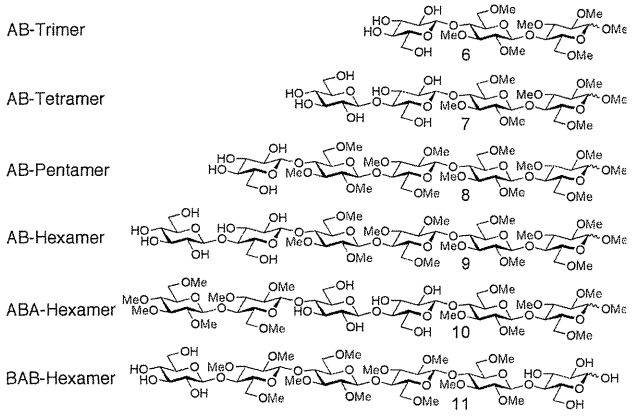


Fig. 5 Chemical structure of AB-trimer **6**, -tetramer **7**, -pentamer **8**, -hexamer **9**, ABA-hexamer **10**, and BAB-hexamer **11**.

3－2．メチルセルロース系ジブロックコポリマーの合成とゲル化特性

そこで次に、重合度の高い位置選択的置換メチルセルロース部（236MC, 23MC, 26MC, 3MC, 6MC）と無置換のグルコースグルコースおよびセロビオース部からなるブロックコポリマー10種類を合成し、メチルセルロースのブロック構造が界面活性能、熱応答性、ゲル化などの物性に与える影響を詳細に検討した．例えば、トリメチルセルロース合成ブロックは市販のメチルセルロース（DS = 1.8）の完全メチル化後、硫酸メタノリシスにより調製し、次いで生成する非還元末端グルコース残基の４位水酸基にセロビオース誘導体を反応させジブロックコポリマーを得た[27]．その結果、トリメチル化誘導体のみが熱ゲル化するという重要な知見を得た[28]．

この研究の延長線上で、親水部のセルロース分子鎖長を伸長させた化合物の性質に興味を持った．上記研究[27,28]では親水性ブロックの導入にグリコシル化反応を利用しており、セロオリゴ糖をグリコシル化ドナーとした場合に反応性の低下が懸念される．そこで、親水性セグメントである無保護セロオリゴ糖と疎水性セグメントであるトリメチルセルロース間の結合に反応性が高いと期待出来るアジド基とアルキン基間の1,3-双極子環化付加反応（クリック反応）を利用したメチルセルロース類縁体の合成を次に行った．その結果、得られた化合物**22**の水溶液はゲル化能を示したことから、トリメチル化セルロース部と無保護セルロースからなるジブロック構造がゲル化に大きな影響を与えていることを確認した[29]．このメチルセルロース類縁体の合成研究により真のメチルセルロースの構造－物性相関をより深く理解することが可能となった．

3－3．新しい界面活性剤としてのセルロース系ジブロックコオリゴマー

単分散なジブロックコオリゴマー **6**, **8**, **9**水溶液の界面活性能を市販のメチルセルロース（SM-4)やジブロックコオリゴマーの混合物水溶液と比較したところ、単分散な化合物の方が高い界面活

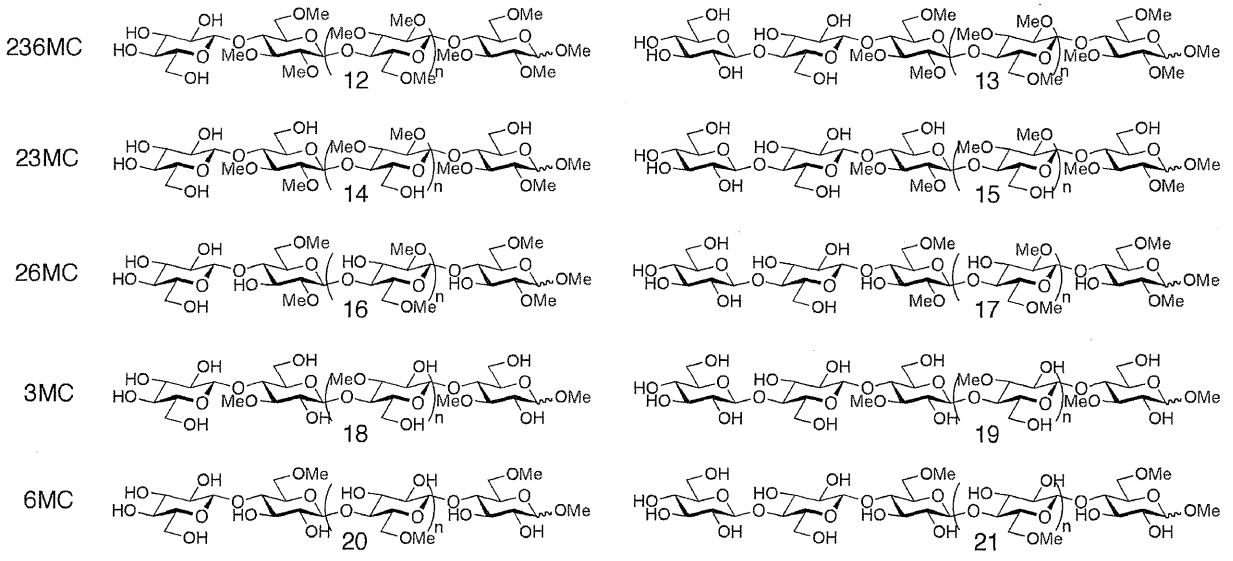
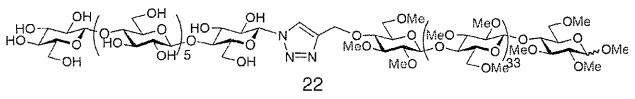


Fig. 6 A series of diblock methylcellulose derivatives with regioselective functionalization patterns.



22

Fig. 7　Chemical structure of diblock methylcellulose analogue.

性を有することがわかった[23]．市販の界面活性剤は炭化水素鎖を疎水部としているが、我々が新しく合成した界面活性剤は糖鎖自体が嵩高く比較的剛直な疎水部となっており性質の違いに興味が持たれる．そこで、この新しいタイプの界面活性剤の簡便な合成法を追求するため、化学構造が比較的単純な三糖誘導体の構造－界面活性相関を詳しく検討した．疎水部としてメチル化およびエチル化グルコースを、親水部としてセロビオースを選択した．疎水部-親水部間のグリコシド結合の違い、分子鎖末端部のグリコシド結合の違い、メチル基とエチル基との違いにより界面活性能は明らかに影響を受けた．高い界面活性を有する三糖化合物**23**は市販の中性、アニオン性、カチオン性界面活性剤と同等の性能（CMC: 0.48 mM; γ_{CMC}: 34.5 mN/m）を示すことが判った[30]．

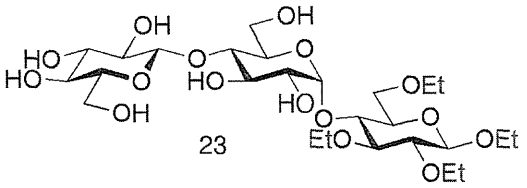


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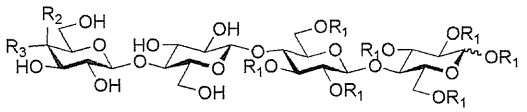
Fig. 8　Chemical structure of ethyl -D- glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranoside (**23**)

4. 両親媒性ブロック的アルキル化四糖-量子ドット複合体による細胞表面の蛍光標識[31]

両親媒性のブロック的アルキル化四糖**7**, **24**-**26**は、既存の非イオン性糖質界面活性剤であるオクチルグルコシドと比較して細胞毒性が顕著に低いことが判った．また、その両親媒性オリゴ糖は自己組織化により水中で直径約200nmのナノミセルを形成した．我々はそれら化合物の生物学的な機能に興味を持ち、様々な培養細胞株にブロック的アルキル化四糖と有機量子ドットとの複合体を作用させた結果、細胞生存率が高い状態で細胞表面を高効率で蛍光ラベルすることに成功した．この結果は低毒性という特徴を活かしメチルセルロース系低分子界面活性剤の応用可能性を広げる成果である．



7: $R_1 = CH_3$; $R_2 = H$, $R_3 = OH$
24: $R_1 = CH_3$; $R_2 = OH$, $R_3 = H$
25: $R_1 = CH_2CH_3$; $R_2 = H$, $R_3 = OH$
26: $R_1 = CH_2CH_3$; $R_2 = OH$, $R_3 = H$

Fig. 9　Chemical structures of methylated or ethylated tetrasaccharides having glucosyl or galactosyl residues at the non-reducing end.

5. おわりに

我々は新しい分子の形に注目して新規なセルロース誘導体を合成し、その構造－物性相関を解明する研究を推進してきた．その過程で、セルロース誘導体分子が分子同士あるいは無機物質、細胞などと相互作用することにより様々な機能を発現し得ることが明らかになった．また、合成した化合物が予想外の性質を示すこともしばしば経験した．セルロース誘導体の研究において、そのような予想外の興味深い結果を得るためには精密合成手法を駆使することが極めて重要である．今後、バイオマスの有効利用の観点から、精密合成手法を用いてコモディティ、スペシャリティユース両方向を指向したセルロース研究を推進する必要性を感じている．

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参考文献

1）森裕之、*TACのLCD構成材料としての応用*、「セルロース利用の最先端」、磯貝明 編、シーエムシー出版、2008, p. 177.
2）日吉辰夫、*バイオメディカル材料*、「セルロースの辞典」、セルロース学会 編、朝倉書店、2000, p. 546.

3) 熊野淳夫、*海水淡水化装置用逆浸透膜（酢酸セルロース）*、「セルロース利用の最先端」、磯貝明 編、シーエムシー出版、2008, p. 166.
4) Teramoto, Y., Nishio, Y., *Polymer*, **44**, 2701 (2003).
5) Ikeda, I., Washino, K., Maeda, Y., *Sen-I Gakkaishi*, **59**, 110 (2003).
6) Enomoto-Rogers, Y., Kamitakahara, H., Takano, T., Nakatsubo, F., *Biomacromolecules*, **10**, 2110 (2009).
7) Enomoto-Rogers, Y., Kamitakahara, H., Yoshinaga, A., Takano, T., *Carbohyd. Polym.*, **87**, 2237 (2012).
8) Ceresa, R. J., *Polymer*, **2**, 213 (1961).
9) Steinmann, H. W., *Polym. Prepr., Am. Chem. Soc. Div. Polym. Chem.*, **11**, 285 (1970).
10) Kim, S., Stannett, V. T., Gilbert, R. D., *Journal of Polymer Science, Polymer Letters Edition*, **11**, 731 (1973).
11) Feger, C., Cantow, H. J., *Polym. Bull.*, **3**, 407 (1980).
12) Mezger, T., Cantow, H. J., *Angew. Makromol. Chem.*, **116**, 13 (1983).
13) de Oliveira, W., Glasser, W. G., *Cellulose*, **1**, 77 (1994).
14) Kamitakahara, H., Nakatsubo, F., *Cellulose*, **12**, 209 (2005).
15) Kamitakahara, H., Enomoto, Y., Hasegawa, C., Nakatsubo, F., *Cellulose*, **12**, 527 (2005).
16) Enomoto, Y., Kamitakahara, H., Takano, T., Nakatsubo, F., *Cellulose*, **13**, 437 (2006).
17) Enomoto-Rogers, Y., Kamitakahara, H., Yoshinaga, A., Takano, T., *Cellulose*, **17**, 923 (2010).
18) Enomoto-Rogers, Y., Kamitakahara, H., Yoshinaga, A., Takano, T., *Cellulose*, **18**, 1005 (2011).
19) Kamitakahara, H., Koschella, A., Mikawa, Y., Nakatsubo, F., Heinze, T., Klemm, D., *Macromolecular Bioscience*, **8**, 690 (2008).
20) Kamitakahara, H., Funakoshi, T., Takano, T., Nakatsubo, F., *Cellulose*, **16**, 1167 (2009).
21) Kamitakahara, H., Funakoshi, T., Nakai, S., Takano, T., Nakatsubo, F., *Cellulose*, **16**, 1179 (2009).
22) Kamitakahara, H., Funakoshi, T., Nakai, S., Takano, T., Nakatsubo, F., *Macromolecular Bioscience*, **10**, 638 (2010).
23) Kamitakahara, H., Nakatsubo, F., Klemm, D., *Cellulose*, **14**, 513 (2007).
24) Kamitakahara, H., Nakatsubo, F., *Cellulose*, **17**, 173 (2010).
25) Kamitakahara, H., Nakatsubo, F., Klemm, D., *Cellulose*, **13**, 375 (2006).
26) Kamitakahara, H., Yoshinaga, A., Aono, H., Nakatsubo, F., Klemm, D., Burchard, W., *Cellulose*, **15**, 797 (2008).
27) Nakagawa, A., Fenn, D., Koschella, A., Heinze, T., Kamitakahara, H., *J. Polym. Sci., Pol. Chem.*, **49**, 4964 (2011).
28) Nakagawa, A., Fenn, D., Koschella, A., Heinze, T., Kamitakahara, H., *J. Polym. Sci., Pol. Phys.*, **49**, 1539 (2011).
29) Nakagawa, A., Kamitakahara, H., Takano, T., *Cellulose* (2012) in press. DOI 10.1007/s10570-012-9703-7.
30) Nakagawa, A., Kamitakahara, H., Takano, T., *Carbohydr. Res.*, **346**, 1671 (2011).
31) Kamitakahara, H., Murata-Hirai, K., Tanaka, Y., *Cellulose*, **19**, 171 (2012).