Monolayers of Reactive Cellulose Derivatives

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Summary: Functional cellulose derivatives are very versatile materials for the creation of mono- and multilayer systems. Hydrophobic alkyl and trimethylsilyl celluloses form highly ordered Langmuir-Blodgett multilayers on hydrophobic substrates. Cellulose thiosulfates and methyl thio ethers were self-assembled on gold and silver surfaces to form hydrophilic monolayers. Cellulose layer systems are capable for chemical transformations under conservation of the structural order. They are suitable platforms for the investigation of molecular recognition at surfaces and the construction of sensor devices. Both biological ligands, e. g. biotin, and enzymes, e. g. horse radish peroxidase, could be attached to cellulose under conservation of their biological function.

Keywords: cellulose; enzymes; molecular recognition; nanolayers; self-assembly

Introduction

Cellulose derivatives are well suited for the formation of ultra-thin films on various substrates. This has several reasons: chains of cellulose derivatives are semi-rigid, their persistence lengths are in the range of 5-15 nm,[1, 2] cellulose can be functionalized by polmer analogous reactions[3] and cellulose is highly biocompatible. Lipophilic alkyl ethers, especially *i*-pentyl ethers, of cellulose form monolayers at the air water interface. These monolayers can be repetitively transferred onto planar hydrophobic substrates to form highly ordered Langmuir-Blodgett (LB) multilayers. The thickness per cellulose layer is 0.9 nm.[4] LB multilayers from trimethylsilyl-cellulose can be regenerated by gaseous HCl.[5] The resulting cellulose multilayers show a inter layer spacing of 0.4 nm which is similar to the one of native crystalline cellulose.[6] Biological receptor molecules, e. g. antibodies, were immobilized at regenerated cellulose LB multilayers functionalized by cyanur chloride for the construction of immunosensors using evanescent wave guide techniques.[7]

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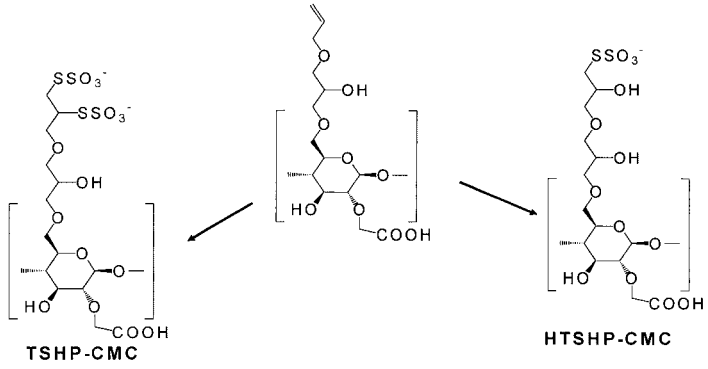
Beside the LB technique the so-called self-assembly method is used for the creation of nanoscopically defined monolayers. Alkane thiols and disulfides irreversibly form monolayers of two dimensional crystallinity onto gold and silver surfaces via Au-S bonds or Ag-S bonds, respectively.[8] These self-assembled monolayers (SAMs) offer several advantages to LB layers: there is no sophisticated instrumentation necessary, and the deposition of the monolayers can be controlled by *in situ* methods like surface plasmon resonance spectroscopy,[9] atomic force microscopy or impedance measurements,[10] and patterned SAMs can easily be produced by mico contact printing[11] or dip pen lithography.[12] SAMs are not only known from monomeric thiols but also from polymeric ones, such as thio derivatives of polystyrene and polyacrylates.[13] We synthesized water-soluble thiosulfate derivatives of cellulose and investigated the cellulose SAMs on gold and silver surfaces by ellipsometry, FT-IR and X-ray photon spectroscopy (XPS).[14-16] In the following we report on the synthesis of new highly water soluble cellulose thio derivatives and their formation of SAMs and describe coupling techniques for the immobilization of biomolecules to those cellulose SAMs.

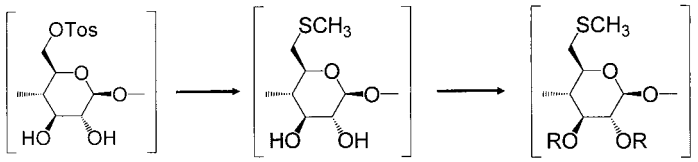
Synthesis of thio derivatives of carboxymethyl cellulose

Commercial carboxymethyl cellulose (CMC) of a degree of substitution (DS) of 1.1 and a degree of polymerization (DP) of 925 was reacted with allyl glycidyl ether in NaOH solution. The partial DS values of the attached allyl-hydroxypropyl groups were in the range of 0.2 – 0.4 depending on the reaction conditions. Partial addition of tetrathionate to the allylic double bonds yielded the cellulose 2'',3''-bis-thiosulfate **TSHP-CMC**, while bromination in water and substitution by thiosulfate afforded the 2''-hydroxy-3''-thiosulfate **HTSHP-CMC** (s. eq. 1).



(1)

Both compounds are highly water soluble. In addition, 6-*O*-tosylcellulose[17] was reacted with sodium methyl sulfide. The resulting methyl thioether was carboxymethylated to afford the water soluble cellulose derivative **MTh-CMC** (s. eq. 2).



(2)

R = H, CH_2COONa

MTh-CMC

Formation self-assembled monolayers (SAMs) on gold

The formation of SAMs on gold was detected *in situ* by surface plasmon resonance (SPR) spectroscopy using a Bio-Suplar 2 instrument from Analytical μ-Systems, Regensburg, Germany (http://www.micro-systems.de) equipped with a continuous flow cell.[18] Both the cellulose thiosulfates, **TSHP-CMC**, **HTSHP-CMC**, and the methyl thioether **MTh-CMC** spontaneously form SAMs on gold. A typical film thickness of 3 nm was derived from the change of the surface plasmon resonance angle. This thickness is in accordance with a multiple attachment of the cellulose chain. As a straight conformation of the chain would lead to much thinner monolayers with an estimated thickness of about 1 nm, we assumed that the polymer chains are bound in a curved conformation. SAM formation was completed within a few minutes for the cellulose thiosulfates, **TSHP-CMC** and **HTHP-CMC** (s. fig. 1). On the other hand, it took some more time (1 h) for the methyl thioether **Mth-CMC**.

The cellulose SAMs were hydrophilic, the contact angles vs. water were in the range of 15-30°. The cellulose SAMs showed little unspecific interactions with blood proteins, e. g. bovine serum albumin. Therefore they are very promising platforms for biosensor devices.

Molecular recognition at cellulose SAMs

As a first example of a detection of a molecular recognition event at a cellulose SAM, we choose the ligand/receptor system biotin/streptavidin. Streptavidin is a protein consisting of 123 amino acids which has a very high and selective affinity to biotin.[19] An amino-terminated biotin derivative was linked by standard N,N'-dicyclohexylcarbodiimide (DCC) coupling to **HTSHP-CMC**. The resulting conjugate formed monolayers on gold. Subsequent addition of a streptavidin solution leads to the built-up of a streptavidin layer on top of the cellulose layer. The streptavidin layer had a thickness of 6 nm, in good agreement with the thickness of a streptavidin molecule.[20] We concluded from this finding, that a dense streptavidin monolayer had been formed. Molecular recognition of antibodies by immobilized antigens will be investigated at cellulose SAMs in the future. The immobilization of ligands at functional cellulose monolayers appears advantageous to the well-known Biacore system,[21] as fewer reaction steps have to be performed on the surface for the creation of a sensor chip.

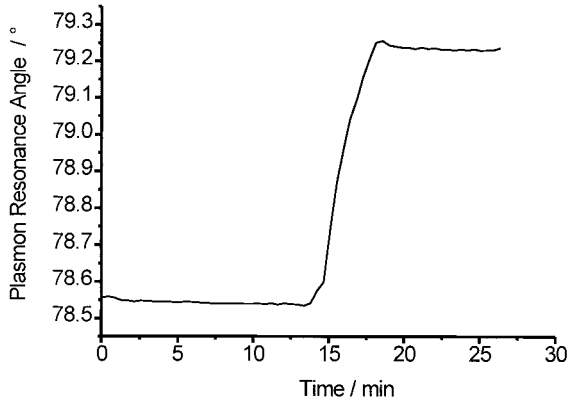


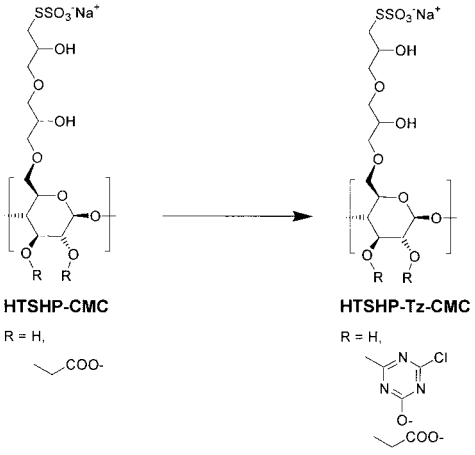
Fig. 1. Kinetics of the SAM formation from a solution of 0.1 % **TSHP-CMC** in 0.1 N HCl on a 50 nm gold layer on glass monitored by SPR at a wavelength of 670 nm.

Enzyme activity at cellulose SAMs

For the attachment of enzymes the cellulose thiosulfate **HTSHP-CMC** was reacted with cyanur chloride to the chlorotriazinyl derivative **HTSHP-Tz-CMC** (s. eq. 3). This compound is stable in

aqueous solution and reacts selectively with amines at pH 8. It spontaneously forms monolayers on gold as shown by SPR. Horseradish peroxidase was immobilized to these reactive monolayers by incubation of the monolayer for 20 minutes in an aqueous solution of the enzyme at room temperature. Presumably lysine residues of the enzyme couple to the chlorotriazinyl groups of the monolayer.

As no further coupling reagents are necessary in the aqueous phase ("reagent free" coupling), adverse side reactions of the enzyme are minimized. Consequently, the enzyme remains still active after the immobilization, as proven by activity tests using the ABTS assay.[22] This example shows in principle, that enzymes can be tested in the nanoscopic environment of a cellulose monolayer. Interesting applications such as highly efficient screening tests for enzyme inhibitors are conceivable.



(3)

Conclusion

Cellulose monolayers offer several advantages to classical SAMs. They are rapidly formed with uniform quality. They can be functionalized for the coupling of ligands and proteins. Biomolecules can be immobilized under gentle "reagent free" coupling conditions. The coupling density can be controlled with high reproducibility. As unspecific interactions are low, cellulose SAMs are suitable platforms for the construction of biosensors.

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[1] K. Kamide, M. Saito, *Macromol. Rapid Commun.* **1983**, *4*, 33.
[2] C. W. Hoogendam, A. De Keizer, M. A. C. Stuart, B. H. Bijsterbosch, J. A. M. Smit, J. A. P. P. Van Dijk, P. M. Van der Horst, J. G. Batelaan, *Macromolecules* **1998**, *31*, 6297.
[3] D. Klemm, B. Philipp, T. Heinze, U. Heinze, W. Wagenknecht, *Comprehensive Cellulose Chemistry II: Functionalization of Cellulose, Vol. 2*, Wiley-VCH, Weinheim, 1998.
[4] M. Schaub, C. Fakirov, A. Schmidt, G. Lieser, G. Wenz, G. Wegner, P. A. Albouy, H. Wu, M. D. Foster, C. Majrkzak, S. Satija, *Macromolecules* **1995**, *28*, 1221.
[5] M. Schaub, G. Wenz, G. Wegner, A. Stein, D. Klemm, *Adv. Mater.* **1993**, *12*, 919.
[6] A. A. Baker, W. Helbert, J. Sugiyama, M. J. Miles, *Biophys. J.* **2000**, *79*, 1139.
[7] F. Löscher, T. Ruckstuhl, T. Jaworek, G. Wegner, S. Seeger, *Langmuir* **1998**, *14*, 2786.
[8] C. D. Bain, E. B. Troughton, Y. T. Tao, J. Evall, G. M. Whitesides, R. G. Nuzzo, *J. Am. Chem. Soc.* **1989**, *111*, 321.
[9] D. K. Kambhampati, W. Knoll, *Curr. Opin. Coll. Interf. Sci.* **1999**, *4*, 273.
[10] M. W. J. Beulen, J. Bugler, M. R. De Jong, B. Lammerink, J. Huskens, H. Schonherr, G. J. Vancso, B. A. Boukamp, H. Wieder, A. Offenhauser, W. Knoll, F. C. J. M. Van Veggel, D. N. Reinhoudt, *Chem. Eur. J.* **2000**, *6*, 1176.
[11] B. D. Martin, S. L. Brandow, W. J. Dressick, T. L. Schull, *Langmuir* **2000**, *16*, 9944.
[12] J.-H. Lim, D. S. Ginger, K.-B. Lee, J. Heo, J.-M. Nam, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2003**, *42*, 2309.
[13] C. Erdelen, L. Häussling, R. Naumann, H. Ringsdorf, H. Wolf, J. Yang, M. Liley, J. Spinke, W. Knoll, *Langmuir* **1994**, *10*, 1246.
[14] US 6,245,579 B1 (2001), Universität Karlsruhe, invs.: G. Wenz, D. F. Petri, S. W. Choi.
[15] S. Choi, H. Lauer, G. Wenz, M. Bruns, D. F. S. Petri, *J. Braz. Chem. Soc.* **2000**, *11*, 11.
[16] D. S. Petri, S. W. Choi, H. Beyer, T. Schimmel, M. Bruns, G. Wenz, *Polymer* **1999**, *40*, 1593.
[17] K. Rahn, M. Diamantoglou, D. Klemm, H. Berghmans, T. Heinze, *Angew. Makromol. Chem.* **1996**, *238*, 143.
[18] S. A. Zynio, A. V. Samoylov, E. R. Surovtseva, V. M. Mirsky, Y. M. Shirshov, *Sensors* **2002**, *2*, 62.
[19] P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, *Science* **1989**, *243*, 85.
[20] B. A. Katz, *J. Mol. Biol.* **1997**, *274*, 776.
[21] W. Jager, *Carbohydr. Chem. Biol.* **2000**, *2*, 1045.
[22] K. Monde, H. Satoh, M. Nakamura, M. Tamura, M. Takasugi, *J. Nat. Prod.* **1998**, *61*, 913.