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Synthesis and Characterization of Cellulose α-Lipoates: A Novel Material for Adsorption onto Gold

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Summary

Novel α-lipoic acid esters of cellulose (cellulose α-lipoates) were synthesized homogeneously in *N,N*-dimethylacetamide (DMAc)/LiCl using different methods for the *in situ* activation of the carboxylic acid. Thus, cellulose α-lipoates with degrees of substitution (DS) in the range 0.11 to 1.45 were accessible with *N,N'*-carbonyldiimidazole and *p*-toluenesulphonyl chloride as *in situ* activating agents for the α-lipoic acid. The reactions proceeded totally homogeneous with high yields giving cellulose α-lipoates soluble in DMSO. The α-lipoate moiety containing a S-S function stays intact during the reaction as revealed by FTIR and ^{1}H NMR spectroscopy. The cellulose α-lipoates showed self-assembly onto gold surface yielding layers with a thickness of 2.9-4.9 nm, which can be confirmed by surface plasmon resonance. The perpropionylated cellulose α-lipoates form films with a comparably low thickness of 0.9 nm.

Introduction

Esterification of cellulose under homogeneous reaction conditions using *in situ* activation steps provides access to a variety of bio-based derivatives with valuable properties [1-6]. Efficient and selective synthetic methods for the homogeneous conversion of cellulose are indispensable for the preparation of defined biocompatible self-assembly materials, e.g. the thin film formation onto gold surface. Such thin films are mechanically and solvolytically stable and can serve as model systems to study fundamental interfacial properties [7], such as wetting [8,9], friction [10], adhesion [11], pattern definition [12] and biomineralization [13]. Thus, Rodziguez has studied the growth of hydroxyapatite crystals on a cellulose matrix using titanium alkoxide as a coupling agent [14]. Cellulose derivatives can also be employed to study the enzyme immobilization on surfaces [15]. Tanaka has demonstrated that thin films (5-10 nm) of regenerated cellulose could serve as ideal inter layers to deposit model and native cell membranes [16].

Here we describe the homogeneous preparation of novel cellulose α-lipoates as tailored cellulose derivatives for self-assembly onto gold surfaces, which can be the basis for biomineralization, crystal growth and enzyme immobilization on surfaces. The advantage of this approach is that both the polymer backbone and the substituent introduced are naturally occurring and biocompatible substances with a high tendency for self assembly.

Experimental

Materials

Microcrystalline cellulose (Avicel® PH-101, DP 280) obtained from Fluka was dried under vacuum at 110°C for 8 h before use. LiCl was dried for 6 h at 105°C in vacuum prior to use. Pyridine was distilled over CaH_2. All other chemicals supplied by Fluka, were used without further purification.

Measurements

^{1}H NMR spectra of the esters were acquired in dimethylsulphoxide (DMSO)-d_6 after peracylation of the unmodified hydroxyl groups [17,18]. FTIR spectra were measured on a Bio-Rad FTS 25 PC using the KBr pellet technique. Elemental analyses were performed by CHNS 932 Analyzer (Leco).
Preparation of gold slides for thin films of cellulose α-lipoates were carried out according to ref. 19: The glass slides (3.5 x 2.5 cm) were cleaned with aq. $NH_3/H_2O_2/H_2O$ (1:1:5) for 10 minutes at 80°C, washed with H_2O and isopropanol and dried in a flow of N_2. These glass slides were coated with gold using a Balzer BAE 250, vacuum coating unit under pressure of less than 5x10-6 hPa, typically depositing 50 nm of gold after first depositing 2 nm of chromium. The gold-coated glass slides were placed for 12 h in DMSO solution (2 mmol) of cellulose α-lipoate, rinsed with ethanol to remove unbound cellulose α-lipoate and dried in a stream of N_2.
Surface plasmon resonance (SPR) measurements were performed in the Kretschmann prism configuration [20] against ethanol. Optical coupling was achieved with a LASFN 9 prism, n= 1.85 at λ = 632.8 nm and index matching fluid n = 1.70 between prism and the BK270 glass sildes. The plasmon was excited with plane-polarized radiations using a He/Ne laser (632.6 nm, 5 mW).

Synthesis of cellulose α-lipoate with α-lipoic acid/p-toluenesulphonyl chloride (Tos-Cl) in N,N-dimethylacetamide (DMAc)/LiCl

To the solution of 1.0 g of cellulose in DMAc/LiCl, 3.53 g Tos-Cl was added, followed by 3.82 g of α-lipoic acid under stirring. The reaction mixture was stirred for 16 h at 60°C under N_2. The homogeneous reaction mixture was precipitated in 600 mL ethanol, washed with 250 mL ethanol three times and the polymer was collected by filtration. The polymer was dried at 50°C under vacuum to yield product **1**.
Yield: 2.50 g (yellowish powder)
Elemental Analysis (EA): 48.20% C, 6.04% H, 21.32% S
DS_{EA} = 1.45
FTIR (KBr): 3481 ν (OH), 2931, 2862 ν (CH), 1238 ν (COC-Ester), 1742 ν (CO-Ester) cm^{-1}

Synthesis of cellulose α-lipoate with α-lipoic acid/N,N'-carbonyldiimidazole (CDI) in DMAc/LiCl

1.5 g CDI was dissolved in 30 mL DMAc followed by 1.91 g α-lipoic acid to obtain the imidazolide of the α-lipoic acid. The mixture was stirred overnight then added to the solution of 1.0 g cellulose dissolved in DMAc/LiCl. The reaction mixture was stirred for 16 h at 60°C under N_2. The homogeneous reaction mixture was precipitated in 500 mL acetone and the polymer was collected by filtration. After washing with 250 mL acetone three times, the polymer was dried at 50°C under vacuum to yield product **3**.
Yield: 1.31 g
DS_{EA}: 0.18
EA: 34.81% C, 6.27% H, 5.94% S (results of EA are summarized in Tab. 1)
FTIR (KBr): 3469 ν (OH), 2919 ν (C-H), 1234 ν (C-O-CEster), 1731 ν (COEster) cm^{-1}

*Perpropionylation of cellulose α-lipoate **3***

Perpropionylation of the unmodified hydroxyl groups was carried out. For this purpose, 0.6 g of sample **3** was allowed to react with 8.0 mL propionic anhydride and 8.0 mL pyridine in the presence of 20 mg of 4-dimethylamino pyridine as catalyst for 24 h at 60°C in N_2 atmosphere under stirring. The polymer was precipitated and washed with 250 mL ethanol four times and then dried at 60°C under vacuum to yield product **3.1**.
Yield: 0.16 g
FTIR (KBr): no ν(OH), 2984, 2946, 2889 ν(CH), 1738 ν(CO_{Ester}), 1440 (Cyclic C-H bending vibrations) cm^{-1}
^{1}H NMR ($CDCl_3$) after perpropionylation: δ (ppm) = 5.01 (H-3), 4.85 (H-2), 4.31 and 3.96 (H-6), 3.62 (H-4), 3.46 (H-5), 2.10-2.16 (H-7, 14), 3.04-3.13 (H-12, 13), 1.35-1.88 (H-8, 10), 0.99, 1.08, 1.18 (H-15 at C-3, 2, 6 respectively)

Results and discussion

The homogeneous esterification of cellulose with α-lipoic acid (thioctic acid) had to be carried out via *in situ* activation of the carboxylic acid. This is due to the fact that there are no reactive derivatives of the acid available resulting from the instability of the disulphide function in the five-membered ring under drastic reaction conditions. Thus, homogeneous modification of cellulose was performed in DMAc/LiCl using *p*-toluenesulphonyl chloride (Tos-Cl) as well as *N,N'*-carbonyldiimidazole (CDI) for the *in situ* activation of α-lipoic acid. In a first experiment cellulose dissolved in DMAc/LiCl was allowed to react with 3 equivalent α-lipoic acid and Tos-Cl (Sample **1,** Tab. **1**). Reaction at 60°C for 16 h yielded organo-insoluble cellulose α-lipoate. The formation of the cellulose derivative was confirmed by FTIR spectroscopy.
The most important information obtained from the FTIR (KBr) spectrum in case of sample **1** was that the cyclic ring of the α-lipoate moiety remains intact during the reaction. This can be concluded from the absence of a band at about 2560-2570 cm^{-1} for a S-H stretching. Signals for CH_2 moieties (C-H bending vibrations) in a cyclic system appeared at 1438 cm^{-1}, which is in the same region as for a comparable CH_2 group in a cyclopropane molecule. The carbonyl group of the ester function was determined at 1742 cm^{-1}. FTIR spectroscopy gave no information concerning the structural reasons for the insolubility of the derivative.

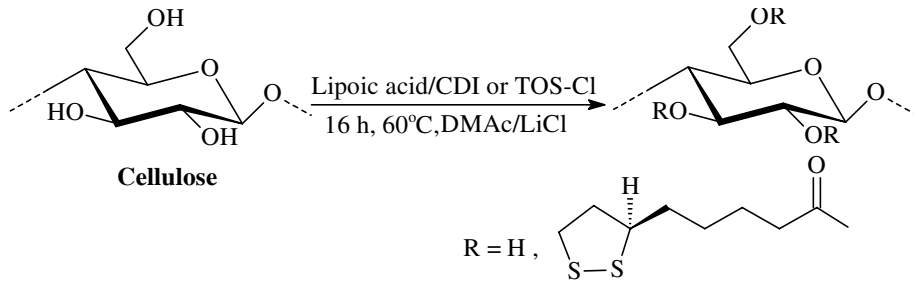

Figure 1. Schematic plot of the conversion of cellulose with α-lipoic acid *in situ* activated with *p*-toluenesulphonyl chloride (Tos-Cl) or *N,N'*-carbonyldiimidazole (CDI)

For the following experiments (esters **2-5**, Tab. 1) we applied *in situ* activation of α-lipoic acid with CDI, which can diminish side reactions [4]. The FTIR spectra obtained for these esters were comparable to the spectrum of sample **1** discussed above. DS-values calculated on the basis of elemental analyses (Tab. 1) were in the range of 0.11 to 0.50. Remarkably high yields can be achieved with the method (up to 98%). Samples **3-5** were soluble in DMSO. Perpropionylation of the samples with propionic anhydride/pyridine was carried out to analyze the cellulose derivatives with ^{1}H NMR spectroscopy [18]. Chloroform soluble mixed esters can be prepared in this way.

Table 1. Conditions and results of esterification of cellulose dissolved in DMAc/LiCl with α-lipoic acid *in situ* activated with *p*-toluenesulphonyl chloride (Tos-Cl, **1**) as well as *N,N'*-carbonyldiimidazole (CDI, **2-5**)

Samples	Molar ratio[a]	% S	DS[b]	Solubility
1	1:3:3	21.32	1.45	Insoluble
2	1:3:3	12.55	0.50	Insoluble
3	1:1.5:1.5	5.94	0.18	DMSO
4	1:1:1	5.29	0.16	DMSO
5	1:0.5:0.5	3.78	0.11	DMSO

[a] = AGU:α-lipoic acid :Tos-Cl or CDI
[b] = DS calculated by elemental analysis

A ^{1}H NMR spectrum of sample **3** ($CDCl_3$, DS 0.18, Fig. 2) after perpropionylation (sample **3.1**) is given in Figure 2. The well-resolved spectrum shows a separate signal for all the AGU protons in the region δ = 3.46-5.01 ppm. The protons on the heterocyclic system appear at 3.10 ppm (H-11 and H-13) and at 1.88 ppm (H-12) as multiplet signals. Signals for the CH_2 moieties in direct neighborhood to the carbonyl of the lipoate (H-7) and the propionate (H-14) overlap and give a broad multiplet in the range from 2.05 to 2.45 ppm. Protons of the aliphatic chain of the α-lipoate moiety (H-8-10) yield peaks at δ = 1.35-1.88 ppm. The propionate methyl group (H-15) gives separate signals at 0.99, 1.08 and 1.18 ppm for propionylation in position 3, 2 and 6, respectively. The peaks of the three protons at the ring carbons in the α-lipoate (H-11 and H-13) can be used to calculate the DS from the spectral integrals versus the spectral integrals of the AGU protons. A value of 0.22 was obtained which is in rather good agreement with the value given in Table 1.

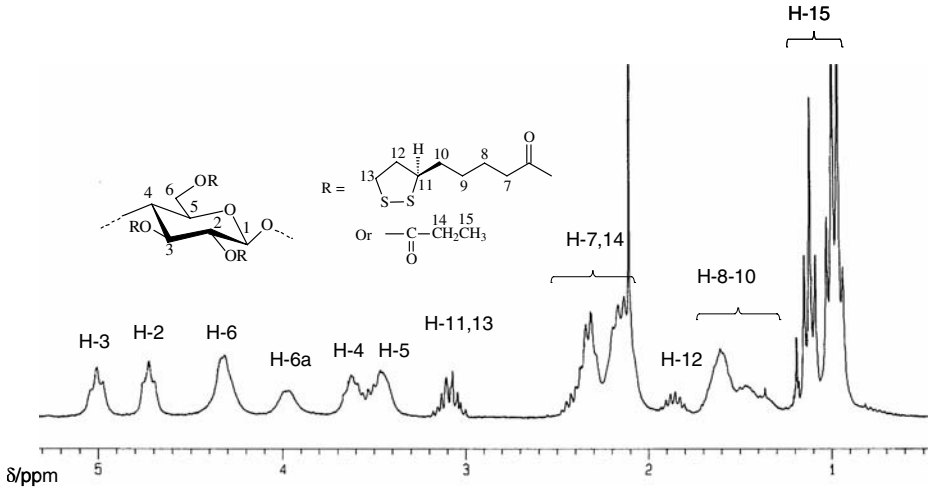

Figure 2. ^{1}H NMR ($CDCl_3$, NS 16) spectrum of cellulose α-lipoate propionate **3.1** (starting polymer **3**)

The binding onto gold surfaces was studied for samples **3-5** using SPR measurements. For this purpose gold-coated glass slides were placed for 12 h in DMSO solution (2 mmol) of cellulose α-lipoate, rinsed with ethanol to remove unbound cellulose α-lipoate and dried in a stream of N_2. The angular changes in plasmon curves indicate binding of the cellulose α-lipoates onto the gold surface. The largest shift of the plasmon curve was observed for the films of cellulose α-lipoate **5** (DS 0.11) corresponding to angular change of 0.560° compared to bare gold (Fig. 3).

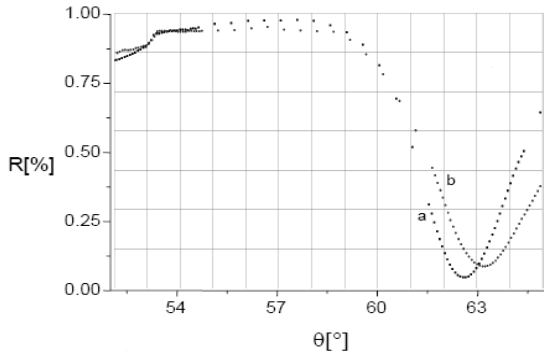

Figure 3. SPR spectrum as function of reflectivity (R[%]) vs coupling angle (θ[°]) of the bare gold (a) and coated with cellulose α-lipoate **5** (b)

The simulated film thickness was calculated to be 4.9 nm for cellulose α-lipoate **5**. A film prepared from cellulose α-lipoate **4** (DS 0.16) yielded a shift of the plasmon curve corresponding to angular change of 0.480° (Fig. 4). The simulated film thickness was determined to be 2.9 nm. Even the perpropionylated cellulose α-lipoate **3.1** (DS 0.18) showed a small shift of the plasmon curve of 0.160° (Fig. 5). For this sample a film thickness of 0.9 nm was calculated.

Consequently, the surface binding can be influenced by changing the DS and the molecular structure, e.g. by subsequent functionalization. Comparably small degrees

of functionalization as in case of cellulose α-lipoate **5** with a DS 0.11 yielded rather thick layers in contrast to samples with a higher DS or more hydrophobic sample. A reason could be the higher tendency of the less substituted cellulose derivatives to form hydrogen bonds leading to a thicker layer on the gold surface. This would explain the formation of a thin monolayer-like film for the perpropionylated cellulose α-lipoate **3.1**.

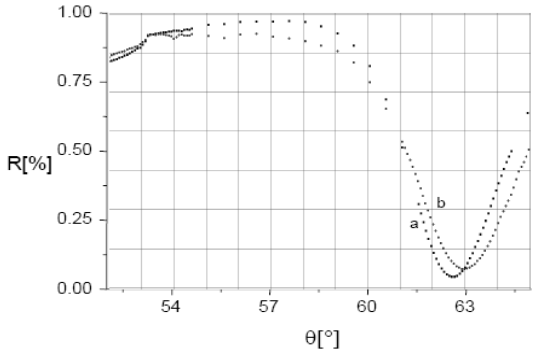

Figure 4. SPR spectrum as function of reflectivity (R[%]) vs coupling angle (θ[°]) of the bare gold (a) and coated with cellulose α-lipoate **4** (b)

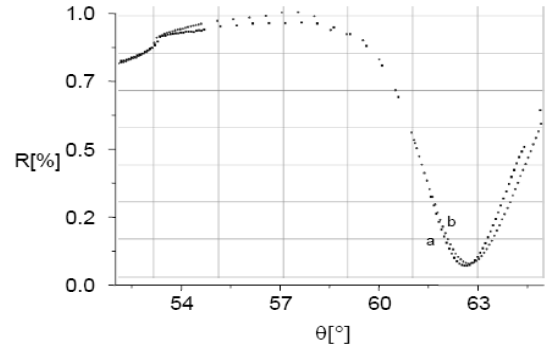

Figure 5. SPR spectrum as function of reflectivity (R[%]) vs coupling angle (θ[°]) of the bare gold (a) and coated with perpropionylated cellulose α-lipoate **3.1** (b)

Conclusions

Cellulose α-lipoates with a variety of DS values can be prepared homogeneously in DMAc/LiCl via *in situ* activation of the α-lipoic acid with *N,N'*-carbonyldiimidazole and *p*-toluenesulphonyl chloride. During the conversions the heterocyclic ring stays intact. Nevertheless, in case of higher DS values insolubility of the samples is observed. The reasons are not clear. Intermolecular cross-linking via S-S bridges can not be excluded. Soluble, lower substituted cellulose α-lipoates show self-assembly behavior onto a gold surface. Adsorption studies by means of SPR showed that a small degree of functionalization gives a comparably thick film on the gold surface. The film thickness decreases if the DS is increased or a subsequent functionalization is carried out to transfer the remaining OH-functions into propionate moieties. This observation leads to the assumption that the hydrogen bond system of the cellulose is responsible for the layer thickness meaning that the degree of functionalization can be applied to adjust the amount of polysaccharide derivative bound to the gold surface. Preliminary experiments with human fibroblasts have shown no significant influence

on their growth on a cellulose lipoate surface, i.e., giving a first hint for the biocompatibility. Future aspects of the work with the new cellulose derivatives are experiments towards biomineralization, crystal growth and enzyme immobilization on the surfaces.

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