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Synthesis and Characterization of Sulfur Containing Dextran- and β-Cyclodextrin Derivatives

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Summary

Sulfur containing dextran and β-cyclodextrin derivatives were synthesised as alternitive coating materials for gold surfaces. The esterification of the carbohydrates with thiophene carboxylic acids and α-lipoic acid was carried out in DMSO by *in situ* activation of the acids with *N*,*N*'-carbonyldiimidazole. The thiophene carboxylic acids vary in the position of the sulfur atom and the spacer between the thiophene and the carboxylic group. DS values ranging from 0.44 to 2.04 were accessible depending both on the carbohydrate and the acid used. Insoluble derivatives were obtained by the conversion of α-lipoic acid with the carbohydrates. The structure of the derivatives soluble in DMSO and DMF was examined by IR- and NMR spectroscopy. Furthermore, subsequent acylation reveals a useful tool for the determination of the degree of substitution of the thiophene derivatives additionally to elemental analysis.

Introduction

Immobilization of sulfur containing substances at metal substrates is of considerable interest to functionalize the surface, especially for biointeraction analyses based on surface plasmon resonance (SPR). It is necessary to functionalize gold surfaces with substances that may interact specifically with various biomolecules and other ligands [1]. In particular oligo- and polysaccharides are of increasing interest because of their natural origin, biocompatibility, and structural versatility for biomedical applications [2]. Dextran, a α-(1→6) linked polyglucan, is conveniently used as a coating material to prevent non-specific protein adsorption [3,4]. β-Cyclodextrin, a cyclic oligosaccharide of seven α-(1→4) linked D-glucose units, is an attractive host molecule for sensing purposes, as it can accommodate a variety of organic guest molecules [5]. The derivatization of these carbohydrates with sulfur containing substituents results in the formation of supramolecular structures at the surfaces via self-assembling processes forming mono- and multilayers or aggregates. The structure of the created surface is responsible for the reduction of non-specific protein adsorption and electrokinetic effects [6]. A high degree on sulfur containing compounds leads to an improved coverage of gold substrates [7].

Thiolated dextran can be produced by the reaction of a 4-nitrophenylchloroformate-activated dextran sample with cystamine and subsequent reduction yielding 2-mercaptoethyl-carbamoyl-dextran with a degree of substitution (DS) up to 0.012. Furthermore, the reaction of 2-iminothiolane with amino dextran leads to thiolated dextran possessing a DS of 0.95, which is used for the preparation of polysaccharide-protein conjugates [8]. The modification of cyclodextrins with sulfur containing compounds was studied more extensively regarding their adsorption onto gold surfaces. XPS measurements showed that β-cyclodextrin containing disubstituted sulfides (-SR) binds more efficiently on gold than thiol-based ones (-SH) [9]. Therefore, several dialkylsulfides are accessible via reaction of the carboxylic acids with the amino functions of previously functionalized carbohydrates or unmodified ones by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as coupling agent [10,11]. Especially the preparation of aminated dextran and cyclodextrin as starting material is a very time-consuming process. Hence, a simple and efficient synthesis strategy is required for the introduction of various sulfur containing substituents in carbohydrates. An interesting approach for the defined esterification of polysaccharides is the *in situ* activation of carboxylic acids with *N*,*N*'-carbonyldiimidazole (CDI) [12,13]. Highly functionalized derivatives are accessible bearing the desired substituent.

Carbohydrates functionalized with α-lipoic acid are interesting compounds for adsorption studies as possessing two sulfur atoms per substituent. Furthermore, unconventional materials like thiophene carboxylic acids may result in different structures after chemisorption depending on the position of the sulfur atom and the alkyl spacer between the thiophene and the carbonyl moiety. The known formation of intermolecular complexes by host-guest interactions of cyclodextrins can influence the shape of the surface as well [14]. By esterification via CDI activation, novel sulfur containing dextran- and β-cyclodextrin derivatives will be synthesized, which may be of interest in various fields where surface modification is needed.

Experimental

Materials

All chemicals were purchased from Fluka and used without further purification. *N*,*N*'-Carbonyldiimidazole, thiophen-2-acetic acid, and thiophene-2-butyric acid were received from Aldrich. Dextran was produced by *Leuconostoc ssp.* strain no. 10817 and possess a M_w of 5,400 g · mol^{-1} (polydispersity index 2.49).

Measurements

NMR spectra were acquired on a Bruker AMX 250 and DRX 400 spectrometer with 16 scans for ^1H NMR (room temperature) and 200,000 scans for ^{13}C NMR (70°C) measurements (25 mg sample · mL^{-1} for ^1H NMR and 100 mg sample · mL^{-1} for ^{13}C NMR studies). FTIR spectra were recorded on a Nicolet AVATAR 370 DTGS spectrometer with the KBr-technique. Elemental analyses were performed by CHNS 932 Analyzer (Leco).

Synthesis of Sulphur-containing Dextran and β-Cyclodextrin Derivatives

As a general procedure, the synthesis of the dextran and β-cyclodextrin derivatives was carried out by esterification of the carbohydrate via *in situ* activation of the carboxylic acid (thiophene-2-carboxylic-, thiophene-3-carboxylic-, thiophene-2-acetic-, thiophene-2-butyric-, α-lipoic acid) with *N*,*N*'-carbonyldiimidazole (CDI). Typically, 3.1 mmol (0.5 g) carbohydrate was dissolved in 10 mL DMSO, and each 9.3 mmol (3 mol per mol AGU) CDI and carboxylic acid were added to the solution. The mixture was allowed to react at 80°C for 24 h under stirring. The product was isolated by precipitation in 250 mL ethanol (water for samples **7** and **8**, isopropanol for sample **10**), washed two times with 50 mL ethanol and dried at 60°C under vacuum. The degree of substitution (DS) was determined by means of ^{1}H NMR spectroscopy after perpropionylation (samples **3** and **5**) and elemental analysis.

Perpropionylation

To determine the DS of the carbohydrate esters by means of ^{1}H NMR spectroscopy, peracylation with propionic anhydride was carried out. For this purpose, 0.2 g of the sample was allowed to react with 6 mL of propionic anhydride in 6 mL pyridine in the presence of 50 mg of *N*,*N*-dimethylaminopyridine as catalyst at 80°C for 24 h. The polymer was precipitated, washed with ethanol (250 mL) two times and dried at 60°C. The sample was reprecipitated from 3 mL chloroform in 100 mL ethanol followed by filtration and drying at 60°C under vacuum. FTIR (KBr): no ν (OH).

Results and Discussion

The synthesis of the dextran and β-cyclodextrin derivatives was carried out via *in situ* activation of the carboxylic acids. The homogeneous esterification was performed in a one-pot reaction in DMSO using *N*,*N*'-carbonyldiimidazole (CDI) as activating agent. α-Lipoic acid and thiophene carboxylic acid with varying position of the sulfur atom and the spacer between the thiophene and carboxylic group were used for the conversion of the carbohydrates to yield thiolated derivatives as shown in Figure 1.

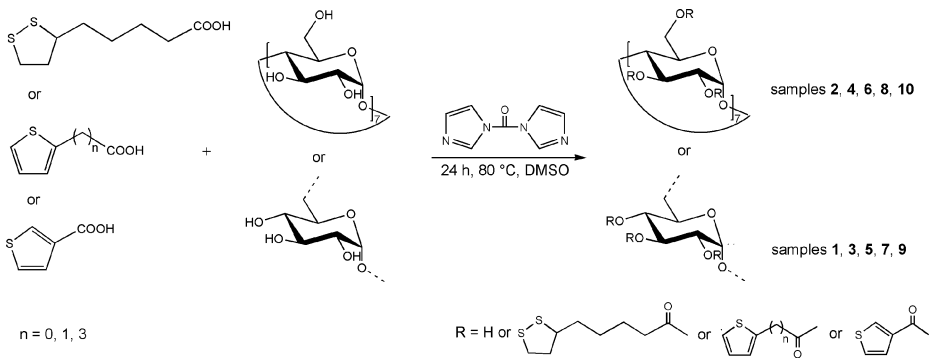

Figure 1. Schematic plot of the conversion of β-cyclodextrin and dextran with sulphur containing carboxylic acids *in situ* activated with *N*,*N*'-carbonyldiimidazole (CDI).

In a set of experiments, dextran and β-cyclodextrin were allowed to react with 3 equivalents of the corresponding acid and CDI at 80°C for 24 h. The formation of the ester was confirmed by FTIR spectroscopy yielding a signal at 1715-1745 cm^{-1} for the carbonyl group of the ester function. A degree of substitution (DS) ranging from 0.44 to 2.04 was calculated on the basis of elemental analyses (Tab. 1). The reaction of the carbohydrates with the thiophene carboxylic acids leads to derivatives soluble in DMSO and DMF whereas the esterification with α-lipoic acid yields insoluble products. Similar results were observed for reaction products of cellulose and α-lipoic acid using toluenesulphonyl chloride as activating agent [15]. Highly substituted cellulose lipoates (DS 0.50 and 1.45) are insoluble whereas low substituted derivatives (DS 0.11-0.18) show solubility in DMSO. Cross-linking reactions due to S-S cleavage of the dithiane may not cause the insolubility because of the missing S-H stretching at about 2550-2600 cm^{-1} in FTIR spectroscopy. An explanation for the occurrence of insoluble β-cyclodextrin lipoates may be the formation of strong inclusion complexes resulting in supramolecular structures [16]. A comparison of the DS values (except the thiophene-2-carboxylic acid derivatives) leads to the assumption that β-cyclodextrin is more reactive. This might be due to primary hydroxyl groups in cyclodextrins whereas dextran only exhibits secondary ones except the low content of primary hydroxyl groups of the non-reducing end groups and at the branches.

Table 1. Results of the esterification of dextran and β-cyclodextrin with sulphur containing carboxylic acids (CA) using *N*,*N´*-carbonyldiimidazole (CDI) as activating agent with a molar ratio of 1:3:3 (AGU:CA:CDI) in DMSO at 80°C for 24 h.

No.	Glucan	Carboxylic acid	DS[a]	Solubility
1	Dextran	Thiophene-2-carboxylic acid	1.23	DMSO, DMF
2	β-Cyclodextrin	Thiophene-2-carboxylic acid	0.87	DMSO, DMF
3	Dextran	Thiophene-3-carboxylic acid	1.59[b]	DMSO, DMF
4	β-Cyclodextrin	Thiophene-3-carboxylic acid	1.70	DMSO, DMF
5	Dextran	Thiophene-2-acetic acid	1.14[b]	DMSO, DMF
6	β-Cyclodextrin	Thiophene-2-acetic acid	2.04	DMSO, DMF
7	Dextran	Thiophene-2-butyric acid	1.25	DMSO, DMF
8	β-Cyclodextrin	Thiophene-2-butyric acid	1.66	DMSO, DMF
9	Dextran	α-Lipoic acid	0.44	Insoluble
10	β-Cyclodextrin	α-Lipoic acid	1.99	Insoluble

[a] Calculated from elemental analysis. [b] Calculated by means of ^{1}H NMR spectroscopy after perpropionylation.

NMR spectroscopy was applied for the structural analyses of the functionalized carbohydrates. Figure 2 shows the ^{13}C NMR spectra of the β-cyclodextrin thiophene-2-carboxylic acid ester (sample **2**), β-cyclodextrin thiophene-3-carboxylic acid ester (sample **4**), and dextran thiophene-3-carboxylic acid ester (sample **3**) recorded in DMSO-d_6. The broad signals of the atoms of the β-cyclodextrin derivatives may results from the formation of intermolecular complexes leading to a reduced mobility of the molecules in solution. Resonances assigned to the carbon atoms of the thiophene moiety are found at 134.5-127.4 ppm. The carbonyl atom of the ester linkage gives a signal at 161.5 ppm. The signals from 69.2 to 77.6 ppm of the β-cyclodextrin esters (Fig. 2a and 2b) result from the carbon atoms C2 to C6 of the anhydroglucose unit (AGU). The peak for C1 appears at 102.0 ppm in Figure 2a. In

addition, the spectrum shows a signal at 97.8 ppm (C1') which corresponds to C1 adjacent to a C2 atom bearing a thiophene moiety. Almost all hydroxyl groups of sample **4** (Fig. 2b) are modified in position C2 because there is only one peak at 97.8 ppm. The functionalization in C6 is indicated by a down-field shift of the signal (C6') respectively to the carbon atom bearing an unmodified hydroxyl group (C6) [17]. Resonances of C6 and C6' in sample **2** were found at 60.0 ppm and 64.3 ppm whereas sample **4** gives one signal at 63.3 ppm (C6'). These results correspond to the DS values of **2** (DS 0.87) and **4** (1.70). Sample **2** is partially substituted in C2 and C6, however, a complete functionalization of **4** in C2 and C6 is indicated by ^{13}C NMR spectroscopic data. Small peaks are not visible because of the minor resolution. Additionally, no selective substitution could be observed for β-cyclodextrin under the reaction conditions used.

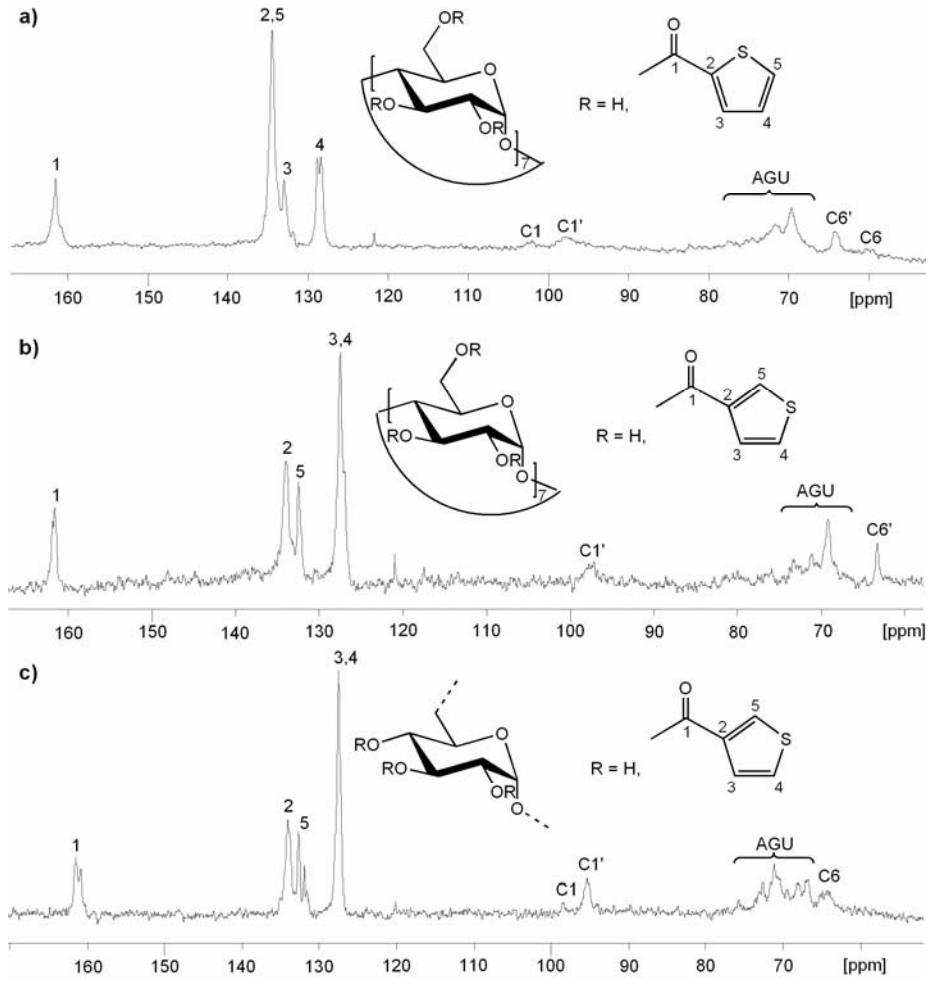

Figure 2. ^{13}C NMR spectrum of thiophene-2-carboxylic cyclodextrin ester (a, sample **2**, 400 MHz), thiophene-3-carboxylic cyclodextrin ester (b, sample **4**, 250 MHz), and thiophene-3-carboxylic dextran ester (c, sample **3**, 250 MHz) in DMSO-d_6.

The carbon atoms of the thiophene moiety of the dextran thiophene-3-carboxylic acid ester (sample **3**) give identical signals as the β-cyclodextrin derivative. Additionally, the spectrum shows the carbon atoms from C2 to C6 of the AGU in the region from 75.7 to 64.4 ppm. The presence of signals representing both C1 (98.4 ppm) and C1' (95.3 ppm) indicates an incomplete substitution in C2 although the DS value is 1.61 and OH in position 2 is the most reactive one [17]. The small signal at 120 ppm in the spectra of all derivatives and a nitrogen content of 0.3 to 1.1 evidences the presence of residual imidazole, which could not be removed by precipitation and washing processes. If there is a need for nitrogen free derivatives, dialysis may be a feasible method.

A supplementary technique to determine the DS of polysaccharides is ^{1}H NMR spectroscopy after perpropionylation [18]. Completely substituted derivates of the dextran thiophene-3-carboxylic acid ester (sample **3**) and –thiophene-2-acetic ester (sample **5**) were achieved by treatment of the samples with propionic anhydride/pyridine and *N*,*N*-dimethylaminopyridine as catalyst. The absence of hydroxyl groups was proven by FTIR spectroscopy; no ν(OH) signal appears. A representative ^{1}H NMR spectrum recorded in DMSO-d_6 of a perpropionylated dextran thiophene-3-carboxylic acid ester (sample **3**) is shown in Figure 3. The DS is calculated from the ratio of the spectral integrals of thiophene protons at 8.2 (H1), 7.6 (H2) and 7.3 (H3) ppm versus the CH_3 protons of the propionate moiety at 0.7 ppm. The resulting DS of 1.59 is in the range of the DS determined by elemental analysis (DS_{EA} 1.42).

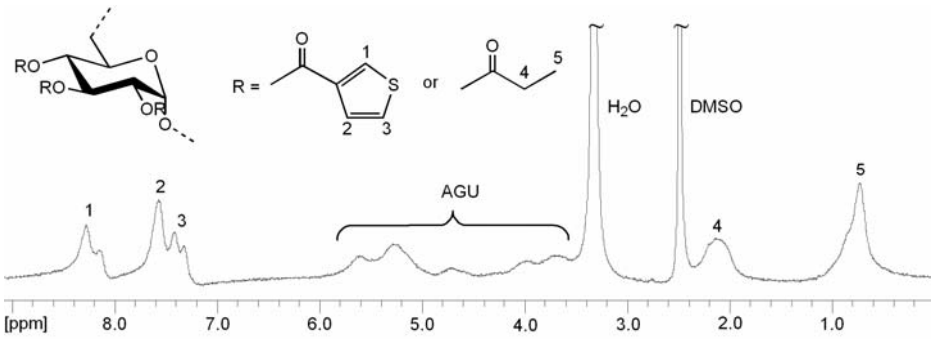

Figure 3. ^{1}H NMR spectrum of a perpropionylated dextran thiophene-3-carboxylic ester obtained from sample **3** (DS 1.59) in DMSO-d_6.

Conclusions

A variety of sulfur containing dextran and β-cyclodextrin derivatives with different degrees of substitution was prepared via *in situ* activation of the carboxylic acid with *N*,*N*'-carbonyldiimidazole. Insoluble derivatives were obtained by the conversion of α-lipoic acid. However, the thiophene carboxylic acids yield DMSO and DMF soluble carbohydrate derivatives. Preliminary experiments have shown that the thiophene functionalized dextran and β-cyclodextrin samples self-assemble onto gold surfaces from DMSO and DMF solutions [20]. In the matter of surface modification with oligo- and polysaccharides, e.g., for the interaction with biomolecules or other organic

compounds, it is necessary to examine the functionalized surfaces. The thickness of the layers and the structure of the resulting surfaces are supposed to depend on the degree of substitution, the carbohydrate used, the position of the sulfur atom in the thiophene moiety, and the spacer length between the thiophene and the carboxyl moiety.

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