Carbohydrate Self-Association

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Protein-like Oligomerization of Carbohydrates

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Many proteins form noncovalent and thermodynamically reversible oligomers, and the state of self-association can dictate a protein's functionality. DNA-binding proteins are very often dimeric, while other proteins exist as trimers (e.g. chloramphenicol transacetylase), tetramers (e.g. hemoglobin), or higher-order reversible association products (tubulin, viral coat proteins, sickle cell hemoglobin), with clear functional roles that have never been observed for carbohydrates. Although weak self-association in a polysaccharide has been shown,[1] we show for the first time the presence of multiple oligomeric forms in a whole class of polymeric carbohydrates, 6-deoxy-6-aminocelluloses, using the hydrodynamic technique of analytical ultracentrifugation as a probe.

Water-soluble aminocelluloses were prepared by the reaction of tosyl cellulose with an excess of di- or trifunctional amines, namely with tris(2-aminoethyl)amine yielding 6-deoxy-6-(2-(bis(2-aminoethyl)aminoethylamino) (BAEA) cellulose (**1**–**3**), as depicted in Figure 1. Similar structures were obtained from reactions of tosyl cellulose with 1,2-diaminoethane and 4,7,10-trioxa-1,13-tridecandiamine to give 6-deoxy-6-(2-aminoethyl)amino (AEA) cellulose **4** and 6-deoxy-6-(13-amino-4,7,10-trioxatridecaneamino) (ATOTA) cellulose **5**, respectively (Supporting Information Figure 1). The degree of substitution (DS) ranged from 0.60 to 0.85 (Supporting Information Table 1). NMR spectroscopic studies revealed that the nucleophilic displacement takes place at the primary position 6 of the anhydroglucose unit (AGU).[2]

Sedimentation coefficient distributions for the five different 6-deoxy-6-aminocelluloses were obtained from sedimentation velocity experiments in the analytical ultracentrifuge for six different solute loading concentrations (from 0.125 to 2.0 mg mL^{-1}) in 0.1 M phosphate-buffered saline (pH 6.8). Astonishingly, for every aminocellulose studied, the sedimentation coefficient distributions show between four and five discrete species with a stepwise increase in sedimentation coefficient. For example, the lowest sedimentation coefficient of the BAEA cellulose **1**, prepared from cellulose with a degree of polymerization (DP) of 450, was detected at 1.8 Svedbergs (S). Additional species sedimenting at peak maxima of approximately 2.8, 4.0, 5.1, and 6.5 S were also clearly found using the SEDFIT algorithm of Dam and Schuck[3] (Figure 1).

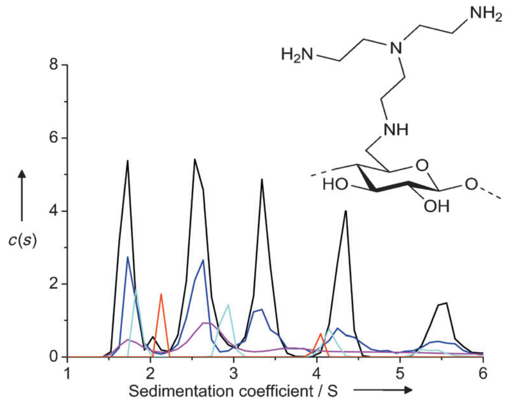

Figure 1. Representative sedimentation coefficient distributions of 6-deoxy-6-aminocelluloses. Distribution shown is for BAEA cellulose **1**, DS$_{\text{Amine}}$ = 0.60, at various concentrations: 2.0 (black), 1.0 (blue), 0.5 (pink), 0.25 (cyan), 0.125 mg mL^{-1} (orange). $c(s)$ = population of species with a sedimentation coefficient between s and ds.

The measurements were repeated for two other BAEA celluloses prepared from cellulose with DP = 250, and comparable results were obtained (Figure 2b,c). Peaks with sedimentation coefficients of approximately 1.6, 2.3, 3.1, 4.3, and 5.3 S have been found for an AEA cellulose (**4**) prepared from cellulose with DP = 450. Furthermore, the ATOTA cellulose **5** shows peaks sedimenting with maxima at approximately 1.7, 2.9, 4.3, and 5.5 S (Figure 2; Supporting Information Table 2). To check that the peaks were not artifacts of the analysis procedure, least-squares $g(s)$ versus s distribution data were also obtained (Supporting Information Figure 3a, s = sedimentation coefficient).[3] Multi-Gaussian analysis resolved the separate peaks (Supporting Information Figure 3b), similar to the $c(s)$ versus s plots.

To ascertain whether the higher-order species are different oligomers, a simple logarithmic relationship between sedimentation coefficient s and molecular weight M can be utilized, namely $s \approx M^b$ or, equivalently, $s_i/s_1 \approx (M_i/M_1)^b$, where 1 denotes the monomer, i denotes the ith species, and b is a power-law coefficient that depends on the conformation (ca. 0.2 for rods, 0.5 for coils, and 0.7 for spheres).[4] Taking the first observable species in each case as the monomer, all five of the 6-deoxy-6-aminocelluloses follow the power-law relation with $b \approx 0.7$, a value consistent with

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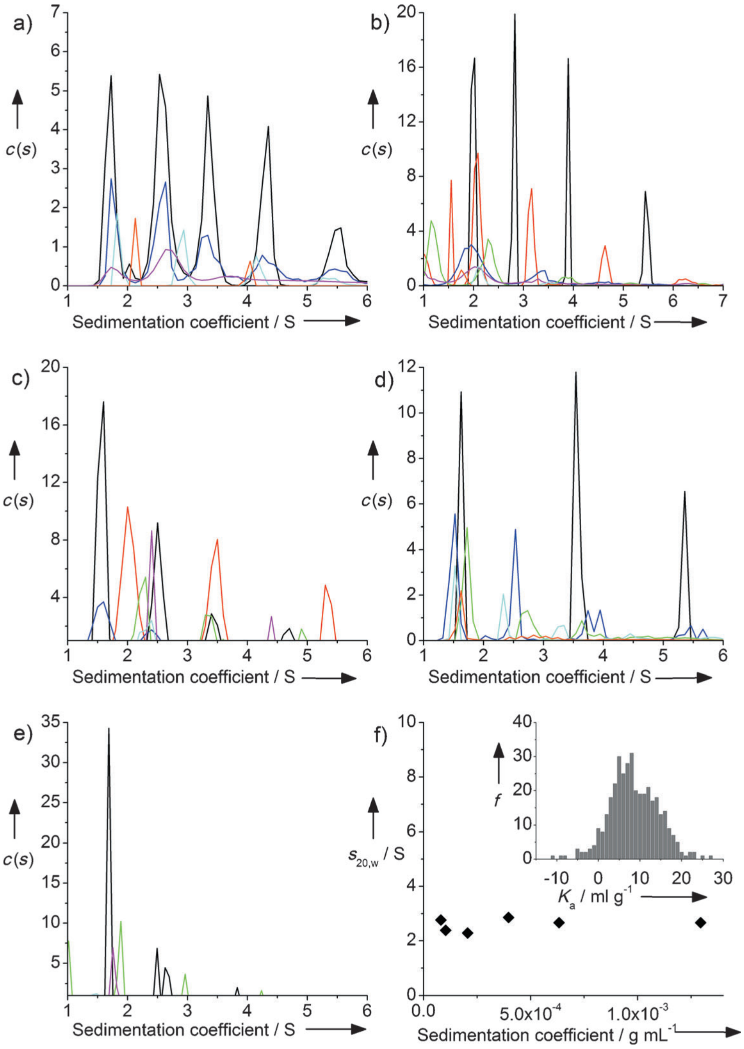

Figure 2. Representative sedimentation coefficient distributions $c(s)$ of a) BAEA cellulose **1** ($DS_{Amine} = 0.60$), b) **2** ($DS_{Amine} = 0.77$), c) **3** ($DS_{Amine} = 0.72$), d) AEA cellulose (**4**, $DS_{Amine} = 0.83$), and ATOTA cellulose (**5**, $DS_{Amine} = 0.85$) at various concentrations: 2.0 (black), 1.5 (red), 1.0 (blue), 0.75 (green), 0.5 (pink), 0.25 (cyan), 0.125 mg mL^{-1} (orange). f) Weight-average sedimentation coefficient as a function of the concentration of BAEA cellulose **1**. Analysis of this distribution via the function monomer–dimer yielded an estimate of $K_d \approx 2$ mM for the (very weak) self-interaction. The statistical distribution of 500 estimates for the parameter K_a (in mL g^{-1}) makes it clear that the value for this parameter, although small, is nonzero (see inset in (f), f = frequency).

that observed for globular proteins rather than the values between 0.2 and 0.5 more typical for polysaccharides (Supporting Information Figure 4). This estimate is subject to some systematic uncertainty because of approximations made concerning hydrodynamic non-ideality and the assertion that the first observable species is the monomer.

In an attempt to estimate the molar mass M_1 of the principal monomeric species from which the "ladder" of oligomers originates, sedimentation velocity analysis was performed, employing both the $c(s)$ and the $c(M)$ options in SEDFIT. The latter option takes an average frictional ratio across all the species in a heterogeneous sample, which is clearly inappropriate for these systems. However, by focusing on scans recorded near the end of the experiment (at these closing stages most of the heavier material will have pelleted), it is possible to obtain an estimate, and values for M_1 of between 12000 and 18000 are obtained, depending on the sample and the number of scans. For BAEA cellulose **2** it was possible to estimate M_1 using sedimentation equilibrium analysis, yielding a value of approximately 13000 (see the Supporting Information).

According to the well-known principle of Le Chatelier, for a reversible association the proportion of the monomer should decrease relative to the higher-order species as the loading concentration is increased. This behavior is observed in reversibly associating protein systems, for example hemoglobin, which exists as discrete monomers below a concentration of approximately 0.1 mg mL^{-1} but forms stable tetramers at physiological concentrations.[5] By stark contrast, for irreversible association phenomena such as antibody aggregation, the degree of oligomerization does not change with increasing concentration. In the case of the 6-deoxy-6-aminocelluloses, we see evidence for at least partial reversibility, with a change in the relative proportion of each species (most notably a drop in the relative proportion of monomer) when the total concentration is changed (Figure 2; Supporting Information Figure 2 and Table 3).

For example, at 20 °C and pH 6.8 the slowest compound present in the BAEA cellulose **1** found at 1.8 S has a relative area of 20 % for the highest concentration and increases to 30 % when the concentration is decreased to 0.25 mg mL^{-1}. In contrast, the relative area under the second peak at about 2.8 S increases from 28 to 34 % when the concentration is lowered from 2.0 to 0.25 mg mL^{-1}, whereas the area under the third peak remains at approximately 18 % when the concentration is decreased from 2.0 to 0.25 mg mL^{-1}. However, for the lowest concentration of 0.125 mg mL^{-1}, only four peaks can be detected, with 59 % for the lowest peak (2.1 S), 26 % for the second lowest (4.0 S), 9.5 % for the third (6.3 S), and 4.1 % for the last (7.8 S).

These results indicate that a dissociation of the oligomeric species in sample **1** occurs below a concentration of 0.25 mg mL^{-1}. A similar increase of the relevant amount of the slowest ("monomer") peak with a decrease in total loading concentration is observed for the other four samples studied. The slowest peak at the second BAEA cellulose **2** accounts for 36 % of the total amount of material at a loading concentration of 2.0 mg mL^{-1}, increases to approximately 70 % for loading concentrations between 1.0 to 0.25 mg mL^{-1}, and is as high as 77 % for the lowest concentration (0.125 mg mL^{-1}). For the BAEA cellulose **3** we find a small increase for the slowest peak from 51 % at 2.0 mg mL^{-1} to 60 % for the lowest concentration studied (0.25 mg mL^{-1}). The amount of the slowest component in the AEA cellulose **4** increases with decreasing concentration. Thus, for a concentration of 2.0 mg mL^{-1} it amounts 29 %, while it amounts 65 % for a concentration of 0.125 mg mL^{-1}.

For a concentration of 2.0 mg mL^{-1} of the ATOTA cellulose **5**, it amounts 67 % for the peak with a sedimentation coefficient of 1.7 S. This value increases to 76 % for a concentration of 0.50 mg mL^{-1}. In the cases of aminocelluloses **2** and **4** a pentamer species was only observed for the highest loading concentration 2.0 mg mL^{-1}.

The effect of temperature and pH value on two of the samples was also examined. For ATOTA cellulose **5** the proportion of monomers clearly dropped (from ca. 70 to 50 %) as the temperature was increased from 10 to 30 °C, which suggests that the interaction was hydrophobic in nature, although for BAEA cellulose **2** no significant change was observed. Both samples showed the maximum proportion of oligomers at pH 4–6.

A new and promising trend is thus disclosed for the structural modeling of interfacial material surfaces with biological recognition functions at the molecular and cellular level. The partially reversible interactions of 6-deoxy-6-aminocellulose can be translated to their interactions with other biological macromolcules, that is, 6-deoxy-6-aminocellulose structures bind preferentially to glycoproteins and proteoglycans with sugar chains arranged like antennas, which act as receptor structures and could potentially act as an extracellular matrix (ECM) such as laminin, poly-L-lysine, or polyornithine. Another promising path is the covalent immobilization of a recognition molecule such as a protein[6] or an aptamer[7] through NH_2 groups on custom-designed aminocellulose self-assembled monolayers by using NH_2-reactive bifunctional reagents. The observation may change the whole perception of carbohydrate molecular interaction phenomena.

Experimental Section

Tosyl cellulose was prepared according to Rahn et al.[8] Typically, aminocelluloses were prepared from tosyl cellulose (4.2 g, DS = 1.02, 13.2 mmol modified AGU) in DMSO (40 mL) by addition of 1,2-diaminoethane (22 mL, 406.7 mmol). The mixture was allowed to react for 6 h at 100 °C and was precipitated in 600 mL acetone. A white product (**4**) was obtained after washing with isopropyl alcohol and vacuum drying. Yield: 2.6 g (92 %), N 10.3 %, S 2.8 % (DS_{Amine}: 0.83, DS_{Tos}: 0.20).

Sedimentation velocity experiments were performed using an Optima XL-I AUC (Beckman Instruments, Palo Alto, USA) . The data were analyzed using the least-squares $c(s)$ method included in the SEDFIT software.[3] Sedimentation coefficients were extrapolated to zero concentration to correct for non-ideality effects.[1]

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