Molecular Recognition by Hydrogen Bonding in Polyelectrolyte Multilayers

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Abstract: Functional polyanions were prepared by copolymerization of sulfopropyl acrylate and sulfopropyl methacrylate with monomers bearing triaminopyrimidine or barbituric acid functionalities, respectively. Functionalized polyelectrolyte multilayers were assembled from these copolymers by stepwise alternating adsorption with poly(choline methacrylate). These multilayers are suited for molecular recognition of substrates that are complementary to the functional groups incorporated. Thus, multilayers containing triaminopyrimidine moieties selectively bind barbituric acid, and vice versa, when exposed to solutions of the 1:1 complex of barbituric acid and triaminopyrimidine. The molecular recognition process was monitored by UV/Vis spectroscopy, time-of-flight secondary ion mass spectroscopy (ToF-SIMS), and photoelectron spectroscopy (XPS). Remarkably, after successful recognition and binding of the complementary substrates to the mutilayers, the stepwise layering could be continued.

Keywords

hydrogen bonds · molecular recognition · polyelectrolyte multilayers · surface chemistry · thin films

Introduction

Molecular recognition is an important prerequisite for various processes in Nature, such as enzyme catalysis and binding to membrane receptors.[1] One of the most prominent examples of this is base pairing in DNA, which is fundamental to life.[2] In the past years great efforts have been made to understand molecular recognition in natural and in synthetic systems.[3] The majority of systems are based on hydrogen bonding, which are more difficult to examine in aqueous systems. So far, experiments have preferentially been carried out in aprotic organic solvents, although aqueous systems are closer to the natural systems. Whereas most experiments are performed in solution, there are examples of molecular recognition phenomena at interfaces, too. In particular, the fixation of receptor units to solid substrates allows molecular recognition to be exploited in a number of applications. Recently, Langmuir–Blodgett multilayers and self-assembled monolayers (SAMs) have been employed in this way.[4] Both of these techniques are useful, but have several disadvantages: for Langmuir–Blodgett films, the sample geometry is limited to flat surfaces and their preparation requires complicated equipment; chemical self-assembly is usually a slow process and is prone to defects, since chemical reactions with quantitative yield are required if defect-free layers are to be formed.

Alternatives to these thin-layer techniques are being searched for. It has been known for a few years that thin, defined multilayers can also be built up by alternating adsorption of oppositely charged polyelectrolytes.[5–7] This method can overcome some of the typical Langmuir–Blodgett and SAM problems: there are no limits concerning the surface geometry or size, and no complicated equipment is required. Also, the deposition process is relatively fast compared to chemical self-assembly. Owing to the polymeric character of the components used for the assembly, problems with defects are minimized.

Recently it was shown that polyelectrolytes with functional groups can be incorporated into multilayer assemblies, which can be modified by chemical reaction on the surface.[8] In analogy, molecular recognition reactions on the outermost layer of an appropriately functionalized polyelectrolyte multilayer should be feasible.

With this aim in mind, we prepared polyanions bearing triaminopyrimidine (TAP) or barbituric acid groups, as the molecular recognition process in the system TAP/barbituric acid is well known. These systems are based on multiple H-bonding, which gives rise to selective and strong interactions, analogous to the pairing of nucleobases.

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Experimental Procedure

Materials (see Scheme 1): The water for all experiments was deionized and purified by an Elgastat Maxima from Elga (resistance 18.2 MΩ). Triaminopyridine was purchased from Fluka, and barbituric acid and branched polyethylenimine (**1**) were purchased from Aldrich. 3-Sulfopropyl acrylate and 3-sulfopropyl methacrylate were gifts from Raschig (Germany). The sodium salt of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) was a gift from Lubrizol (UK), which was polymerized to give polymer **2** according to ref. [9]. Poly(choline methacrylate) (**3**) was prepared by analogy to ref. [10]. Copolymer **4** was synthesized by radical copolymerization of a functionalized monomer containing barbituric acid with equimolar amounts of potassium 3-sulfopropyl acrylate in the presence of 1 mol% of azobisisobutyronitrile as thermal initiator, and **5** by a similar procedure using monomers containing 2,4,6-triaminopyrimidine groups and potassium 3-sulfopropyl methacrylate. The synthesis of the comonomers bearing a TAP or a barbituric acid functionality will be described elsewhere [11]. The copolymerizations were carried out for 5 d at 80 °C in dimethyl sulfoxide (DMSO). The polymers were purified by several precipitation steps. A tenfold excess of toluene was used as precipitation agent. The polymer precipitated, while the monomers stayed in solution. The absence of low molar mass species after precipitation was checked by TLC. The copolymer compositions of the resulting statistical copolymers are given in Scheme 1 and were determined by elemental analysis of sulfur and nitrogen. The samples for elemental analysis were recovered after ca. 5% conversion; the results obtained therefore provide evidence for a statistical copolymerization. Owing to the ionic nature of the polymers, the usual methods for determining molar masses fail. In ^{1}H NMR groups no end groups are visible; therefore, the DP (degree of polymerization) should at least exceed 10.

Preparation of the Polyelectrolyte Multilayers: The polyions **1**–**3** were dissolved in water at a concentration of 0.02 mol of repeating unit per liter. Copolymers **4** and **5** were dissolved in a mixture of 90 vol% of water and 10 vol% of DMSO (p.a.). As supports for the multilayer assemblies, quartz plates and silicon wafers were used, which were cleaned and pretreated as described in ref. [12]. An initial pair of buffer layers, namely, **1** and **2**, were deposited onto all supports prior to deposition of the various polyelectrolytes. Multilayers were assembled by successively dipping the supports in a solution of polycation **3** for 20 min and then in the polyanion solution for 20 min, except in the case of polyanion **4**, where the adsorption time had to be prolonged to 50 min. Before changing the polyelectrolyte solutions, the supports were dipped into three beakers with pure water for 1 min each, to remove excess adhering solution.

Recognition experiments: For the recognition experiments, multilayer assemblies with polymer **4** on top and assemblies with polymer **5** on top were dipped into the same 2×10^{-2} mol L^{-1} solution of a 1:1 complex of TAP and barbituric acid.

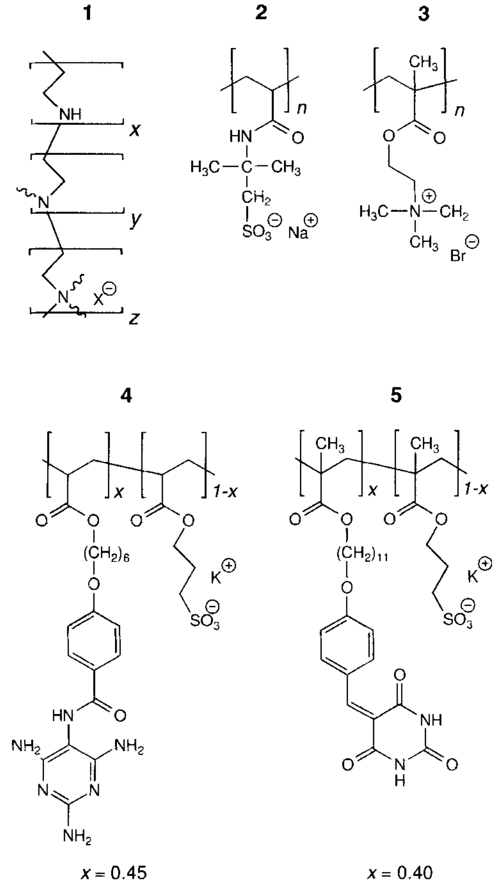
Methods: The UV/Vis spectra of the multilayer assemblies on quartz were recorded with a SLM-AMINCO DW-2000 spectrophotometer in the double-beam mode. A cleaned quartz plate served as reference. Silicon wafers covered with multilayer assemblies were analyzed by ToF-SIMS (Charles Evans) with a 5 kHz pulsed Ga$^+$ beam (15 keV, 400 pA) and by XPS (SSI-X-Probe: SSX-100/206 from Fisons, equipped with an aluminum anode and a quartz monochromator).

Results and Discussion

The polymers shown in Scheme 1 were used for the assembly process. The copolymerizations were nearly ideally statistical: with equimolar monomer feeds, copolymer compositions (determined by elemental analysis of sulfur and nitrogen) with only a slight excess of ionic units were obtained.

UV/Vis Spectroscopic Studies of the Multilayers:

Multilayers containing TAP groups: Alternating multilayers could be succesfully prepared from polycation **3** and polyanion **4**. As the times needed for adsorption of a sufficiently thick polyelectrolyte layer depends sensitively on the chemical structure of the polyion to be adsorbed, the kinetics of the adsorption of **4** on a layer of **3** was investigated by UV/Vis spectroscopy before performing further experiments.



Scheme 1. Chemical formulae of the polymers employed in this study.

Figure 1 shows the increase in absorbance as a function of adsorption time for one layer of polyion **4** on a surface covered with polycation **3**. Saturation is reached after about 90 min. However, the adsorption need not be driven to saturation in multilayer assembly. After approximately 50 min, sufficient polymer is adsorbed to enable the deposition of the next layer of **3**. As the adsorption of polycation **3** requires 20 min, one complete deposition cycle lasts 70 min, which is relatively fast com-

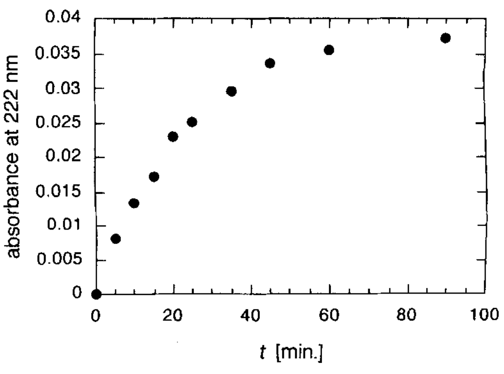


Fig. 1. Growth in the absorbance of a layer of polyanion **4** as a function of adsorption time

pared to the typical times of several hours to one day needed per layer of chemically assembled multilayers.

To study molecular recognition, three double layers of polycation **3** and polyanion **4** were deposited on a quartz substrate. Then, the multilayer with the TAP-functionalized polymer **4** on top was exposed to a solution of the 1:1 complex of barbituric acid and TAP. The multilayer deposition and the binding of barbituric acid were followed by UV/Vis spectroscopy.

Figure 2 shows the plot of the absorbance at 220 nm versus the number of deposition cycles for multilayers of **3** and functionalized **4**. The absorbance increases linearly for the first three layers; this demonstrates that reproducible deposition is taking place. After deposition of the third double layer, exposure to the barbituric acid/TAP complex causes an additional increase in absorbance. This strongly suggests that barbituric acid is successfully bound to the multilayer. Remarkably, further polyion layers can be deposited after the binding step, and the increase in absorbance is again linear (Figure 2).

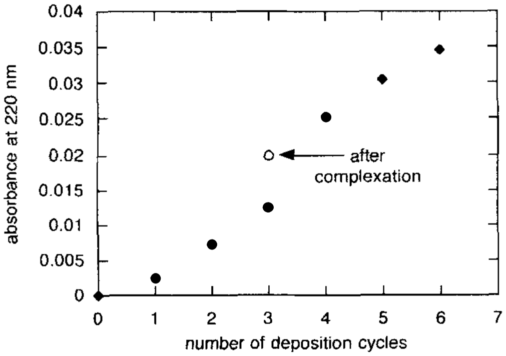


Fig. 2. Growth in the absorbance of a multilayer assembly of **3** and **4** as a function of the number of adsorption cycles.

In Figure 3 , the full UV/Vis spectra of the multilayers are shown after three deposition cycles of **3**/**4** (curve A), after three deposition cycles plus complexation (curve B), and after three further deposition cycles of **3**/**4** (curve C). When the spectra are compared for the sample before and after the complexation step, the above discussed increase in absorbance is evident. In addition, a hypsochromic shift in the maximum wavelength from 275 (curve A) to 265 nm (curve B) is observed. A similar shift in λ_{max} is also observed when **4** and barbituric acid are mixed in solution. This behavior is analogous to the hypsochromic shift upon helix formation of two complementary DNA strands.[2] These results indicate the successful complexation and binding of barbituric acid to the multilayer.

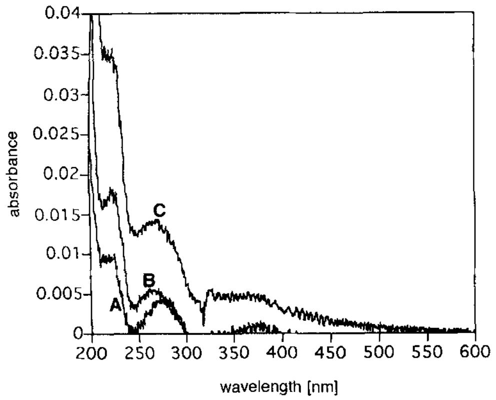


Fig. 3. UV/Vis spectra of an assembly of **3** and **4**: A) after three deposition cycles, B) after three deposition cycles and complexation, C) assembly B after three more deposition cycles.

Multilayers containing barbituric acid residues: Alternating multilayers could be succesfully prepared from polycation **3** and polyanion **5**, too. The adsorption kinetics are somewhat faster than for polyanion **4** (Figure 4). The equilibrium state is reached after approximately 1 h. Again, a sufficient amount of polyanion to allow the deposition of a further layer of polycation **3** is bound after shorter periods. As 20 min proved to be sufficient, the adsorption time for a complete deposition cycle for one double layer is reduced to 40 min.

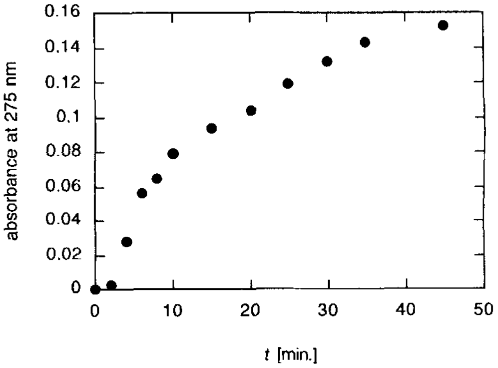


Fig. 4. Growth in the absorbance of a layer of polyanion **5** as a function of the adsorption time.

In Figure 5, the absorbance of a multilayer assembly of **3** and **5** is plotted versus the number of deposition cycles. Here, the increase of absorbance versus the number of cycles is not linear but parabolic. As such a phenomenon is frequently encountered for polyelectrolytes carrying hydrophobic groups,[13] this behavior may be due to the long hydrophobic spacer groups separating the barbituric acid from the polymer backbone in **5**. Nevertheless, the fact that the absorbance increases after each step demonstrates that the multilayer system is growing.

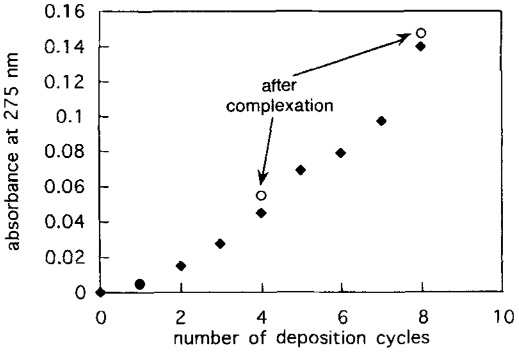


Fig. 5. Growth in the absorbance of a multilayer assembly of **3** and **5** as a function of the number of adsorption cycles.

To study the molecular recognition process by UV/Vis spectroscopy, the same type of experiments were performed for the multilayer system **3**/**5** as described above for the system **3**/**4**: four double layers of polycation **3** and polyanion **5** bearing barbituric acid residues were deposited on quartz; the multilayer with the functional polymer **5** on top was then exposed to a solution of the 1:1 complex of barbituric acid and TAP. Since polyanion **5** bears barbituric acid functionalities, the partner for molecular recognition is now TAP.

After four double layers had been deposited, the exposure of polyanion **5** to the solution of the TAP/babituric acid complex caused an increase in the absorbance. This result strongly suggests that TAP is being bound. As for the system **3**/**4**, the multilayer build-up could be continued after the binding step, and the absorbance grew in a nonlinear fashion like for the first four double layers. Also, a second exposure of the multilayer assembly to the solution of the TAP–babituric acid complex after the eighth deposition cycle again induced an increase in the absorbance (Figures 5 and 6).

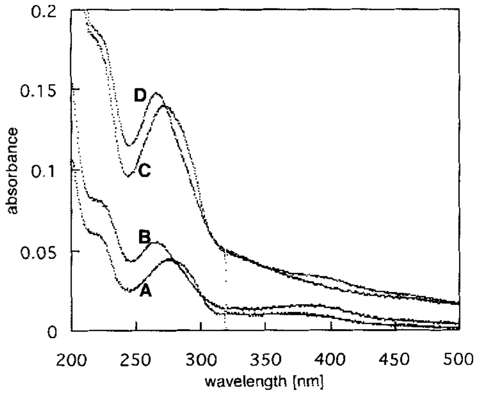


Fig. 6. UV/Vis spectra of an assembly of **3** and **5**: A) after three deposition cycles, B) after three deposition cycles and complexation, C) assembly B after three more deposition cycles, and D) after a second complexation step on assembly C.

The multilayer spectra before (curves A and C) and after (curves B and D) exposure to TAP are shown in Figure 6. In addition to the increase in absorbance after complexation, indicating the binding of additional material, a hypsochromic shift in λ_{max} from 277 (curve A) to 265 nm (curve B) is observed. Like the hypsochromic shift that occurs in the multilayer system **3**/**4** after exposure to the 1:1 barbituric acid–TAP solution, this indicates the binding of TAP to the multilayer. A similar hypsochromic shift is observed after the second exposure of the multilayers to the barbituric acid–TAP solution. Since curve C is already derived from a superposition of complexed and uncomplexed layers, the shift of λ_{max} from curve C to D is less pronounced than that from curve A to B.

Studies of Molecular Recognition by Time-of-Flight Secondary Ion Mass Spectroscopy (ToF-SIMS): The binding of babituric acid to multilayers prepared with polyanion **4** was also studied by ToF-SIMS. The binding is indicated in the negative ion spectra by a significant increase of the CNO^- intensity (Table 1). This signal can be attributed to a fragmentation of the bound babituric acid. Whereas the intensity of the fragments sputtered from the sulfur-containing side-chains varies weakly with complexation, the signal related to the nitrogen-containing side-chain of **4** ($C_{11}H_{13}N_6O^+$) decreases drastically after complexation. This observation suggests a shielding of the TAP moiety in polyanion **4**, which points to a high complexation yield. Hence, the ToF-SIMS analysis corroborates the UV/Vis studies of the recognition process.

Table 1. ToF-SIMS results for the system **3**/**4**/barbituric acid.

Ion	Mass (Da)	**4** before complexation % of total intensity	**4**/barbituric acid complex % of total intensity
$C_{11}H_{13}N_6O^+$	245	0.10 ± 0.05	0.0046 ± 0.0007
CNO^-	42	0.20 ± 0.002	0.36 ± 0.03
SO_3^-	80	0.72 ± 0.03	0.75 ± 0.02
$CH_2SO_3^-$	94	0.15 ± 0.007	0.13 ± 0.004
$C_3H_7SO_4^-$	139	0.012 ± 0.001	0.008 ± 0.001

The binding of TAP to multilayers prepared with polyanion **5** is accompanied by an increase in the intensity of the small nitrogen-containing ions at the surface (Table 2). The drastic increase in the $C_2H_4N^+$ intensity of more than one order of magnitude cannot be explained by a change in the surface concentration of polycation **3**, because the characteristic peak intensities of **3** are increased only by less than a factor 2 ($C_5H_{12}N^+$ and $C_5H_{12}NO^+$). Therefore, this signal increase must be a direct result of the complexation of the barbituric acid residues with TAP. Indeed, a similar pattern with high intensities of the CH_2N^+ and $C_2H_4N^+$ peaks is characteristic for multilayer samples with polyanion **4** on top, in which TAP moieties are inherently bound. The marked decay of the oxygen-containing peak intensities after the complexation step is also consistent with a partial screening of the polymer by the TAP molecules. As for the system **3**/**4**, the ToF-SIMS analysis of the system **3**/**5** corroborates the UV/Vis studies of the recognition process.

Table 2. ToF-SIMS results for the system **3**/**5**/TAP.

Ion	Mass (Da)	**5** before complexation % of total intensity	**5**/TAP complex % of total intensity
CH_2N^+	28	0.13 ± 0.02	0.95 ± 0.2
$C_2H_4N^+$	42	0.49 ± 0.1	7.5 ± 1.5
$C_5H_{12}N^+$	86	0.43 ± 0.06	0.75 ± 0.04
$C_5H_{12}NO^+$	102	0.34 ± 0.03	0.61 ± 0.03
$C_3H_7O^+$	59	5.9 ± 1.7	0.16 ± 0.02
$C_4H_7O_2^+$	87	0.80 ± 0.25	0.02 ± 0.006
$C_5H_9O_2^+$	101	0.46 ± 0.11	0.01 ± 0.001

Studies of Molecular Recognition by XPS: The binding of TAP to the multilayer system **3**/**5** was analyzed by XPS, which shows the increase of the nitrogen content with the complexation step: 3.2 atom% N are found before complexation and 4.2 after complexation. Moreover, the deconvolution of the N 1s peak shows that the intensity of the component due to **3** (at 402.5 eV) increases slightly after complexation (from 2.1 to 2.3 atom%), which is consistent with the SIMS analysis, and that the main relative increase (from 1.1 to 1.9 atom%) is observed for the component corresponding to the phenyl-NH_2, at 400.0 eV. This component cannot be clearly separated from the pyridine-type nitrogen or from the aliphatic-type nitrogen in the spectrum. On the other hand, the oxygen content decreases only slightly, in agreement with the larger information depth and the lower sensitivity to the chemical environment of this technique (24.5 atom% O before complexation and 24.0 after complexation).

Conclusions

Polyelectrolyte multilayers can be assembled from polycation **3** with the anionic copolymers **4** or **5** bearing functional moieties for molecular recognition. Both UV/Vis spectroscopy and ToF-SIMS show the successful binding of barbituric acid and TAP, respectively, to the complementary complexation partner in the multilayers. The binding of TAP to polyanion **5** can also be monitored by XPS. The successful molecular recognition is remarkable for these systems, as they are based on H-bonding and the binding takes place in an aqueous environment. Also, it has to be kept in mind that the low molar mass component is complexed by its low molar mass partner in solution prior to its transfer to the polymer-bound complexation sites. This demonstrates the strength and the selectivity of the recognition in the system TAP/barbituric acid.

At present it is not clear whether the recognition process takes place only in the topmost layer, or whether the components may penetrate into underlying layers and bind there to the functional polyanions, too. This will require further experiments.

Remarkably, the complexation of the polyanion layer does not hinder the deposition of further polyelectrolyte layers. As the recognition process does not interfere with further layering, the deposition of protective coating layers or of polymers with other functionalities might be envisaged. Since polyelectrolyte multilayer assembly is a facile and rapid process, it not only offers a convenient method to study molecular recognition phenomena, but also a possible approach to chemical sensors.

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