Assembly of Thin Films by Means of Successive Deposition of Alternate Layers of DNA and Poly(allylamine)

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ABSTRACT: We have recently introduced a new method of creating ultrathin films based on the electrostatic attraction between opposite charges. Consecutively alternating adsorption of anionic and cationic polyelectrolytes leads to the formation of multilayer assemblies. Here, we extend our concept to the procedure of ultrathin (100-500-Å) film growth with alternation of DNA and poly(allylamine) molecular layers. Multilayer buildup was monitored by small-angle X-ray reflectivity; the step of growth for layer pairs of DNA/poly(allylamine) was 40 Å. The relationship between this growth mechanism and the process of natural complex formation between DNA and polyamines (spermine, spermidine, etc.) may be of interest.

Introduction

Ultrathin organic films are currently gaining interest in many areas such as integrated optics, sensors, friction-reducing coating, and surface orientation layers.[1] Most of these tasks require the preparation of well-defined films composed of molecules in a unique spatial arrangement with respect to each other and to the substrate. It is most interesting to construct films with a supramolecular architecture in which the individual organic molecules are macroscopically oriented and in which molecules with different functionality can be incorporated into individual layers. Of special interest is the deposition of biological macromolecules such as proteins and DNA. The DNA-containing thin films, for example, may be used in sensor devices for various specific reagents for DNA, which might even be interesting for ecological problems.

A new technique of constructing multilayer assemblies by consecutively alternating adsorption of anionic and cationic polyelectrolytes was recently developed in our laboratory.[2,3] An advantage over the classic Langmuir-Blodgett technique[4] is that adsorption processes are independent of the substrate size and topology. In this technique, as opposed to the Langmuir-Blodgett technique, one can work with water-soluble molecules, which is required for many biological macromolecules.

The principle of the multilayer assembly is shown in Figure 1 and is described as follows. A solid substrate with a positively charged planar surface is immersed in the solution containing the anionic polyelectrolyte, and a layer of polyanion is adsorbed (step A). Since the adsorption is carried out at relatively high concentration of polyelectrolyte, a number of ionic groups remains exposed to the interface with the solution, and thus the surface charge is effectively reversed. After rinsing in pure water the substrate is immersed in the solution containing the cationic polyelectrolyte. Again a layer is adsorbed, but now the original surface charge is restored (step B). By repeating both steps (A, B, A, B, ...) in a cyclic fashion, alternating multilayer assemblies are obtained. The complete reversal of surface charge is the crucial factor for a regular stepwise growth of the multilayer films. There is recent additional evidence that this charge reversal takes place at polymer concentrations above approximately 5 mg/mL.[5]

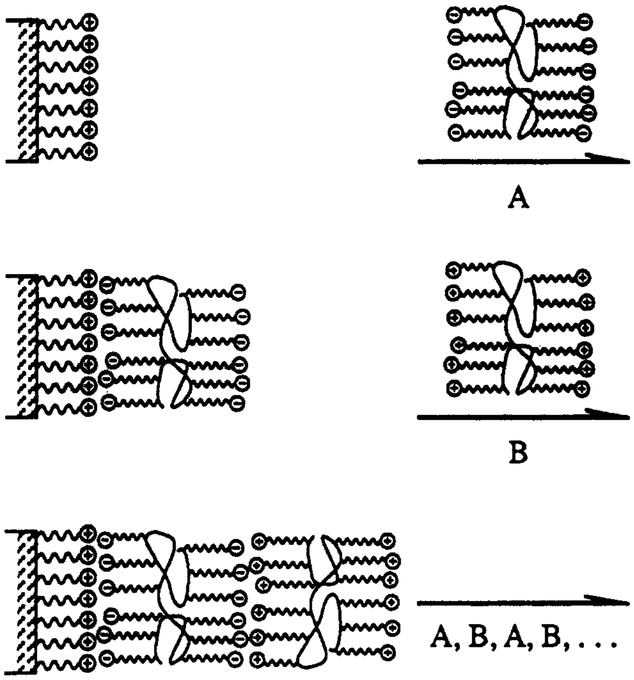

Figure 1. Side view schematically depicting the buildup of multilayer assemblies by consecutive adsorption of anionic and cationic polyelectrolytes. For reasons of simplicity the counterions were omitted in these oversimplified graphic representations; poly(styrenesulfonate), poly(allylamine). It is not implied that the symbols used for the polyelectrolytes represent their actual structure in solution or after the adsorption.

We have previously reported on the construction of alternating multilayer films using poly(styrenesulfonate) (PSS) and poly(allylamine) (PAH).[6,7] It was established that films composed of at least 60 layers can be grown and that the thickness of the films increases linearly with the number of layers. Furthermore, the thickness of each individual layer and thus also the total thickness of the film can be adjusted very precisely by changing the ionic strength of the solution from which the polyions are adsorbed.[7] We believe that in principle all polyelectrolytes should be suitable for the incorporation into multilayer assemblies; as an additional example, we have demonstrated the assembly of films of a poly(vinyl sulfate) potassium salt and poly(allylamine) hydrochloride.[8]

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The main idea of the method consists of alternation of the terminal charge after every next layer deposition. This implies that there is principly no restriction to certain polyelectrolytes and that the construction of multilayer assemblies should also be possible by using DNA as a polyanion and poly(allylamine) as a polycation. Except an interest in such films from the point of view of thin organic film technology there might even be biological meaning in our work: the interaction between DNA and protoamines and polyamines plays an important role in compactization and packing of DNA *in vivo*.[9]

We have taken a DNA solution and used it for DNA adsorption in alternation with the "positively charged" poly(allylamine) above a 270-Å thick precursor polymeric film. We consider DNA as a polyanion for our procedure, due to the negative charge of the sugar–phosphate backbone of DNA. Moreover, there are the experiments of DNA adsorption from solution to positively charged lipid monolayers.[10]

Multilayer buildup and structural analysis are completely based on X-ray reflectivity data and its fitting to film models. From earlier work on films constructed by consecutive adsorption of polycations and polyanions it is well established that X-ray reflectivity is a powerful tool for the analysis of film growth and structure.[7,8] From the evaluation of the X-ray results we obtain the total thicknesses of the films (L) and an estimate for their roughness (σ) and the thicknesses of a pair of DNA/PAH layers, the DNA layer, and pairs of PSS/PAH layers, which will be compared and discussed. For convenience of the X-ray analysis the DNA-containing multilayer films were grown on precursor films consisting of 10 layers of PSS/PAH.

Materials and Methods

Poly(styrenesulfonate) (sodium salt, M = 100 000) (PSS) was obtained from SERVA; poly(allylamine) (hydrochloride, M = 50 000–65 000) (PAH) was obtained from Aldrich. All polyelectrolytes were used without further purification. The ultrapure water used for all cleaning steps and as a solvent for the adsorption was obtained by reversed osmosis (Milli-RO 34TS, Millipore GmbH) followed by ion-exchange and filtration steps (Milli-Q, Millipore GmbH).

DNA was extracted from sturgeon sperm and purified by dialysis against EDTA for 4 days in 10^{-2} M NaCl (Institute of Biophysics, Puschino, Moscow reg., Russian Academy of Sciences). The molecular weight of DNA is 1.5×10^7; it contains less than 1% of admixed proteins and RNA. The purification and double-helix structure of DNA was checked with the help of UV and IR spectral analysis. A giperchromic effect at 260 nm was 38–39%, melting temperature T = 85 °C in a 0.1 M NaCl phosphate buffer. For deposition a 0.1 mg/mL DNA solution in 0.02 M NaCl, pH 5.5, was used.

Polyelectrolyte films were deposited onto glass slides of the size 12 × 38 mm². These substrates were washed for 30 min in an ultrasonic bath at 60 °C in a solution containing 1% KOH in a mixture of water/ethanol (3:7). After cleaning the substrates were carefully washed in Milli-Q water and immersed in boladication solution for 30 min.[2,3] After this procedure the substrate is positively charged and used for precursor film deposition beginning with the polyanion PSS.

All polyelectrolytes were adsorbed from aqueous solutions containing 0.02 monomol/L of polymer (monomol refers to the molar concentration of monomer residues) and 0.01 mL of HCl. The adsorption was carried out as follows. The substrates were immersed in 10 mL of a polyelectrolyte solution for 20 min at room temperature. After deposition of each polyelectrolyte layer the substrates were rinsed three times in pure water. The PSS/PAH layers were deposited from solutions containing 0.02 monomol/L of PSS, 0.01 mol of HCl, and additionally 0.5 M $MnCl_2$ for the polyanion layers and 0.02 monomol/L of PAH, 0.01 mol of HCl, and additionally 2.0 mol/L of NaBr for the polycation layers. Due to this salt content a film thickness of 270 ± 5 Å was reached after deposition of only 10 alternating layers. DNA adsorption was carried out in the same way, but the adsorption time was 1 h.

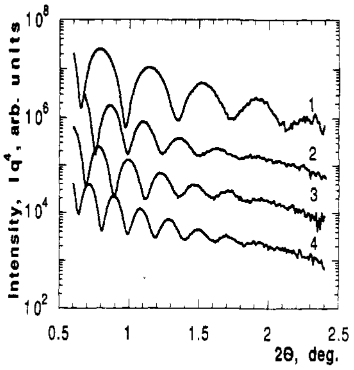

Figure 2. Corrected X-ray reflectivity versus scattering angles 2θ for successive steps of deposition: (1) Precursor film consisting of 10 layers of PSS/PAH; (2) 4 layers of DNA/PAH on the precursor film; (3) 6 layers of DNA/PAH; (4) 8 layers of DNA/PAH on the precursor film.

The multilayer buildup was monitored by small-angle X-ray reflectivity (SAXR). Measurements were performed with a Siemens D-500 powder diffractometer equipped with a graphite monochromator on the detector side using Cu Kα radiation with a wavelength of 1.54 Å. Data were acquired via a DACO-MP interface connected to a personal computer using a step-width of 0.01° (in 2θ) and counting intervals of 5 s. The dependence of the SAXR spectra on layer numbers was recorded from a single multilayer specimen on a glass substrate that was dried in a stream of nitrogen in between the deposition cycles. We monitored the film thickness during film growth by evaluation of Kiessig fringes according to refs 8 and 11.

Results

PSS/PAH multilayers were grown on glass substrates as schematically depicted in Figure 1. The preparation of a precursor film proved necessary in order to detect the adsorption of a single DNA layer. When DNA/PAH layers are adsorbed onto a precursor film with a thickness of approximately 270 Å, many strong Kiessig fringes are observed and adsorption of the additional single layer is detected easily. From our experience we consider it a valid assumption that the electron densities of the precursor film of PSS/PAH and the DNA film are close enough to be treated as being identical for the evaluation of the observed fringes. This allows us to determine the thickness of the DNA/PAH layers as the difference of the total film thickness and the thickness of the precursor film.

We deposited 10 PSS/PAH layers (terminal PAH layer is positive) on a glass, recorded the X-ray reflectivity curve, and calculated the film thickness (270 ± 5 Å); the film surface roughness is σ = 6 Å. At this step of heterostructural growth the film gave strong and well-defined Kiessig fringes (Figure 2, curve 1).

Then we changed the polyanion (replaced PSS with DNA) and further performed alternate adsorption with the DNA/PAH pair. The process of a film growth proceeded, but the step of growth became smaller. Figure 2 demonstrates the X-ray reflectivity curves for the successive stages of film growth. One can see that the periodicity of Kiessig fringes decreased, reflecting the increasing film thickness. Figure 3 shows the dependence of calculated film thicknesses on the number of adsorbed layers. At the first stage of preparation (the precursor film formation) we have linear growth with a step d = 60 Å, and at the second stage (DNA/PAH deposition) it is linear too, but with a smaller step: d = 40 ± 1 Å. Usually we controlled the film thickness at every even number of

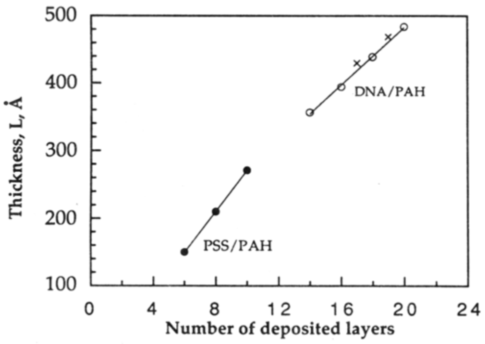

Figure 3. Dependence of the film thickness (L) on the number of deposited molecular layers: $(PSS/PAH)_{1-10} + (DNA/PAH)_{11-20}$. Crosses mark the odd steps of deposition.

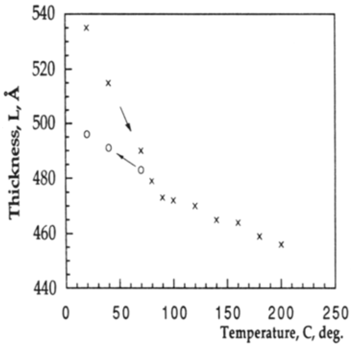

Figure 4. Dependence of the $\{(PSS/PAH)_{1-10} + (DNA/PAH)_{11-20}\}$ film thickness (L) on temperature. Arrows show the directions of heating and cooling.

layers, but in two cases (for 17 and 19 "steps") we measured the thickness after deposition of the DNA layer alone. Comparison of steps of growth for the DNA/PAH pair and only for DNA gives us the thickness of the adsorbed DNA layer (33 ± 2 Å) and the PAH layer (7 ± 2 Å). Due to the DNA helix and in contrast to our experience with PSS/PAH and PVS (poly(vinyl sulfate))/PAH self-assembled films (were the steps of growth for even and odd layers were the same[8]) here the step of growth for DNA is much bigger than the one for PAH. We attribute this observation to the structural rigidity of the DNA macromolecules. The cross section of the DNA double helix is approximately 18 Å, so the thickness of the DNA layer in the film corresponds either to two layers of DNA or to one layer of supercoiled DNA.[12]

Temperature Behavior of DNA Containing Multilayers. Figure 4 shows the dependence of the film thickness on temperature. One can see in the process of film heating the breakpoint at a temperature of 85 ± 5 °C, which may be interpreted as a phase transition. As follows from the cooling branch of the dependence this phase transition is irreversible. The phenomena may be due to the unpairing of double-stranded DNA to single-stranded DNA which takes place in solution at 82 °C.[12] This effect is not observed in polyelectrolyte multilayers in the absence of DNA.

Deposition of DNA Stained by Ethidium Bromide. In addition we have studied the buildup of films using DNA stained with ethidium bromide.[13] The concentration of ethidium bromide was 1 mg/mL (i.e., 1 mg of dye/0.2 mg DNA). This dependence of the thickness of the film on the number of deposition cycles differs from the one in Figure 3. First of all, the process of growth is not linear: at the 8 and 10 stages the step of growth is about 40 Å; then at the 12–14 stages it is about 25 Å, and at the 15–16 stages the growth process was stopped and no further film thickness increase was achieved. At present we do not completely understand these data, but they may be due to the uncoiling of DNA supercoils, which takes place at high ethidium bromide concentration. The investigation of this film (with a total thickness of 400 Å) by fluorescence microscopy shows that the films grow very homogeneously (Figure 5). The scratch was made on purpose in order to enhance and visualize the film uniformity.

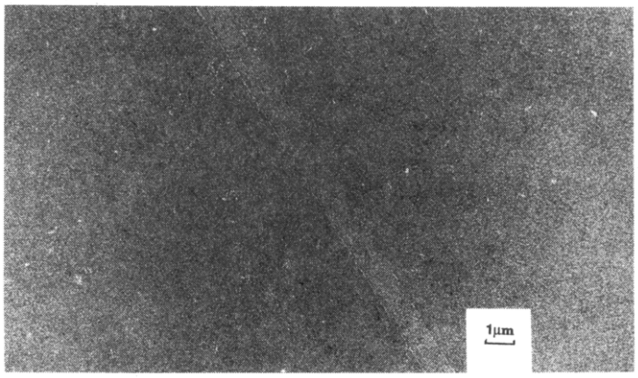

Figure 5. Fluorescence micrograph of the $DNA_{ethid.}$/PAH multilayer. Magnification ×750. The scratch was made on purpose in order to help visualize the homogeneity of the film.

Summary and Discussion

Here we have reported on a self-assembly procedure for the fabrication of thin films with alternation of DNA and poly(allylamine) molecular layers. The total thickness of the multilayer film assembly increases linearly with the number of deposited layers; the step of growth for a pair of DNA/PAH is 40 Å. This result is interesting for thin organic film technology. But the outcome of our study may also be related to the process of complex formation between DNA and polyamines in nature. Polyamines are present in the phage heads, chromosomes, ribosomes, RNA polymerase–DNA complexes, etc.[9]

Data on the intracellular location and their abundance in embryonic and proliferating tissues suggest that the polyamines may participate in the regulation of template-dependent synthesis. Spermine and spermidine promote transcription and translation *in vivo* and *in vitro*. The cationic nature of these compounds suggests that they may directly interact with nucleic acids. In analysis of this interaction a correspondence of a distance between the positive charges of the polyamines and the negative charges of the DNA phosphates was emphasized: in spermine, spermidine, and cadaverine NH_2 groups are separated by the different numbers (3–5) of methylene groups.[9]

In poly(allylamine) used in this work the amines are separated by two methylene groups, but they are attached as side groups which gives them some spatial freedom. At the present stage of the work we do not suggest a specific interaction between the positive charges in PAH and the negative charges in the DNA double helix. But the idea to copy the natural principle of DNA packing (for example, as in the spermine–DNA complex) for the future development of the DNA/polycation self-assembly procedure is currently being evaluated in our laboratory.

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References and Notes

(1) Ulman, A. *An Introduction to Ultrathin Films, from Langmuir–Blodgett to Self-assembly*; Academic Press: Boston, 1991; p 440.

(2) Decher, G.; Hong, J. D. European patent 0472 990 A2, 1992.

(3) Decher, G.; Hong, J.-D. Buildup of Ultrathin Multilayer Films by a Self-Assembly Process: 2. Consecutive Adsorption of Anionic and Cationic Bipolar Amphiphiles and Polyelectrolytes on Charged Surfaces. *Ber. Bunsenges. Phys. Chem.* **1991**, *95*, 1430–1434.

(4) Roberts, G. G. *Langmuir–Blodgett Films*; Plenum Press: New York, 1990; p 420.

(5) Berndt, P.; Kurihara, K.; Kunitake, T. Adsorption of poly(styrenesulfonate) onto an ammonium monolayer on mica: a surface forces study. *Langmuir* **1992**, *8*, 2486–2490.

(6) Decher, G.; Hong, J.; Schmitt, J. Buildup of ultrathin multilayer films by a self-assembly process: 3. Consecutively alternating absorption of anionic and cationic polyelectrolytes on charged surfaces. *Thin Solid Films* **1992**, *210/211*, 831–835.

(7) Decher, G.; Schmitt, J. Fine-tuning of the film thickness of ultrathin multilayer films composed of consecutively alternating layers of anionic and cationic polyelectrolytes. *Prog. Colloid Polym. Sci.* **1992**, *89*, 160–164.

(8) Lvov, Y.; Decher, G.; Möhwald, H. Growth and Structural Characterization of Layer-by-Layer Adsorbed Films of the Polyelectrolytes Polyvinylsulfate and Polyallylamine. *Langmuir* **1993**, *9*, 481–486.

(9) Minyat, E.; Ivanov, V.; Kritzyn, A.; Monchenkova, L.; Schyolkina, A. Spermine and spermidine-induced B to A transition of DNA in solution. *J. Mol. Biol.* **1978**, *128*, 397–409.

(10) Erokhin, V.; Popov, B.; Samori, B.; Yakovlev, A. Immobilization of DNA fragments by Langmuir–Blodgett technique. *Mol. Cryst. Liq. Cryst.* **1992**, *215*, 213–220.

(11) Rieutord, F.; Benattar, J.; Bosio, L.; Robin, P.; Blot, C.; Kouchkovsky, R. X-ray optics in Langmuir–Blodgett films. *J. Phys.* **1987**, *48*, 679–687.

(12) Lodish, H.; Darnell, J.; Baltimore, D. *Molecular Cell Biology*; Scientific American Books: 1990; p 682.

(13) Bauer, W.; Vinograd, J. Interaction of closed circular DNA with intercalative dyes. *J. Mol. Biol.* **1970**, *47*, 419–432.