Layer-by-layer deposition of avidin and polymers on a solid surface to prepare thin films: significant effects of molecular geometry of the polymers on the deposition behaviour

Jun-ichi Anzai* and Masahiro Nishimura

Faculty of Pharmaceutical Sciences, Tohoku University, Aramaki, Aoba-ku, Sendai 980-77, Japan

An alternate and repeated deposition of avidin and biotin-labelled polymers (polyethyleneimine or polyamidoamine dendrimer) provides the avidin–polymer thin films on a solid surface, in which the loading of avidin in each layer depends significantly upon the molecular geometry of the polymers.

Recently much attention has been devoted to the development of molecular assemblies of thin film based on successive deposition of alternate layers of anionic and cationic polyelectrolytes including synthetic polymers, proteins and nucleic acids, as an alternative procedure to the Langmuir–Blodgett technique.[1] The layer-by-layer deposition technique relies on the electrostatic force of attraction as an origin of the strong adhesion between the anionic and cationic layers. Therefore, the combination of polymers and biomaterials which have the same sign of electric charges and electrically neutral polymers cannot be used in this procedure.

However, it may be possible to construct the layer-by-layer structure by means of polymers and biomaterials which have biological interactions such as protein–ligand, antibody–antigen and lectin–saccharide bindings. This would extend the scope of the layer-by-layer deposition technique in constructing the thin film assemblies composed of proteins and other biomolecules, because non-ionic polymers and even polymeric materials of the same polarity can be built into the same assemblies simultaneously through the biological interactions. In fact, a few reports have appeared on the construction of multilayer films based on such biological interactions.[2] Based on this strategy, we have prepared multilayer thin films composed of avidin (Av) and either biotin-labelled polyethyleneimine (b-PEI) or poly(amidoamine) dendrimer (b-PAMAM). Av is a glycoprotein found in egg white and is known to contain four identical binding sites to biotin (binding constant, K_a = *ca.* 10^{15} M^{-1}).[3] This communication reports the preparation of Av–b-PEI and Av–b-PAMAM multilayer films which were assembled through the strong affinity between Av and biotin. It is emphasized that the loading of Av in the films depends significantly on the molecular geometry of PEI and PAMAM.

PEI (Tokyo Kasei Co., average molecular weight 45 000) and PAMAM (Aldrich Chem. Co., Generation 4, molecular weight 14 215) were labelled with biotin residues by the reaction with an excess of sodium sulfosuccinimidyl-6-(biotinamido)-hexanoate, by which about 10 biotin residues per molecule were introduced randomly to the polymers. PEI is a product of a random polymerization of aziridine and has a random branched structure (the ratio of primary, secondary and tertiary amino groups is nominally about 1:2:1). The PAMAM used assumes approximately a globular conformation (diameter 3–4 nm) composed of an ethylenediamine core and an ideally branched structure of $-CH_2CH_2CONHCH_2CH_2N$ units and contains 64 primary amino groups on the surface.[4] In order to study the formation of multilayer thin films by spectrophotometry, fluorescein-modified Av (F-Av) (Molecular Probe, Inc., nominally 5 fluorescein residues per protein) and either b-PEI or b-PAMAM were deposited alternately on a quartz slide to form the layer-by-layer structure. The quartz slide (5 × 1 × 0.1 cm) was first treated in a 10% dichlorodimethylsilane solution in toluene overnight to make the surface hydrophobic. The silylated quartz slide was immersed in an F-Av solution (10–100 μg ml^{-1}, phosphate buffer, pH 7) for 60 min at room temperature to deposit the first layer of F-Av. After being rinsed with the buffer for 10 min, the quartz slide was immersed in a b-PEI or b-PAMAM solution (10–100 μg ml^{-1}, phosphate buffer, pH 7) for 60 min and rinsed. This treatment would provide a double layer of F-Av and b-PEI or b-PAMAM on both surfaces of the quartz slide. The deposition was repeated 10 times and the absorbance of the quartz slide at *ca.* 500 nm, originating from the fluorescein moiety, was measured after each deposition.

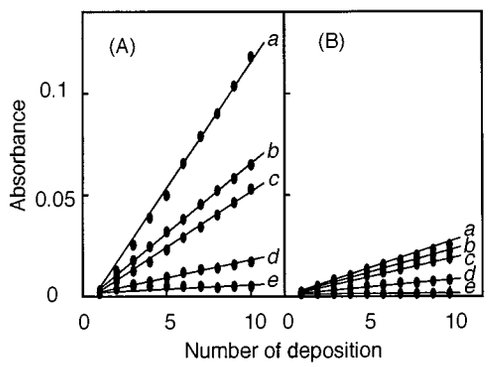

Fig. 1 Absorbance of the multilayer-modified quartz slide at *ca.* 500 nm as a function of the number of deposition; (A) F-Av–b-PEI multilayer and (B) F-Av–b-PAMAM multilayer. Concentration of F-Av and the polymer; (*a*) 100, (*b*) 50, (*c*) 25 and (*d*) 10 μg ml^{-1}. The plots in (*e*) show those using unmodified PEI and PAMAM (100 μg ml^{-1}).

Fig. 1(A) shows an increase in absorbance of the F-Av–b-PEI-modified quartz slide as a function of the number of deposition of the layers. The first layer of F-Av, which is adsorbed directly on the surface of the quartz slide, is known to form a monomolecular layer.[5] After the second deposition, the absorbance increased in proportion to the number of deposition, confirming the formation of a multilayer structure on the quartz slide by the step-by-step deposition. In contrast, the absorbance did not increase when unmodified PEI containing no biotin residue was used in place of b-PEI. This is reasonable because both Av and PEI should be positively charged in the phosphate buffer (the isoelectric point of Av is reported[3] to be in pH 9.0–10) and repel one another electrostatically. These observations demonstrate that the strong affinity between Av and biotin should be responsible for the formation of F-Av–b-PEI multilayers, which cannot be assembled due to the electrostatic force of repulsion unless PEI is modified with biotin. The

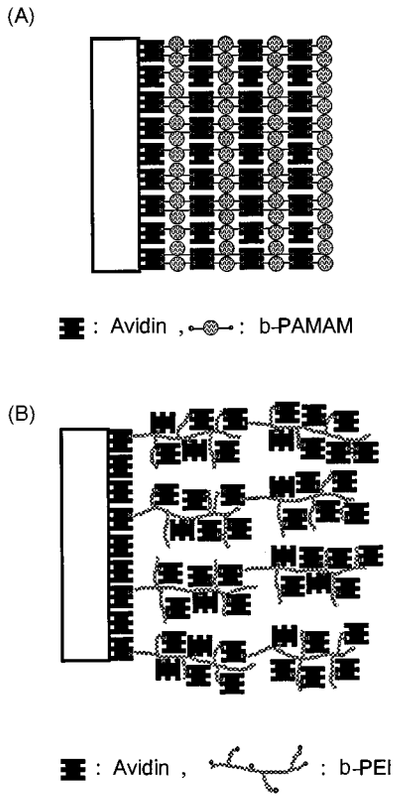

Fig. 2 Possible structure of the Av–polymer multilayer films; (A) F-Av–b-PAMAM multilayer and (B) F-Av–b-PEI multilayer

slope of the plots in Fig. 1(A) depended significantly upon the concentration of F-Av and b-PEI in the bathing solutions (10–100 μg ml^{-1}) from which F-Av and b-PEI were deposited, suggesting that the loading of F-Av in each layer of the thin films is a function of the F-Av and b-PEI concentration in the solutions. Using a molar extinction coefficient of 176 000 M cm^{-1} for F-Av at *ca.* 500 nm and assuming that, upon each deposition, F-Av forms a monomolecular layer in close packing on the surface of the quartz slide, the slope of the plot is calculated to be 0.0022 ± 0.0004 depending upon the orientation of F-Av molecule on the surface (the molecular dimensions of Av are reported to be 6.0 × 5.5 × 4.0 nm).[3] Judging from the data in Fig. 1(A), approximately five layers of F-Av are adsorbed on the slide upon each deposition when the film is prepared using the 100 μg ml^{-1} solutions, while the 10 μg ml^{-1} solution provides nearly a monomolecular deposition of F-Av.

Fig. 1(B) shows the absorbance of F-Av–b-PAMAM multilayer films, prepared by using 10, 25, 50 and 100 μg ml^{-1} solutions, as a function of the number of deposition. The absorbance increased linearly with increasing deposition number in all cases, confirming the formation of a layer-by-layer structure through the Av–biotin binding. Unmodified PAMAM did not form the multilayer film. These results are basically in line with the deposition behaviour of the PEI-based thin films. However, the loading of F-Av did not depend significantly upon the concentration of the bathing solutions. For the F-Av–b-PAMAM films prepared from the 25–100 μg ml^{-1} solutions, the slopes of the plot are in the range of (1.8–2.6) × 10^{-3}. This suggests that approximately a monomolecular layer of F-Av is adsorbed upon each deposition, though the loading depends very slightly upon the Av concentration in the solutions. If the multilayer films are composed of ideal monolayers of F-Av and PAMAM, the thickness of the unit layer (one Av plus one PAMAM layer) should be 7–10 nm. Thus the deposition behaviour of F-Av–b-PAMAM multilayers is distinct from that of the PEI-based multilayers.

The different behaviour in the deposition of the F-Av–b-PEI and F-Av–b-PAMAM multilayers can be explained based on

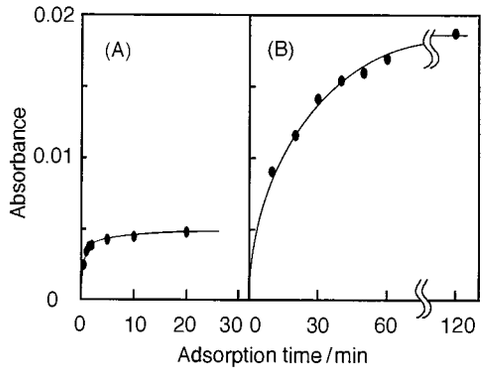

Fig. 3 Time course of the adsorption of F-Av on the surfaces of (A) b-PAMAM and (B) b-PEI layers

the molecular geometry of PEI and PAMAM. It can be envisaged that the globular molecules, Av and PAMAM, are deposited to form a monomolecular layer upon each deposition because the driving force of adsorption is the Av–biotin complexation and non-specific adsorption is prohibited by electrostatic repulsion. An idealized monomolecular deposition of the F-Av–b-PAMAM films is schematically shown in Fig. 2(A). In this type of adsorption, the loading of adsorbate usually exhibits a saturation in the high concentration region. This agrees with the data in Fig. 1(B) that the loading of F-Av depends very slightly upon the concentration in the range of 25–100 μg ml^{-1}. In contrast to the monolayer adsorption in the F-Av–b-PAMAM films, multilayers of F-Av were adsorbed in the F-Av–b-PEI films upon each deposition. The apparent multilayer formation does not mean a successive and non-specific adsorption of F-Av to the surface of the F-Av layer formed already. We have ascertained independently that F-Av cannot be adsorbed onto the surface of F-Av monolayer-modified quartz slide. This discrepancy can be solved by taking account of the molecular geometry of PEI, *i.e.* not a globular but a random branched conformation. The adsorbed PEI probably assumes a shaggy surface due to the branched backbone. The surface roughness may be introduced also by the polydispersed nature of the molecular weight of PEI. For these reasons, several binding sites in PEI (biotin residues) are able to attach themselves to a polymer chain which protrudes vertically from the surface. In this situation, F-Av molecules should be accommodated in the PEI surface to form multilayers and the loading of F-Av depends upon the concentration of F-Av in the bathing solution. A possible structure of the F-Av–b-PEI film is illustrated schematically in Fig. 2(B).

We have also found that the adsorption of F-Av is faster in the F-Av–b-PAMAM films than in the F-Av–b-PEI films (Fig. 3). The completion of adsorption of F-Av onto the surface of the b-PAMAM layer in all solutions tested takes about 10 min, compared with about 60 min in the b-PEI case. This originates probably from the different morphology in the PAMAM and PEI surfaces. It is plausible that the shaggy surface of PEI requires much time to accommodate F-Av due to hindered accessibility of F-Av molecules toward the binding sites in PEI. A higher content of positive charge in PEI may also be responsible for the suppressed rate of adsorption of F-Av due to electrostatic repulsion, because both PEI and F-Av should be positively charged under the experimental conditions. It is clear that PEI contains many more ionizable amino groups (or primary, secondary and tertiary amines) than PAMAM does. We have ascertained that the adsorption of F-Av and the polymers is virtually irreversible due to the strong complexation between Av and biotin; no desorption was observed upon rinsing with buffer.

In summary, we have demonstrated that the multilayer structure of thin films composed of Av and biotin-labelled polymers depends significantly upon the geometry of the polymers;

globular polymer (PAMAM) provides a monolayer deposition of Av, while the Av multilayer is formed by the deposition with randomly branched polymer (PEI). These results would be useful for regulating the loading of biomaterials in the thin films based on the layer-by-layer deposition technique.

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