Alternate Layer-by-Layer Assembly of Organic Dyes and Proteins is Facilitated by Pre-mixing with Linear Polyions

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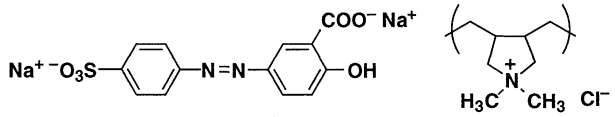
In the preparation of ultrathin films by alternate assembly, prior mixing with linear polyions facilitates stable adsorption of small-molecule dyes and direct assembly of globular proteins and micro-plates.

Alternate layer-by-layer assembly that is based on neutralization and resaturation of surface charge (Figure 1A) is a powerful technique to obtain regularly-layered structures of water-soluble macroions[1-4] such as linear polyelectrolyte, protein, and clay. Versatile molecular architectures created by this method leads to unique functions such as sequential reaction with multi-enzyme films,[5] and charge separation[6] and electroluminescence[7] with films of polymeric dyes. However, we sometimes encounter difficulties in the assembling. Major difficulties exist in immobilization of small molecules and direct assembly of non-flexible components such as proteins and micro-plates. We have found that these difficulties can be eliminated by prior mixing of dye molecules and proteins with linear polyions.



MY10 (MW 366) **PDDA**

Dye molecules such as Congo Red can be assembled with oppositely-charged polyions, whereas assembly of some other dyes is difficult because of higher water-solubility.[8,9] Alternate assembly of one of the latter dyes, 5-(4-sulfophenylazo) salicylic acid disodium salt (MY10), was conducted with

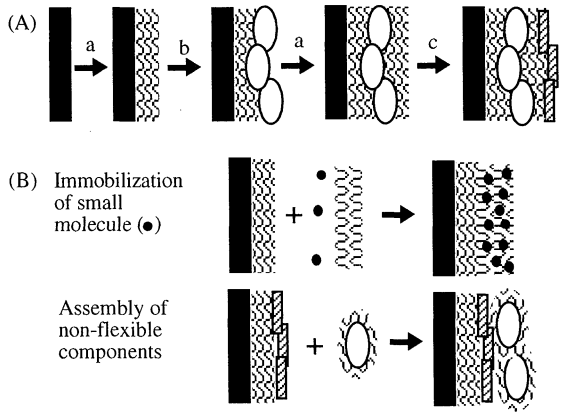


Figure 1. Schematic illustration of alternate assembly
(A) Conventional alternate assembly of a, linear polyion (+); b, protein (–); c, clay (–).
(B) Pre-mixing alternate assembly of small molecules and proteins with polyions.

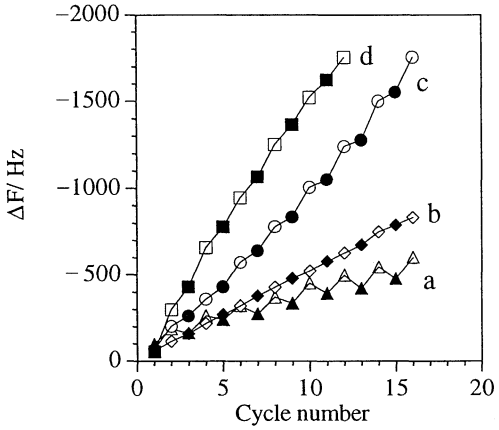


Figure 2. Frequency decrease of QCM upon film assembly (open plot, anion adsorption; filled plot, cation adsorption): a, 1 mM MY10 + 1.9 mM PDDA; b, 15 mM PSS + 19 mM PDDA; c, (1 mM MY10 and 15 mM PSS) + 19 mM PDDA; d, (10 mM MY10 and 15 mM PSS) + 19 mM PDDA. These films were assembled on 4 layers of the PEI-PSS precursor film. Immersion time is 20 min.

poly(diallyldimethylammonium chloride) (PDDA) on quartz crystal microbalance (QCM, AT-cut, 9 MHz, Ag-electrode) by alternately dipping the QCM plate in aqueous solutions of dye and polyion, as described previously.[3,10] The dye adsorption was observed as indicated by mass increase corresponding to frequency change of −105 Hz (open triangle in Figure 2a); however, mass decrease (+48 Hz) was found for the subsequent adsorption step of PDDA. Since the PDDA solution turned yellow slightly during the latter step, MY10 molecules that have been adsorbed on the QCM plate must become dissolved probably by interaction with PDDA. This situation is remedied by pre-mixing of MY10 and linear polyanion, poly(sodium styrenesulfonate) (PSS). The QCM frequency changes observed in this case (Figure 2c and d, for 1 and 10 mM of the dye, respectively) are negative for both steps, and are larger than that of the control, PSS-PDDA assembly (Figure 2b). Dye desorption is now suppressed, and the dye is stably incorporated in the alternate polyion film. Dye incorporation was also confirmed by an increase of UV absorbance at 350 nm for the films prepared on quartz plates under the same conditions as that for Figure 2d. The dye content in the adsorbed layer (0.35 mmol/mg) that is calculated from UV absorbance is smaller than the relative content of dye and polyion in solution (1.47 mmol/mg). It indicates that PSS is adsorbed on PDDA surface better than monomeric MY10. The regular increase in the UV absorbance with number of layers (1—10 layers) confirms constant incorporation of the dye in every layer (Figure 1B).

Direct assembly of oppositely-charged proteins and micro-plates has been difficult, probably because electrostatic

attraction cannot be maximized among globular and plate-shaped species.[3] Glucose oxidase (GOD, pI 4.2) and montmorillonite (Mont), both being negative and non-flexible, can be assembled only with a polyion intermediate layer which acts as an electrostatic glue. It is reported that mixtures of oppositely-charged proteins and polyions give charged, water-soluble complexes.[11] These complexes, in appropriate molar ratios, possess inverted flexible surface charges. Thus, we mixed GOD (0.5 mg/mL) with cationic poly(ethyleneimine) (PEI, 1.0 mg/mL) and used the mixture for assembly with Mont (0.3 mg/mL) at pH 6.8 (Figure 1B). Regular film growth was observed with ΔF of –130 Hz for GOD-PEI step and –230 Hz for Mont step. The former value is smaller in magnitude than ΔF for pure GOD layer but larger than that for pure PEI layer. The same GOD-PEI mixture can also be assembled alternately with anionic PSS (1.0 mg/mL).[12] Interestingly, the activity of GOD in a film where GOD was pre-mixed with PEI and assembled with PSS was 67 times higher than that of GOD directly assembled with PEI.[5] The activity increase may be ascribed to de-aggregation and/or favorable microenvironments for the enzyme.

It is established that pre-mixing of adsorbing species with polyions improves alternate layer-by-layer assembly in several aspects. The improvement extends the technical advantage of alternate assembly considerably. In particular, precious proteins and small functional molecules are assembled more readily.

References and Notes

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1 G. Decher and J.-D. Hong, *Ber. Bunsenges. Phys. Chem.*, **95**, 1430 (1991).

2 M. Sano, Y. Lvov, and T. Kunitake, *Annu. Rev. Mater. Sci.*, **26**, 153 (1996).

3 Y. Lvov, K. Ariga, I. Ichinose, and T. Kunitake, *J. Am. Chem. Soc.*, **117**, 6117 (1995).

4 Y. Lvov, K. Ariga, I. Ichinose, and T. Kunitake, *Langmuir*, **12**, 3038 (1996).

5 M. Onda, Y. Lvov, K. Ariga, and T. Kunitake, *Biotechnol. Bioeng.*, **51**, 163 (1996).

6 S. Keller, S. Johnson, E. Brigham, E. Yonemoto, and T. Mallouk, T, *J. Am. Chem. Soc.*, **117**, 12879 (1995).

7 M. Onoda and K. Yoshino, *Jpn. J. Appl. Phys.*, **34**, L260 (1995).

8 T. Cooper, A. Campbell, and R. Crane, *Langmuir*, **11**, 2713 (1995).

9 K. Ariga, Y. Lvov, and T. Kunitake, *J. Am. Chem. Soc.*, submitted.

10 [MY10] = 1 mM, [PDDA] = 1.9 mM, immersion time of 20 min, and pH 6.8.

11 V. Kabanov, *Polym. Sci.*, **36**, 143 (1994).

12 GOD pre-mixed with negatively-charged PSS can be stably assembled with cationic PEI.