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Preparation of Stable Langmuir-Blodgett Films of Photosynthetic Bacterial Reaction Center from *Rhodopseudomonas viridis* Using Poly-L-lysine

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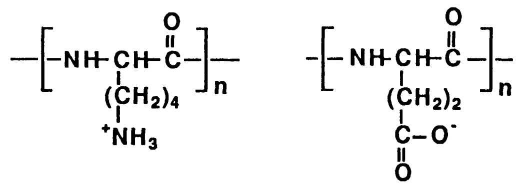
In order to build up stable Langmuir-Blodgett(LB) film of the photosynthetic reaction centers (RC) from *Rhodopseudomonas viridis*, effect of the formation of polyion complex between charged residues of RC and water soluble polyions was examined. Cationic polymer, poly-L-lysine (PLL), enhanced the stability of RC LB films during the film preparing process whereas anionic polymer, poly-L-glutamate, induced complete denaturation of RCs. LB films of RCs prepared with PLL show reproducible surface pressure area isotherm and high transfer ratio of RC monolayer to solid substrate.

Light induced charge separation of the initial reaction of photosynthesis takes place in the transmembrane protein, RC in photosynthetic bacteria.1) Well-organized molecular assembly of chromophores and peptides in the RC is indispensable for effective charge separation.2) RC could be a model of bioelectric devices. Recently, approaches have been done using Langmuir-Blodgett technique to handle RCs for molecular construction.3,4)

Various techniques have been adapted to fabricate protein LB films,5) for example, adsorption into lipid layer,6-8) specific interaction to functional lipid,9,10) and spreading membrane fraction.11-13) To construct these protein LB films, the stability of protein monolayer during film preparation is essential. It is known that structure formation of azobenzene surfactants is attained by the formation of polyion complex.14,15) We applied this technique for preparation of RC-LB films. The stability of RC-LB films was improved by adding cationic polymer to subphase.

Chromatophores, the photosynthetic membrane, were prepared from *Rps. viridis* (ATCC No. 19567) as reported previously.16) RCs were solubilized from the chromatophores in the presence of 10% LDAO (lauryl dimethyl amine N-oxide) in 10 mM Tris-HCl buffer (pH 8.2). RCs were purified through a Sepharose CL-6B column (25ø X 800 mm, Pharmacia, Sweden) with 10 mM Tris-HCl buffer (pH 8.2) containing 0.1% LDAO. RCs were concentrated on ultrafiltration membrane (PM-30, Amicon, USA).

Two kinds of polyions were examined for making polyion complexes : Poly-L-lysine (PLL, Sigma Chemical, USA) as polycation and poly-L-glutamic acid (PLG, Sigma Chemical) as polyanion (Fig. 1). The subphase was pure water from a milli-Q system (Millipore, USA) containing 0.5 mM Tris-HCl (pH 7.0). Ca^{2+} ion or the above polymer of 0.05 mM (1 M = 1 mol monomer unit dm^{-3}) was also added if necessary.



Poly-L-lysine (PLL) $n_{av}=41$ **Poly-L-glutamic acid (PLG)** $n_{av}=48$

Fig. 1. The structures of water soluble polymers used in this report.

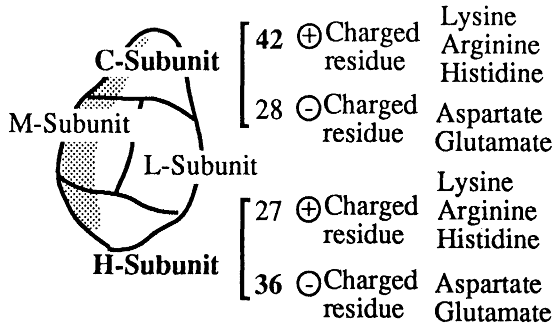


Fig. 2. Schematic diagram of charged residues in reaction center protein of *Rps. viridis*. Only charged residues at pH 8.2 were shown.

The measurement of the surface pressure-area isotherms and automatical deposition of monolayer were carried out with an Langmuir trough (Type LB-3000 with Wilhelmy-type film balance, KSV Instrument, Finland). Solubilized RCs of 60 µl (OD_{830} = 4) were spread on aqueous subphase along tilted glass rod. Monolayer was transferred to hydrophilic glass plate from 2 to 6 layers under a controlled surface pressure at 30 mN m^{-1} by the vertical deposition method. As an index of stability, absorbance spectrum of deposited LB films were measured by using high sensitivity spectrophotometer (JASCO, HSSP-2).

The RC from *Rps. viridis* consists of four different subunits C, H, L and M.[17] M and L-subunits span the membrane and they bind pigment cofactors and an iron atom.[18] C and H-subunits oppositely project from lipid bilayer. C-subunit has relatively positive charge. Contrarily, H-subunit has negative charge (Fig. 2).[19] Anionic and cationic polyions could form polyion complex with charged C and H-subunit, respectively.

The surface pressure-area isotherms of RC monolayers on subphase containing Ca^{2+} ion, PLL and PLG acid are shown in Fig. 3. Due to the presence of LDAO in RC solution, initial surface pressure was high. To reduce the contents of surfactant in the RC monolayer, the compression started after 20 min from the spreading.

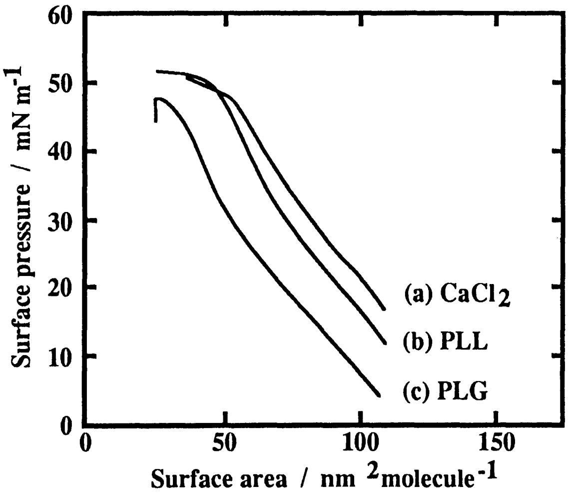


Fig. 3. Surface pressure-area isotherms of RC monolayer on aqueous subphase containing various counter ions with 0.5 mM Tris-HCl buffer (pH 7.0) at 18 °C.

Table 1. Comparison of mean molecular area with different polymers

	Area per molecule (nm^2)	
Counterion	Experimental at 30 mN m^{-1}	Extrapolated to 0 mN m^{-1}
Ca^{2+}	80	122
Poly-L-lysine	69	100
Poly-L-glutamic acid	52	81

The cross sectional area of the steep slope to zero pressure gives mean molecular area of RC. Area per molecule at 30 mN m^{-1} and extrapolated to 0 mN m^{-1} are listed in Table 1. The extrapolated area of RC using polyions are smaller than that using Ca^{2+} ion. Difference of area between the polyions reflects the structure of the polyion complex : PLG might be bound more tightly than PLL. The RC-LB films with these polyions were packed more densely than that with metal ion. The cross sectional area of RC from X-ray crystallography[17,18] is smaller than the experimental data obtained here. This difference could be explained assuming that the M-L subunit region of RC is surrounded by detergent molecules.[5]

In transfer process, RC-LB films with these counter ions were transferred on to the substrate during upward stroke (Z-type structure). In the LB films, H-subunit attached on the substrate surface, because H-subunit faces on subphase at air-water interface.[4] Dependence of transfer ratio on the number of transfer is shown in Fig. 4. At the first deposition, the transfer ratios were identical regardless of counterions (transfer ratio = decrease of monolayer surface area / surface area of immersed substrate). The transfer ratio exceeded 100% at the first deposition because a part of the RC monolayer was transferred to the solid substrate during the first downward stroke. After deposition of 4 times, transfer ratio over 70% was achieved with PLL (Fig. 4(a)). The ratios were lower with PLG and Ca^{2+} ion than PLL with increasing numbers of layers. The absorption spectrum of native RC solution has characteristic peaks at 960 nm and 830 nm derived from bacteriochlorophyll dimer and bacteriochlorophyll monomer, respectively (Fig. 5(a)).[20] Figures 5(b)-(d) show absorption spectra of LB films with 6 layers of RC which was prepared using Ca^{2+} ion or water soluble polyions. From these spectrum, PLG caused complete loss of chromophore peaks. In contrast, the peaks were maintained by using cationic ions (Ca^{2+} ion and PLL). Of the cations, PLL induced less damaging effect than Ca^{2+} as shown in the spectra of Fig. 5.

Results here indicate that the formation of PLL-RC complex gives much improvements for LB film preparation. Transfer ratio was enhanced and the RCs were much stabilized. Complex formation with polymers could give RCs resistance against the stress during the LB film preparation process. However, complex formation might provide negative effect on RCs as they were denatured by PLG binding to C-subunit region.

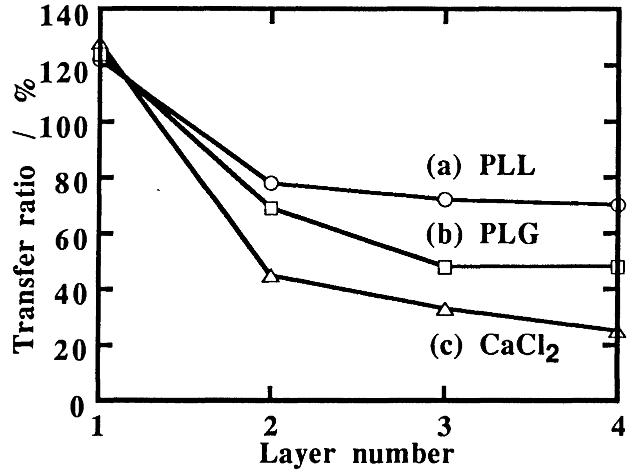


Fig. 4. Dependence of transfer ratio on transfer number of RC monolayer with various counter ions.

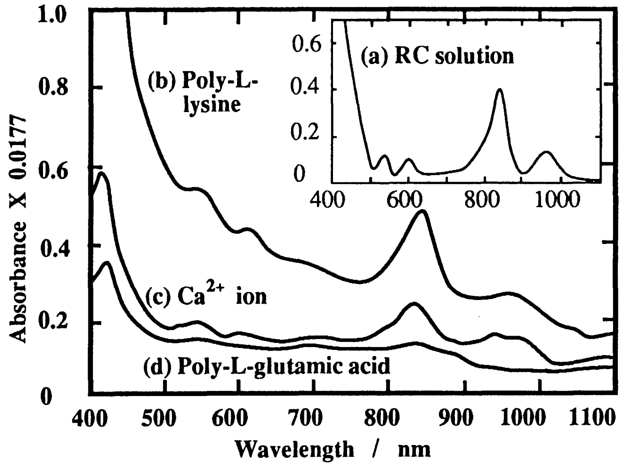


Fig. 5. Absorption spectrum of 6 layers RC-LB films using various counter ions.
Curve a shows absorption spectrum of RC suspension.

In the complex formation between polyions to RCs, appropriate interaction should be required. Further analysis on the interaction of polymers to RCs of the complex formation is now under progress.

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References

1) N. K. Packham, P. Mueller, and P. L. Dutton, *Biochim. Biophys. Acta*, **933**, 70 (1988).
2) C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, and P. L. Dutton, *Nature*, **355**, 796 (1992).
3) G. Alegria and P. L. Dutton, *Biochim. Biophys. Acta*, **239**, 1057 (1991).
4) Y. Yasuda, Y. Hirata, H. Sugino, M. Kumei, M. Hara, J. Miyake, and M. Fujihira, *Thin Solid Films*, **210 / 211**, 733 (1992).
5) V. Erokhin and L. A. Feigin, *Progr. Colloid. Polym. Sci.* , **85**, 47 (1991).
6) D. M. Tiede, *Biochim. Biophys. Acta*, **811**, 357 (1985).
7) M. Deleers, J. Caspers, and J. M. Ruysschaert, *J. Colloid Interface Sci.*, **428**, 61 (1977).
8) D. E. Graham and M. C. Phillips, *J. Colloid Interface Sci.*, **403**, 70 (1979).
9) M. Ahlers, R. Blankenburg, D. W. Grainger, P. Meller, H. Ringsdorf, and C. Salesse, *Thin Solid Films*, **180**, 93 (1989).
10) H. Haas and Möhwald, *Thin Solid Films*, **180**, 101 (1989).
11) T. Furuno, H. Sasabe, and K. M. Ulmer, *Thin Solid Films*, **180**, 23 (1989).
12) T. Miyasaka and K. Koyama, *Chem. Lett.*, **1991**, 1654.
13) D. M. Tiede, P. Mueller, and P. L. Dutton, *Biochim. Biophys. Acta*, **681**, 191 (1982).
14) K. Nishiyama, M. Kurihara, and M. Fujihira, *Thin Solid Films*, **179**, 477 (1989).
15) M. Shimomura and T. Kunitake, *Thin Solid Films*, **132**, 243 (1985).
16) M. Hara, T. Majima, J. Miyake, S. Ajiki, H. Sugino, H. Toyotama, and S. Kawamura, *Appl. Microbiol. Biotechnol.*, **554**, 32 (1990).
17) J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *J. Mol. Biol.*, **180**, 385 (1984).
18) J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, *Nature*, **618**, 318 (1985).
19) H. Michel, *J. Mol. Biol.*, **158**, 567 (1982).
20) A. Vermeglio and G. Paillitin, *Biochim. Biophys. Acta*, **681**, 32 (1982).

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