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Chiral Polypeptide Monolayers from Self-Condensation of Amphiphilic Amino Acid Ester. Effect of Chirality on the Membrane Structure

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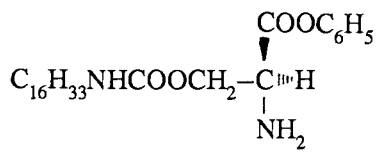
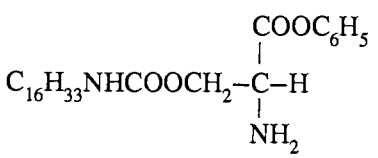
A Chiral polypeptide monolayer was prepared by self-condensation of an amphiphilic (*S*)-serine phenyl ester on an aqueous subphase. The monolayer film gave a two-dimensional structure markedly different from that of racemic analogues, reflecting the effect of molecular recognition of chirality in the lateral self-condensation process.

Stereochemical regulation in ordered molecular assemblies can provide rare functionalities to the close interactions of oriented species. Molecular recognition in chiral monolayers, for instance, has been studied by Harvey et al.[1] where difference of aggregation behavior between enantiomers and racemates were visualized by scanning tunneling microscopy. Ultimate effect caused by the "chiral recognition" was however not clearly shown experimentally with the set of inert monolayers they employed. In this communication the authors like to report an evidence of how the chirality of molecules affects the structure and morphology of fluid monolayers, by taking a look at a reaction-proceeding dynamic medium, i.e., polypeptide formation through a lateral self-condensation reaction.

Amphiphilic phenyl ester of (*S*)-serine **1** was prepared by reacting phenyl ester of optically active *N*-*t*-butoxycarbonyl (*S*)-serine with stearylamine using phosgene as a condensing agent to introduce a urethane linkage. As a reference to this, a racemic isomer **2** was prepared from (*RS*)-serine which is analogous to the racemic phenyl ester used in the previous study.[2] Both amphiphiles formed stable monolayers and their Π-A isotherms at 30 °C are compared in Fig.1. In an expanded temperature range of 10 to 50 °C, film area of the enantiomer **1** was always found to be smaller than that of the racemate **2**. The result immediately shows that a closer packing of molecules are taking place in the enantiometric monolayer in comparison with the racemic monolayer. Such a packing effect can be attributed to a higher regularity in the lateral molecular orientation being established in the enantiometric monolayer than in the racemic monolayer.



1 *S*-



2 *RS*-

The chiral monolayer of **1** was then subjected to polycondensation on a neutral aqueous subphase at room temperature under a controlled surface pressure of 15 mN/m. Rapid and spontaneous condensation reaction took place in the monolayer film, which was confirmed by the IR spectroscopy for deposited films as descried previously.[2] Quantitative estimation of the polymerization rate based on IR data was however not possible due to the existence of a urethane linkage which optically hindered the peptide bond being developed. Reaction rate was thus assessed by monitoring the decrease in film area during the course of the reaction, which is to correlate to the degree of condensation.[2] After a reaction for 2 h, about 50% shrinking in film area occurred, which is similar to the results obtained with phenyl ester analogues of amphiphilic amino acid[2] and indicates a relatively high rate constant (ca. 7×10^{-4} s^{-1}) achieved in the present polycondensation reaction.

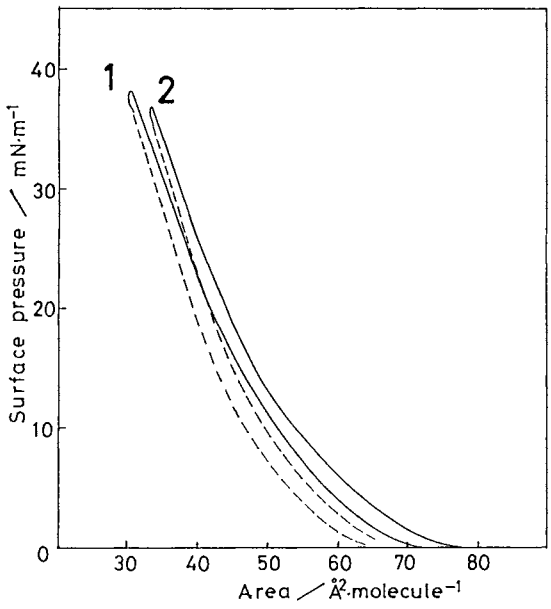


Fig. 1. Surface pressure-area isotherms of **1** and **2** on a neutral aqueous subphase at 30 °C. Dotted lines are results of return way after commpression.

Structures of the polypeptide films thus prepared were investigated by scanning electron microscopy (SEM) using a Langmuir-Blodgett (LB) monolayer deposited on a silicon wafer tip. Figure 2 shows typical morphologies observed for the polypeptide monolayers of **1** and **2**, respectively, after self-condensation at 35 °C for 30 min. Remarkable difference in the membrane uniformity took place between the two monolayers. It is now evident that the lateral homogeneity in the polymerized film was significantly improved by replacing the racemic system by the chiral system. In the racemic monolayer, large independent islands, likely rich in peptide chains, occupy the total film area in a mosaic form with gaps interposed between islands, which are probably of non-reacted monomers. Such a phase separation was not the case for the chiral polypeptide film composed of **1**, in which uniformity is largely improved if there remains somewhat fine structures in the SEM image. In view of the fact that a similar phase separation has alway been observed for polycondensations with optically non-active amino acid esters,[2] it is appropriate to assume that the island formation in the racemic system is a result of the molecular recognition between same enantiomers, i.e., chiral recognition,[1] leading to the formation of an enantio-selected homopolymer. An attempt to examine the degree of the optical purity with respect to the island domains was not successful as the product was hardly solubilized by most organic solvents.[3]

In order to investigate the degree of molecular packing, an electrochemical permeability measurement was carried out for polypeptide LB films built up on a glassy carbon disk electrode. Figure 3 depicts cyclic voltammograms measured for the redox reaction of aqueous ferricyanide on the electrode which had been coated with varied numbers of the **1** monolayer before and after the polymerization. Comparison of the redox currents with an equivalent number of monolayers obviously shows that the suppression of

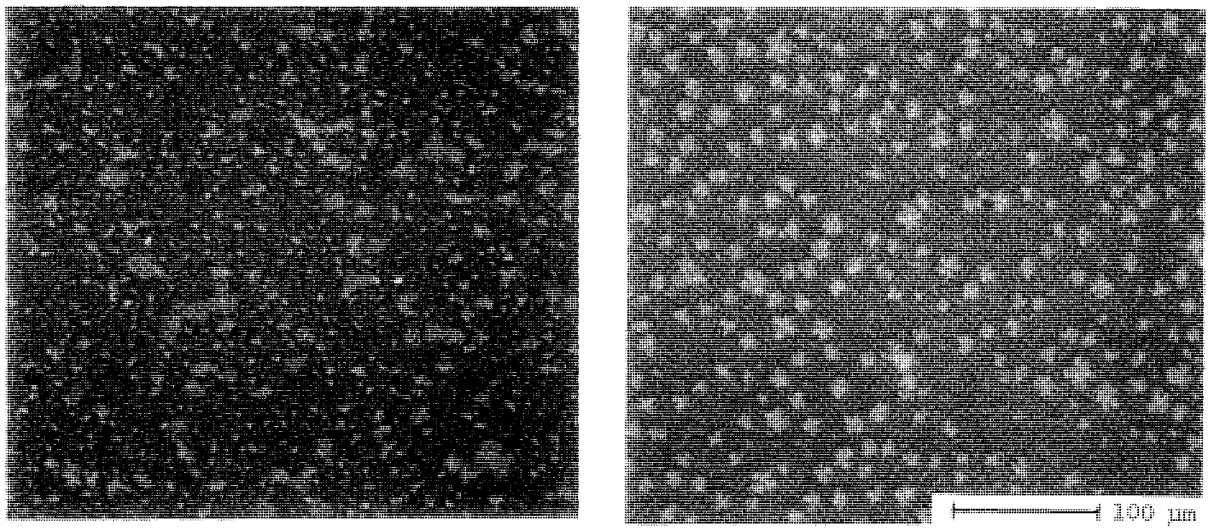


Fig. 2. Membrane morphologies observed by SEM for polymerized monolayers of **1** (left) and **2** (right) after spontaneous polymerization on a pH 7.4 aqueous subphase (35 °C) for 30 min. Surface pressure of monolayers was controlled at 15 mN/m.

the permeability is much more effective with the polymerized LB films than with the monomeric films. Difference in the permeability was also examined between the chiral (**1**) and racemic (**2**) monolayers. This difference, however, was found to be very small falling within the range of an experimental deviation, indicating that the suppression effect depends in large part on the existence of the polypeptide structure itself. In Fig. 3, a reference data (**C**) is added in which a polymeric LB film prepared by radical polymerization of *N*-octadecylacrylamide was examined for comparison. Apparently current suppression

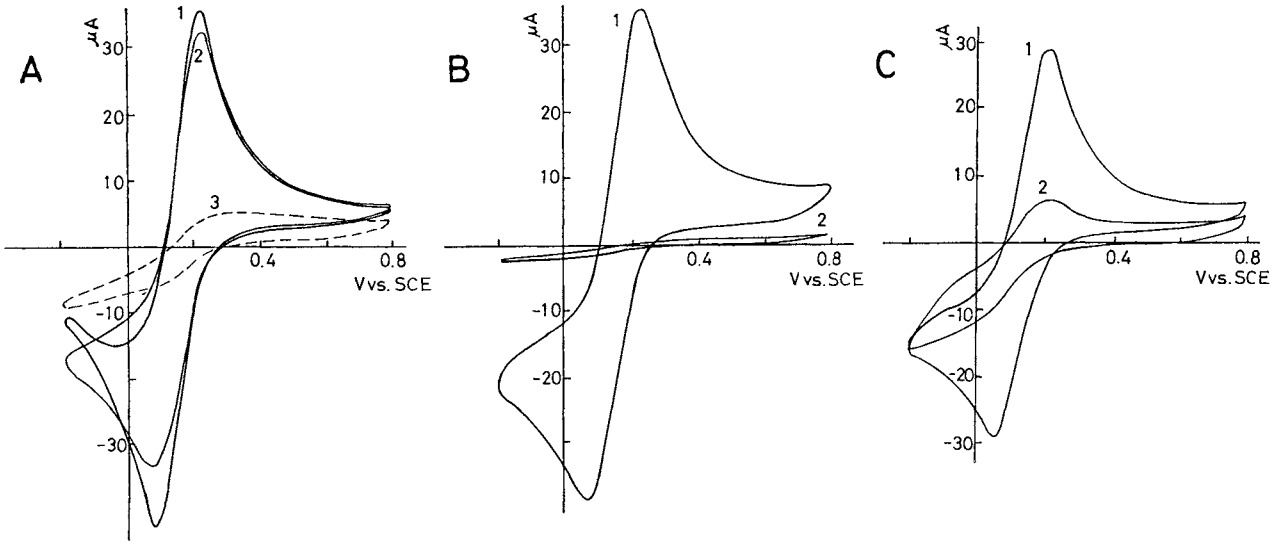


Fig. 3. Cyclic voltammograms for ferric/ferrous cyanide redox reaction (10^{-3} mol dm^{-3} $K_3Fe(CN)_6$ in 0.01 mol dm^{-3} KNO_3) measured on glassy carbon electrodes with surfaces covered with the monomeric LB films (**A**) and the polymerized LB films (**B**) of the enantiomer **1** and, for comparisons, polymerized LB films of *N*-octadecylacrylamide (**C**). 1, without monolayers; 2, with 8 monolayers; 3, with 16 monolayers. Deposition of the LB films was performed under a surface pressure of 30 mN/m after polymerization of 1 h.

with this polymer film is much lower than that achieved by the polypeptide film. Other typical kinds of amphiphiles suited for radical polymerization, e.g., diacetylene derivatives, gave a similar result, most probably due to defect-rich structures frequently brought about by this kind of rapid polymerization.[4] Slower but spontaneously proceeding mild polymerization systems, as typically demonstrated here, are considered more capable to produce a membrane of uniform and dense structures.

The chiral polypeptide monolayer prepared by the present self-condensation technique had a film thickness of ca. 20 Å per monolayer on the basis of elipsometry, showing that the lateral ordering and vertical orientation to the film plane of the amphiphile is retained in the polypeptide form, together with the information of molecular orientation studied by the polarized reflection/absorption IR spectroscopy which is described elsewhere.[2] We have synthesized, besides the enantiomer **1**, other phenyl ester analogues from optically active serine having varied lengths of hydrophobic group and different forms of amide linkage, all of which showed desirable reactivities for the chiral peptide monolayers. Taking an advantage of the ordered and close-packed structure of the monolayers, further study by way of extending the function of the chiral peptide LB film for recognition and enantio-selective permeation of substrate is highly promising for biochemical and medical applications.

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References

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3) The polypeptide Langmuir-Blodgett films were insoluble in such solvents as MeOH, THF, DMF, dioxane, chloroform, Methyl Cellosolve, hexamethylenephosphonamide, etc .
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