CHEMISTRY LETTERS, pp. 619-622, 1991

Rapid Self-Polycondensation of Amphiphilic Amino Acid Esters in Ordered Molecular Assemblies. An Morphological Evidence for Lateral Growth of Polypeptide at the Air-Water Interface

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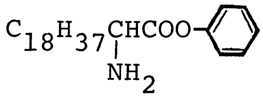
Monolayer-forming amphiphiles of amino acid esters, phenyl and 1,1-dichloroethyl esters of 2-aminoeicosanoic acid, underwent self-condensation to polypeptides in ordered monolayers with their reaction rates much higher than in non-ordered media. Scanning electron microscopy (SEM) revealed rapid progress of lateral polymerization at the air-water interface.

Molecular orientation and its regulation are often considered as an essential factor in biological synthesis. Polypeptide synthesis, which is normally feasible in vigorous conditions, was demonstrated in an ambient condition by Folda et al.$^{1)}$ with use of ordered monolayers comprising long alkyl chain derivatives of amino acid methyl ester capable of self-condensation reaction. Peptide bond formation spontaneously proceeding in Langmuir-Blodgett films has been studied by Fukuda et al.$^{2)}$ who employed amphiphilic long alkyl esters of amino acids for polycondensation. It is noteworthy that these approaches have shown the significance of the topological effect involved in the amide bond formation in relevance to the biological peptide synthesis. However, few studies have then been conducted to improve the reactivity of such spontaneous polymerization systems which had been of considerably low rate needing a reaction time of the order of days. The authors synthesized new amphiphilic amino acid derivatives, phenyl ester (**1**) and 1,1-dichloroethylester (**2**) of 2-eicosanoic acid, which are endowed with appropriately high reactivities for polycondensation by possessing pKa values of the conjugated alcohols released from the ester groups being approximately 10 (for **1**) and 13 (for **2**) .



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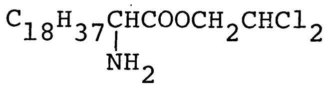

$C_{18}H_{37}CH(NH_2)COOCH_2CHCl_2$

2

The amino acid esters, **1** and **2**, formed stable monolayers on an aqueous subphase when they were spread from chloroform solutions. Figure 1 shows the pressure - area isotherms of the monolayers obtained immediately after the formation. The monolayer of **1** underwent a rapid decrease in area when left on a neutral aqueous subphase at a constant surface pressure. This shrinking effect reflects the progress of polycondensation in the fluid monolayers.$^{1)}$ Monolayer isotherm of **1** thus ultimately settled at the position exhibited in the figure (dashed line), giving about 50% shrinking in area as a result of spontaneous polycondensation reaction.

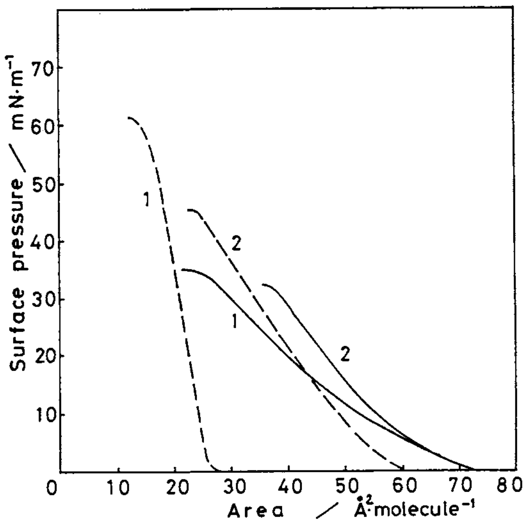

Fig. 1. Surface pressure-area isotherms for monolayer films of **1** (denoted as 1) and **2** (as 2) at 20 °C. pH of subphase was 7.4. Dashed lines represent isotherms obtained after polycondensation.

IR absorptions of the monolayers were measured by depositing the supernatant monolayers onto a silicon wafer by the horizontal lifting method$^{3)}$ and allowing them to form a small condensed deposit by treating with suitable organic solvents. IR spectrum of the deposit was thus obtained on the wafer at time intervals during the course of condensation reaction. Figure 2 depicts the spectra measured for the **1** monolayer just after and 2 hours after the formation, respectively. The spectral change observed corroborates the formation of amide bond (1650 cm^{-1}, 1530 cm^{-1}) along with cleavage of the ester bond (1760 cm^{-1}), which, similar to the observation by Folda et al.,$^{1)}$ gives a primary indication for the progress of polycondensation. A similar absorption change and a concomitant area decrease occurred for the dichloroethylester (**2**) monolayer, the rate of which was however much slow as was expected from its relatively high pKa value.

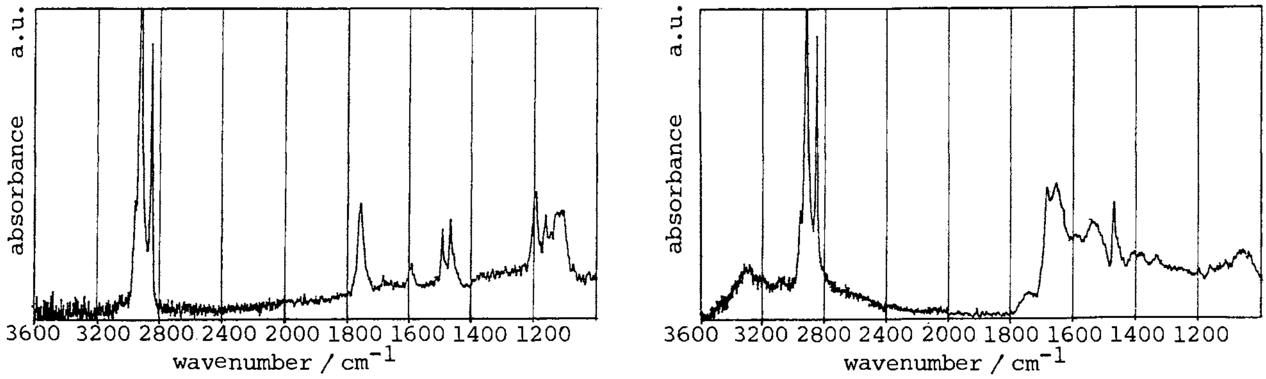

Fig. 2. IR absorptions of **1** before (left) and after (right) the polycondensation at the air-water interface. Monolayer was kept at a pressure of 15 mN·m^{-1} and temperature of 35 °C during a reaction time of 2 hours.

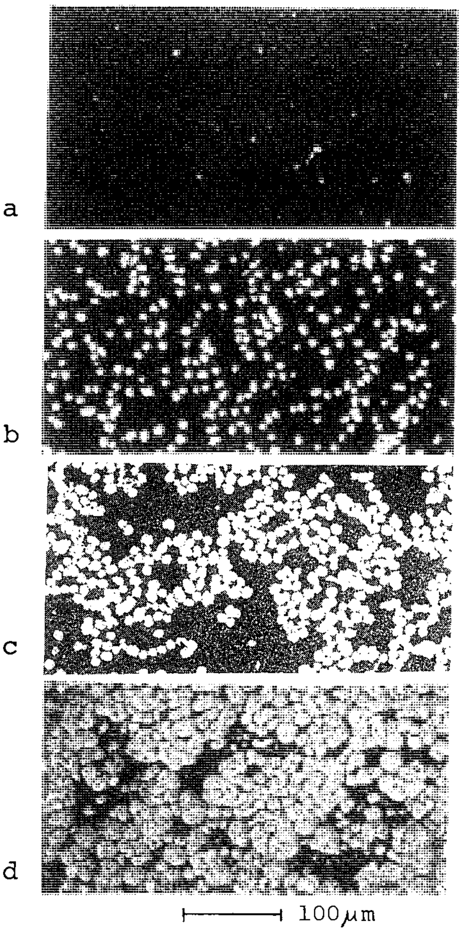

Fig. 3. Morphology of a monolayer film of **1** observed by scanning electron microscopy during the course of polycondensation at 35 °C and 15 mN·m^{-1}. Reaction time, 0, 10, 20, 60, min from the top to bottom (a-d).

Scanning electron microscopy (SEM) was undertaken in an attempt to obtain direct information on the condensation procedure. Figure 3 shows typical features of **2** monolayer varing with reaction time, where the sample consisted of a single monolayer placed on a silicon wafer substrate. It is clearly seen that generation of a small island is followed by its lateral growth in size which is finally dominating the net area of the monolayer. This is the first observation which visualizes the morphological dynamics of self-condensation reaction in a two-dimensional molecular array. To confirm that the reaction proceeds in the lateral direction without causing bulk aggregations of the product (polypeptide), reflection-absorption IR spectroscopy was carried out for a Langmuir-Blodgett multilayer of **1** built up on a gold substrate. This revealed, based on a comparison of absorption strength of bands,3) that the long alkyl chains in a polymerized monolayer (see Fig. 3, d) retain a vertical orientation (normal to the plane) along with the lateral ordering of molecules.

On the basis of IR absorption data, apparent degree of the condensation reaction (p) was estimated in terms of the ratio of absorbance decrease in the ester band (1750 cm^{-1}) to its initial absorbance with the absorbance of alkyl C-H vibration being standardized. This value reached 0.7 to 0.8 depending on the surface pressure employed. Here, it is to be noted that the p value turned out to be directly related to the fraction of total area occupied by the islands observed in the SEM scans. Plot of the function $-\ln(1-p)$ versus reaction time gave a linear relation, with its slope corresponding to the rate constant of the reaction. In Fig. 4, a result for the monolayer **2** at the air-water interface (present method) is presented and compared with those obtained with a Langmuir-Blodgett monolayer and a casted film formed on a solid substrate. Marked difference in reaction rate observed here reflects significant effects of molecular orientation and fluidity of the molecules. This result clarifies that the fluid ordered system, like an air-water interfacial monolayer, is most efficient for the spontaneous polycondensation while non-ordered media like a cast film (as well as liquid solutions) suppress much of it. Ordered arrangement of molecules and their lateral fluidity are thus essential to conduct peptide synthesis by self-condensation of amino acid ester where close arrangement between the ester and the amino groups of adjacent molecules is required. These factors are important in inferring the topological effect involved in the mechanism of biological synthesis.

Reaction rates of polycondensation were estimated to be $6.5 \pm 1.5 \times 10^{-4}$ s^{-1} for **1** and $4.5 \pm 1.5 \times 10^{-5}$ s^{-1} for **2** under conditions of 35 °C and a surface pressure of 15 mN·m^{-1}. Methyl ester analogue of the present amphiphile was also examined and gave a very low reaction rate, i.e., 1.1×10^{-5} s^{-1}, as has been expected due to its high pKa value and also reported earlier.$^{1)}$

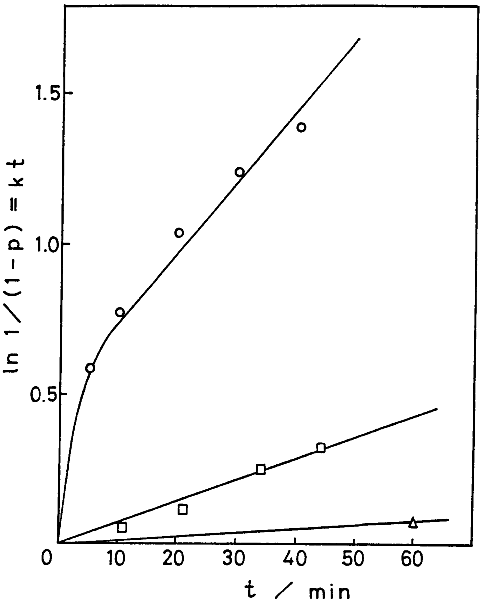

Fig. 4. Plot of ln 1/(1-p) versus reaction time for determination of polycondensation rate. Monolayers were reacted under a pressure of 25 mN·m^{-1} and a temperature of 35 °C. p denotes the degree of polycondensation (see text).

Degree of polymerization in the present self-condensation system has not been determined in unambiguous way because the product was not soluble in any of such solvents as THF, DMF, dioxane, chloroform, Methyl Cellosolve, hexamethylenephosphonamide, etc. End-group analysis applied to partially dissolved suspensions roughly estimated the polymerization degree to be in the range of 10-50.

Extension of the present rapid self-condensation system is expected to enable preparation of highly ordered thin polypeptide films or polymerized liposomes with peptide bonding, which is attracting increased interest in medical applications. Our molecular design for this purpose is presently under progress.

References

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(Received January 10, 1991)