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Effect of Cu(II) Ion on the Polycondensation of Octadecyl Esters of Aromatic Amino Acids in Monolayers at the Air/Water Interface

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Polycondensation of octadecyl esters of L-phenylalanine (L-PheC18), L-tyrosine (L-TyrC18) and L-tryptophan (L-TrpC18) in the monolayers on the subphase containing Cu(II) ion was significantly accelerated in comparison with those in the monolayers on phosphate buffer or in bulk phases. A reaction mechanism including formation of an intermediate complex and reorientation of the aromatic rings was proposed.

Previously, we have found that the polycondensation of long-chain esters of α-amino acids such as glycine, alanine and those containing aromatic rings can be markedly accelerated in monolayers at the air/water interface.[1,2] However, in the case of aromatic amino acid derivatives, because of steric hindrance of the aromatic rings, the rates of polycondensation in the monolayers were still rather slow, although they were ten times or more faster than those in bulk phases. Miyasaka *et al.* have improved the rates of polycondensation in monolayers by using the amphiphilic amino acid derivatives with easily releasable phenyl or 1,1-dichloroethyl groups.[3] Shibata *et al*. have synthesized the *N*-carboxy anhydride derivatives of glutamate and obtained the rapid polycondensation in the monolayers.[4] However, these molecules result in polypeptides with long alkyl side chains at the α-carbon atom. In the present work, we have found that a certain metal ion such as copper(II) dissolved in the subphase can remarkably promote the polycondensation of long-chain esters of aromatic amino acids in the monolayers and discussed a possible mechanism for the accelerating effect.

Octadecyl esters of L-phenylalanine (L-PheC18, mp:37.0-38.0 ℃), L-tyrosine (L-TyrC18, 83.5-84.5 ℃) and L-tryptophan (L-TrpC18, 87.0-88.0 ℃) were synthesized as described previously.[2] Monolayers of these esters were spread from the benzene solutions onto the aqueous subphase containing 1×10^{-3} M of $Cu(II)Cl_2$, and the surface pressure (π)-area (A) isotherms were recorded with a Lauda film balance (compression speed: 5 Å^2/molecule•min). The progress of polycondensation in the monolayers was monitored by measuring the FT-IR spectra of the films scraped from the water surface at different time intervals.

Figure 1 shows the π-A isotherms for the monolayers of the esters of aromatic amino acids spread on the aqueous subphases containing 0.01 M phosphate buffer (pH 8.0) and 0.001 M $Cu(II)Cl_2$ (pH 5.7). The monolayers on phosphate buffer solution exhibited characteristic plateau regions between 30-40 mN/m. On the other hand, when the subphase contained Cu(II) ion, area contraction and expansion occurred in lower and high pressure regions, respectively, leading to more densely packed monolayers with less compressibility, especially in the cases of L-TyrC18 and L-TrpC18, where the plateau regions disappeared completely. These facts suggest that the complexes between Cu(II) ion and the amino acid esters can be formed[5] in the monolayers.

When the monolayers on the subphase containing Cu(II) were kept at the constant pressure of 30 mN/m,

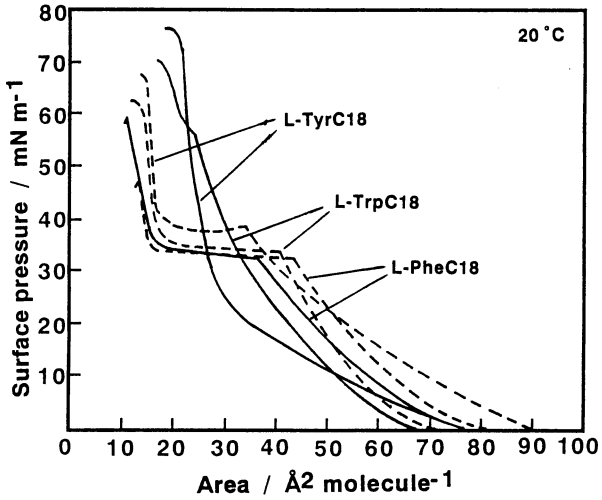

Fig.1. π-A isotherms for the monolayers spread on subphases containing 0.01 M phosphate (dashed lines) and 0.001 M $CuCl_2$ (solid lines).

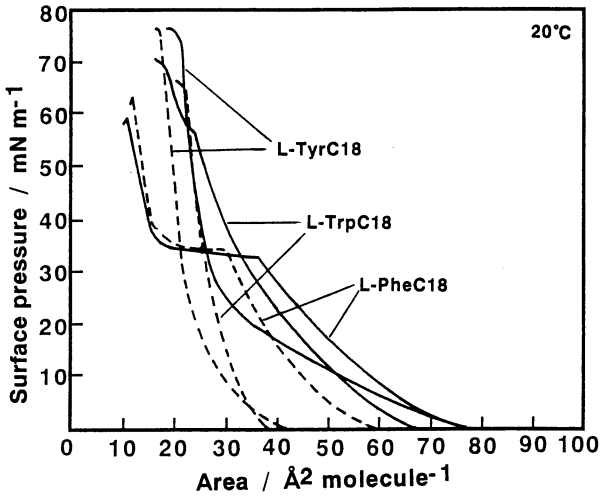

Fig.2. π-A isotherms for the monolayers on subphase containing 0.001 M $CuCl_2$ immediately after spread (solid lines) and after 30 min (dashed lines).

significant shrinkage of the molecular areas was observed within 30 min. Rates of area shrinkage were in the order of L-TyrC18>L-TrpC18>L-PheC18. Figure 2 shows the π-A isotherms measured after keeping the monolayers under compression (below the plateau) for 30 min, in comparison with those recorded immediately after spread. Change in the π-A isotherms from expanded to condensed type accompanied with area contraction in the lower pressure region suggests the progress of polycondensation in the monolayers.[2]

In order to clarify the polycondensation, the monolayers were scraped up onto calcium fluoride plates at different time intervals and the FT-IR spectra of the films were measured. Figure 3 shows FT-IR spectra for the L-TyrC18 films scraped 15 and 60 min after spread (A and B), together with the skeletonized film (C) by dissolving the unreacted monomer and released octadecanol in hexane. For comparison, the spectrum of the film scraped from the monolayer immediately after spread on phosphate buffer (D) is also shown. With time advanced, the ester band at 1736 cm^{-1} decreases, while the bands at 1683-1672, 1653 and 1635 cm^{-1} are enhanced, which can be assigned to the amide I bands for oligopeptides and polypeptides with helix or random coil and β-sheet conformations, respectively[6] (a peak at 1614 cm^{-1} can be assigned to the C=C stretching of phenol group). Similar results were obtained for the other esters. These facts indicate that the long-chain esters of aromatic amino acids are converted to polypeptides in the monolayers with release of octadecanol. On the other hand, a minor peak was found to appear at 1700 cm^{-1}, which can be assigned to the protonated carboxylate groups. This fact suggests that in addition to the polycondensation in the monolayers, a part of the ester undergoes hydrolysis. Therefore, we cannot estimate the conversion of the monomer to polypeptide only from the decrease of ester band. In the LB films of these amino acid esters, however, the hydrolysis of the ester can be neglected because of the absence of water. Using the LB films at different stages of polycondensation, [7] a relation-

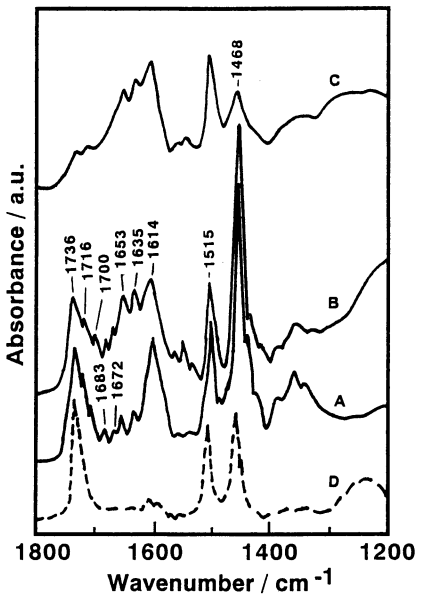

Fig.3. FT-IR spectra of L-TyrC18 films obtained from subphases containing $CuCl_2$, 15 min (A), 60 min (B) after spread and after dissolving the monomer and octadecanol (C), immediately after spread on phosphate buffer (D).

ship between percent conversion and relative intensity of the amide band to the ester band can be obtained. By applying this relation to the films scraped up from the monolayers on the subphase containing Cu(II) ion at different time intervals, the percent conversions of monomer to polypeptide were estimated, as shown in Fig.4. In the initial 50 min, the polycondensation proceeds rapidly, and then the conversions tend to saturate at 60-65% after 200 min.

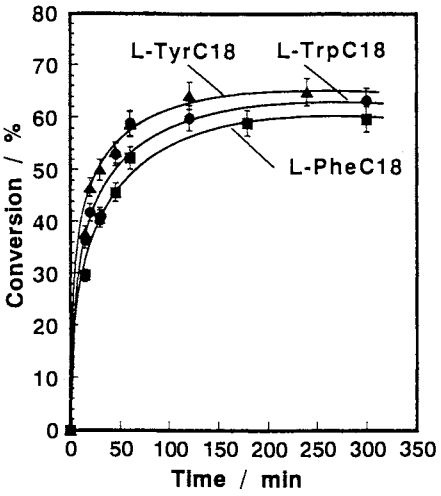

Fig.4. Conversions versus reaction time.

Supposing the polycondensation is the first order reaction in the initial stage, it is possible to evalute the apparent rate constants, which are listed in Table 1, as compared with those for the polycondensation in monolayers on phosphate buffer and in bulk solids. It can be found that the polycondensations are markedly accelerated in the monolayers on the subphase containing Cu(II) ion, which are ten times or more faster than those in the monolayers on phosphate buffer and about hundres times faster than those in the bulk solids. These values are even greater than that in the monolayer of octadecyl ester of alanine without aromatic ring which causes the steric hindrances.[1)]

Table 1. Apparent rate constants of polycondensation (s^{-1})

	L-TyrC18	L-TrpC18	L-PheC18
Monolayer[a)] (20 °C)	4.02×10^{-4}	2.56×10^{-4}	1.93×10^{-4}
Monolayer[b)] (20 °C)	1.58×10^{-5}	1.94×10^{-5}	0.96×10^{-5}
Bulk solid (below mp)	2.57×10^{-6}	1.87×10^{-6}	0.35×10^{-6}

a) 0.001 M $CuCl_2$, b) 0.01 M phosphate buffer

This accelerating effect of Cu(II) ion on the polycondensation in monolayers can be explained by a following mechanism, as illustrated in Fig.5. From the π-A isotherms (Fig.1), it was made clear that the monolayers were significantly condensed on the subphase containing Cu(II) ion. This suggests that intramolecular and/or intermolecular complexes (1:1) can be formed between Cu(II) ion and the amino and ester groups of the aromatic amino acid derivatives. The complex formation in the monolayer not only increases the encounter probability of adjacent amino and ester groups in the layer plane, but also favors the most appropriate arrangement of the functional groups for the formation of polypeptide. It is supposed that the complex formation

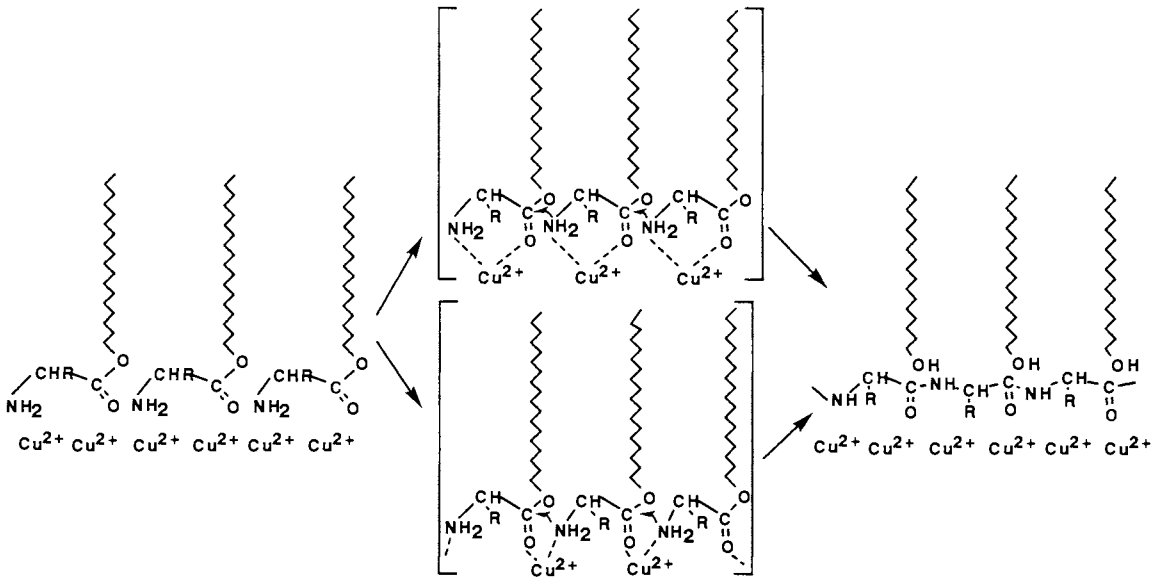

Fig.5. A possible mechanism for polycondensation in monolayers on the subphase containing Cu(II).

causes a change in orientation of the aromatic rings which lead to a more compact packing and a reduction in the steric hindrance for the reaction. Thus, the polycondensation can be remarkably accelerated. This is supported by the complete disappearance of the plateau regions in the π-A isotherms of L-TyrC18 and L-TrpC18 on the subphase containing Cu(II), which is very similar to those after polycondensation (Fig.2). In the case of L-PheC18, the change of orientation is relatively difficult to occur (the plateau didn't disappear), which is reflected in the relativly low polycondensation rate.

Further, the reaction mechanism is supported by the structure of the resultant polypeptides. If the reaction proceeds through the above mechanism, polypeptides with β-sheet predominant conformation are expected. This is reflected in the IR spectrum of the film after dissolving unreacted monomer and released octadecanol (Fig.3 (C)), where the peak intensity of the amide I band at 1635 cm^{-1} is slightly stronger than at 1653, suggesting an abundance of the β-sheet conformation in the resultant polypeptides. Similar results were also found for the other derivatives. This fact is different from the case on the phosphate buffer, in which the polypeptide takes mainly helix or random coil conformation.

Figures 6(a) and (b) show pictures by a scanning electron microscope for the L-TyrC18 monolayer transferred on silicon immediately after spread and after keeping for 24 h at 20 ℃ on the subphase containing Cu(II) ion, respectively. A clear phase seperation involving uniform aggregates with a diameter of 10 μm was observed after the polycondensation in the monolayer. This seems to be caused by the release of the octadecanol through the polycondensation. After removal of the octadecanol by hexane, different image of domains ascribable to the insoluble polymer was obtained. From the fact that any Cu(II) ion could not be detected in elemental analysis of the film by an energy dispersive X-ray spectroscopy with SEM, the complex with Cu(II) is regarded as only an intermediate. Other divalent metal ion, such as nickel, zinc, calcium, cobalt, magnesium or trivalent aluminum ions exhibited almost no accelerating effect on the polycondensation in the monolayers. This can be ascribed to their ability to form the complex according to the Irving-Williams series.[8]

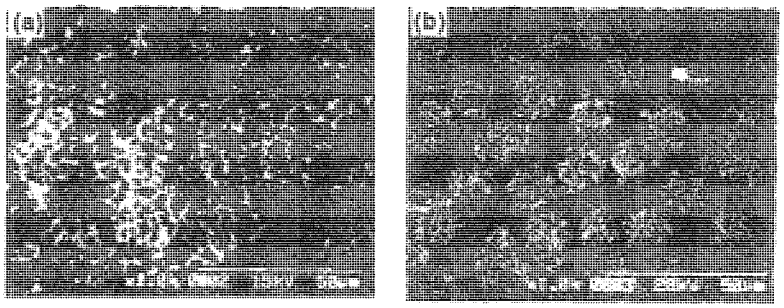

Fig.6. SEM images of L-TyrC18 monolayers transferred on silicon from the subphase containing Cu(II) ion, immediately (a), after 24 h (b).

In conclusion, it has been found that the polycondensation of long-chain esters of aromatic amino acids can be markedly accelerated in the monolayers on the subphase containing Cu(II) ion through an intermidiate complex formation.

References

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