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Polycondensation of Long-chain Esters of Aromatic Amino Acids in Monolayer at the Air/water Interface

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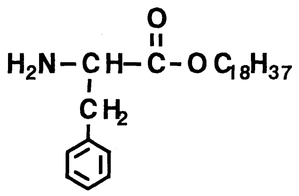
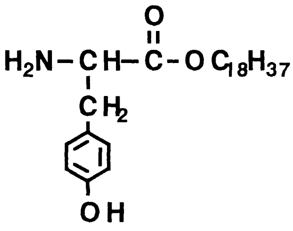
Polycondensation of octadecyl esters of aromatic amino acids (L-TrpC18, L-TyrC18, L-PheC18) can be markedly accelerated in the monolayers on the aqueous subphase of pH 8. The reaction rate was ten times or more faster than in the bulk solid. There exist optimum molecular packing and orientation for the polycondensation. The resultant poly-amino acids take α-helix and/or random coil conformations.

The air/water interface provides an adequate environment for controlling of molecular orientation and packing which are favorable for polycondensation reaction. Previously, we have found that polycondensation of long-chain esters of simple α-amino acids, such as glycine and alanine, proceeds spontaneously in the monolayers at the air/water interface and in the LB films without any initiator.[1)] Similar results were obtained for long-chain esters of glutamic acid and lysine.[2,3)] Recently, Miyasaka et al have reported polycondensation of phenyl esters of amphiphilic amino acids in the monolayers.[4)] On the other hand, aromatic amino acids such as phenylalanine, tyrosine and tryptophan are expected to take various molecular orientation and packing in the monolayers due to the rings differing in size and hydrophilicity. In this study, we synthesized octadecyl esters of these aromatic amino acids to investigate effects of the aromatic rings on the polycondensation of these esters in the monolayers at the air/water interface.

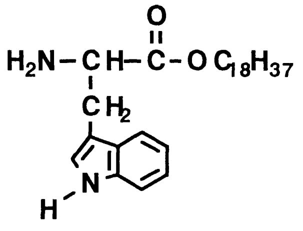
The materials used, together with abbreviations and melting points, are shown as follows:



L-PheC18
mp: 37.0-38.0 °C



L-TyrC18
mp: 83.5-84.5 °C



L-TrpC18
mp: 87.0-88.0 °C

These compounds were synthesized by esterification of the corresponding aromatic amino acids and octadecanol with *p*-toluenesulfonic acid in toluene, followed by desalting with ammonium gas and purified by recrystallization from methanol solution. The monolayers were spread from benzene solution onto the aqueous subphase containing 0.01 M phosphate buffer (pH 8) and the surface pressure-area isotherms were recorded with a Lauda film balance. To trace the polycondensation at the air/water interface, the monolayers were scraped up onto calcium fluoride plates at different time intervals and FT-IR spectra of the scraped films were measured.

Figure 1 shows the π-A isotherms for the monolayers of L-TrpC18, L-TyrC18 and L-PheC18 obtained immediately after spread and those recorded after keeping the monolayers at the definite areas of 43, 38, and 49 Å^2/molecule for 2, 5, and 5 hours, respectively. The pressure for the monolayer of L-TrpC18 arose at 73 Å^2/molecule and exhibited a plateau region from 41 to 21 Å^2/molecule. The extrapolated areas for the expanded and condensed regions were 60 and 20 Å^2/molecule, respectively. After keeping the monolayer at 43 Å^2/molecule (just before the plateau) for 2 hours, the plateau region diminished and the area decreased for the expanded region, while increased for the condensed region. This result suggests that polymerization reaction takes place in the monolayer.[5,6] The monolayers of L-TyrC18 and L-PheC18 exhibited similar behaviors, although the area increase in the condensed region was not observed for L-PheC18.

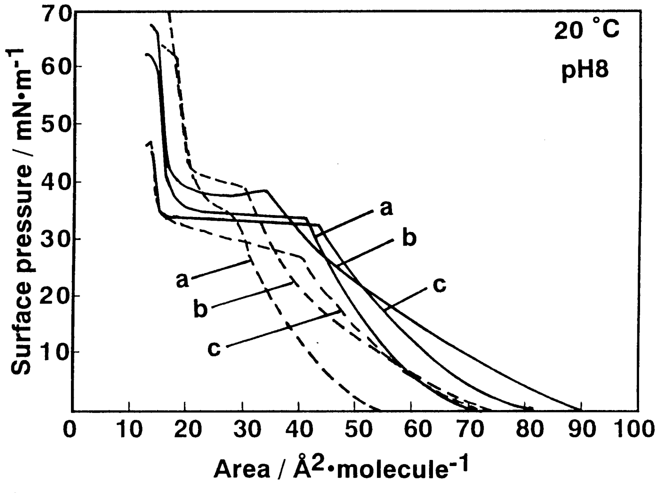


Fig.1. Surface pressure-area isotherms for the monolayers of L-TrpC18 (a), L-TyrC18 (b), and L-PheC18 (c), immediately after spread (full lines) and after 2-5 hours (dotted lines).

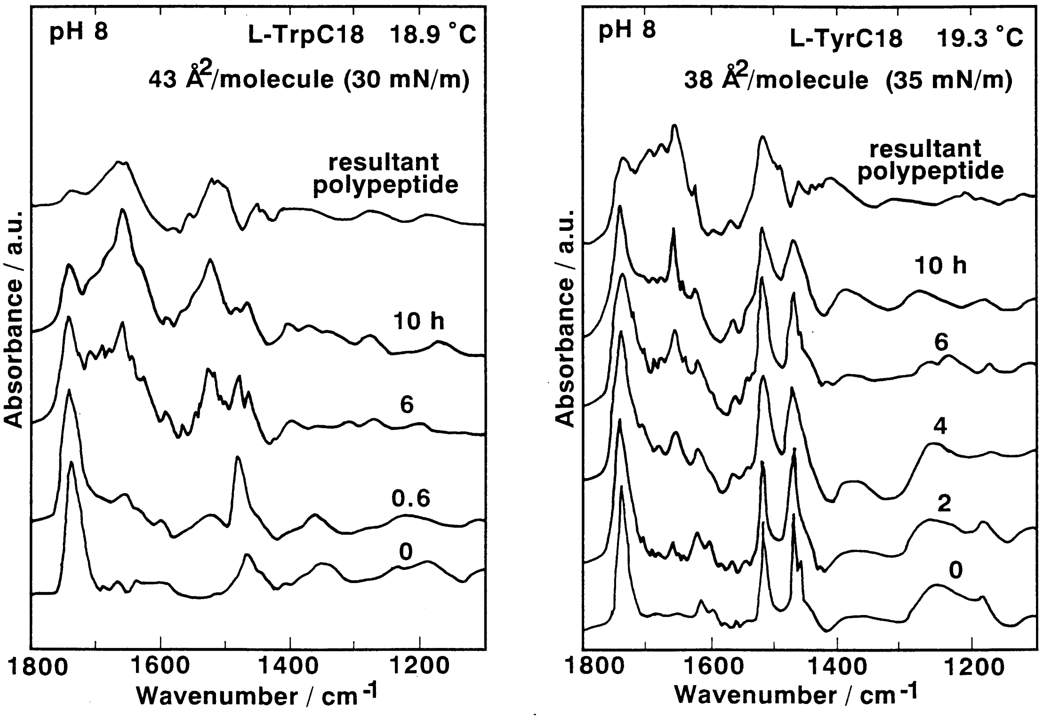


Fig.2. FT-IR spectra of L-TrpC18 and L-TyrC18 films scraped up from the water surface onto CaF_2 at different time intervals (the areas were kept at 43 and 38 Å^2/molecule, respectively) and the resultant polypeptides after removing unreacted monomers and released octadecanol.

Figure 2 shows the change of FT-IR spectra with time for L-TrpC18 and L-TyrC18 films which were scraped up onto CaF_2 plates at different time intervals. The most important features of these spectra are that the ester band at 1736 cm^{-1} decreases with time, while the bands at 1653 and 1516 cm^{-1} increase. The bands at

1653 and 1516 cm^{-1} (overlapped with the vibration of the aromatic ring) can be assigned to the amide I and II bands of peptides, respectively. These results indicate that the esters of aromatic amino acids were converted to polypeptides with release of octadecanol in the monolayer at the air/water interface, supporting the above consideration of the π-A isotherms. The pH of the subphase could have a great influence on the monolayer behaviors. On strong acidic or alkaline aqueous subphase, the hydrolysis of the ester was a main event, while on the nearly neutral subphase of pH 8, the condensation was predominant.

After polycondensation, the octadecanol released in the monolayer is transferred onto the CaF_2 plate together with poly-amino acids. Thus, from the diminish of relative strength of the ester band at 1736 cm^{-1} to the CH_2 stretching band at 2918 cm^{-1}, we can estimate the percent conversion (x) in the monolayer. These results are shown in Fig.3. In the initial 10 hours, the polycondensation proceeds rapidly and the conversions for L-PheC18, L-TyrC18, and L-TrpC18 attained to 30, 40, and 45%, respectively. After that the reaction rate slowed down and tended to saturate. Supposing that the polycondensation is a first-order reaction at the initial stage, then by plotting -ln(1-x/100) against the reaction time, linear relationships can be obtained, as shown in Fig.4. From the slopes of the lines it is possible to get the apparent rate constants, which were 1.94×10^{-5}, 1.58×10^{-5}, and $0.96\times10^{-5} s^{-1}$ for L-TrpC18, L-TyrC18, and L-PheC18, respectively. These values are considerably smaller than that obtained for the alanine derivative.[1)] The difference in the rate constants seems to be due to larger steric hindrance of the aromatic rings. Comparing with L-TrpC18 and L-TyrC18, the slower reaction rate was observed for L-PheC18. This may be ascribed to different orientations of the aromatic rings in the monolayer. It can be seen from the π-A isotherms that L-PheC18 with a benzene ring exhibits the larger area than L-TrpC18 containing a bulky indole ring. Since L-PheC18 has no hydrophilic group in the aromatic ring as in indole (N-H) or in phenol group (-OH), the benzene ring lies flat on water surface and thus makes it difficult for ester and amino groups to approach each other, which results in the lower reaction rate. On the other hand, the apparent rate constants in the monolayers are ten times or more greater than those in the bulk solids. This indicates that the molecular orientation and packing in the monolayer play an important role in acceleration of the

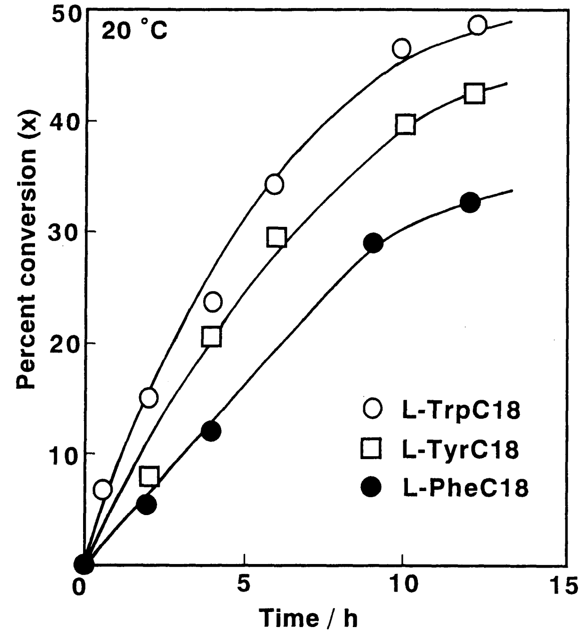


Fig.3. Percent conversion (x) versus reaction time.

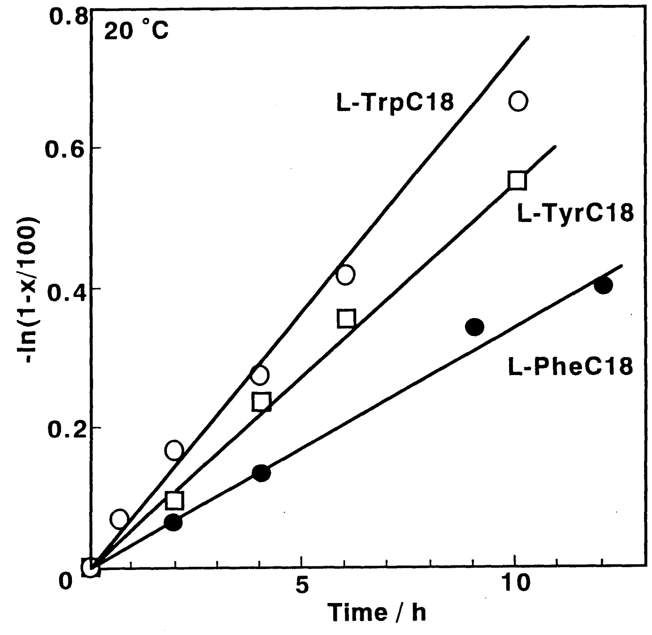


Fig.4. Plot of -ln(1-x/100) versus reaction time.

polycondensaton reaction.

By changing the molecular area in the monolayer, it is possible to vary the molecular packing and orientation which are expected to affect the reaction rate. Figure 5 shows the relationship between the apparent rate constants and the molecular areas. It can be found that the optimum molecular area exists for the polycondensation, which is just before the plateau region. Particular orientation and suitably close packing of the monomer molecules together with some conformational freedom seem to be very favorable for the polymerization reactions. By taking advantage of monolayers at the air/water interface, we can realize such molecular arrangements that the functional groups take the most effective conformation for the polycondensation.

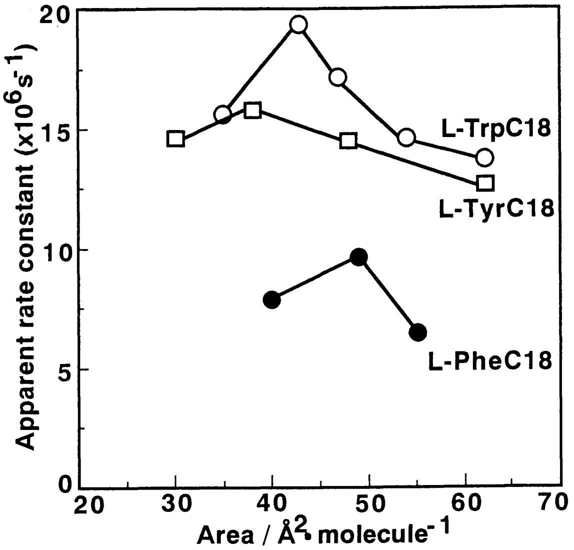


Fig.5. Relationship between apparent rate constants and molecular areas.

After immersing the polymerized films in acetone for 5 minutes to remove the unreacted monomers and released octadecanol, we can get the FT-IR spectra of the skeletonized films, as shown in the top of Fig.2. The amide I band appeared around 1650-1656 cm^{-1} suggests that the poly-amino acids produced in the monolayer take mainly α-helix and/or random coil structures.

References

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