A New Thermal Polycondensation of Alanine in Molten Urea[1]

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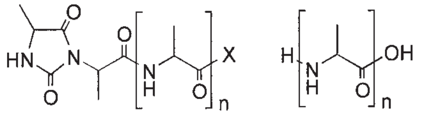
Heating of alanine with urea afforded polyalanine containing a hydantoin ring at the *N*-terminal position. Molecular weight of the polymer was lower than 8000, as estimated by dialysis of the product. Molten urea was considered to facilitate spontaneous polymerization of alanine as well as to act as a dehydrating agent.

Many model experiments have been performed to obtain information on the prebiotic formation of polypeptides.[2–5] These investigations revealed that aspartic acid, glutamic acid, and lysine undergo self-condensation by the action of heat, since heating of these amino acids produces polymerizable intermediates, such as intramolecular anhydrides, imides, or amides. The lactams of glutamic acid and lysine in the molten state had also been appreciated as polar reaction media, facilitating the thermal polycondensation.[2] The thermally thus-formed polypeptides and co-polypeptides gathered special interest as a model of proto-protein on the primitive Earth.[6–8] These protein-like substances were revealed to catalyze the formation of oligonucleotides and peptides[9–11] as well as to form microspheres,[3] which could be regarded as a protocell model.

Recently, we revealed that molten urea is superior to several molten ammonium salts in dissolving some dicarboxylic acids.[12] Therefore, we attempted to polymerize alanine with the aid of molten urea in this study. Alanine is a typical amino acid which had been known to be incapable of undergoing thermal polymerization. The presence of urea was suggested on the primitive Earth,[13–15] and it seems to be significant to examine the role of urea in the prebiotic formation of biomolecules, including polypeptides.

We heated an equimolar mixture of DL-alanine and urea at 140 °C for 1 h by using an autoclave, and analyzed the aqueous solution of the reaction mixture by LC-MS. In the thermal reaction product, three types of alanine oligomers (**1–3**, Chart 1) were successfully identified on the basis of their molecular weights, as shown in Figure 1, which shows the total ion chromatogram and the $[M+1]^+$ mass fragmentograms for each oligomer. Oligomers of type **1** and **2** were revealed to possess a hydantoin ring located at their *N*-terminals. The ring position was confirmed by the fact that dinitrophenylation followed by hydrolysis of the dialyzed reaction product afforded no *N*-dinitrophenylalanine. Oligomers of type **3** consisted only of relatively low molecular weight oligomers that could escape through the dialysis membrane (cutoff molecular weight (CMW): 2000).



1 : X = OH (MW : 186 + 71n) **3** : (MW : 18 + 71n)
2 : X = NH$_2$ (MW : 185 + 71n)

Chart 1.

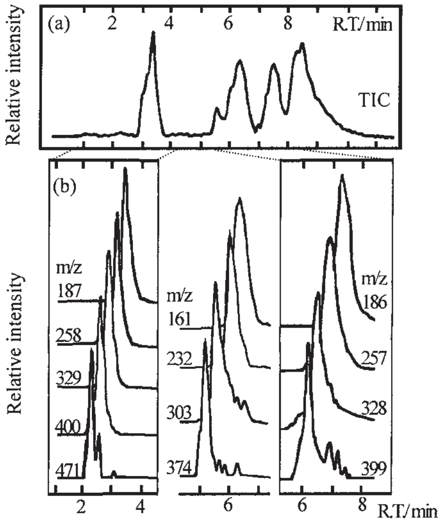


Figure 1. Total ion chromatogram (a) and $[M+1]^+$ mass fragmentograms (b) of thermal reaction product.

Concerning the higher polymer that was obtained after dialysis (CMW: 8000) and dryed at 110 °C under reduced pressure, it was biuret reaction-positive, being characteristic of polypeptides, and its IR spectrum (Figure 2) practically agreed with that of poly-DL-alanine, although the band at amide I and II region (1500–1750 cm^{-1}) was rather broad. Furthermore, amino acid analysis of the polymer hydrolyzate indicated that the polymer contains an alanyl residue at 14 mmol g^{-1}, which is in agreement with the calculated value (14.03 mmol g^{-1}) for polyalanine of polymerization degree of 100. Although the entire structure of the higher polymer was still uncertain, it might be mainly composed of alanyl peptide, and determination of its strict structure will be a future problem.

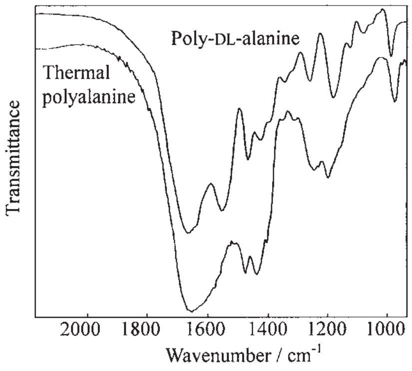


Figure 2. Infrared absorption spectra of polyalanines.

In order to evaluate the effect of reaction time on the polymerization, the products heated at 140 °C for 1 and 10 h were dialyzed using 4 types of membranes (CMW: 500, 2000, 5000, and 8000). The yields of polymers that remained after dialysis were estimated from the amounts of alanine in the hydrolyzates of the dialyzed polymers (Figure 3). The molecular weight of the polymer ranged up to about 8000, which corresponds to a polymerization degree of about 110. As judged by the yields after dialysis using a membrane of CMW 500, heating time had little effect on the total yield of polymers. Longer reaction time, however, proved to increase polymers of higher degree, as observed in the polymer yields after dialysis using membranes of CMW 2000, 5000, and 8000.

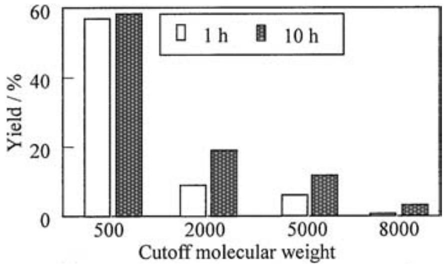


Figure 3. Yield of thermal polyalanine after dialysis.

We then followed the polymerization by means of thermogravimetric analysis, and the results are shown in Figure 4. No change in the quantity of alanine (mp 295 °C (dec)) was observed during the heating at 140 °C for 6 h, whereas heating of urea at the same temperature decreased its weight to about 25% of the original value in 2 h due to the well-known thermal decomposition into ammonia, isocyanic acid, and oligoureas including biuret. The decrease ceased on further heating. The decrease in weight of a heated equimolar mixture of alanine and urea started about 20 min earlier than that of urea alone, which might indicate that the polymerization of alanine proceeds, at least in part, without the aid of any dehydrating agent in molten urea. The weight of the mixture continued to decrease until 6-h heating, though slowly after 2 h, and finally to afford residual weight of about 55% of the original value. Since 32% of the weight loss of the mixture (43% of urea in weight) can be explained by decomposition of urea that caused weight loss of 75% in the case of urea alone, further weight decrease of the mixture might be attributed to release of H_2O due to condensation of alanine. The results of the thermal analysis may indicate that the polymerization proceeded rapidly at an early stage of the heating and slowly after 1- or 2-h heating.

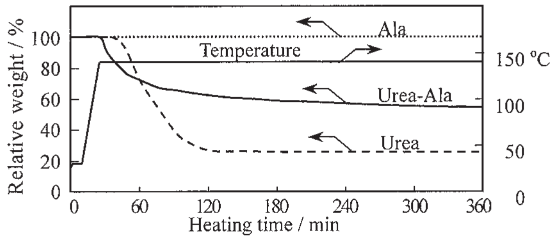
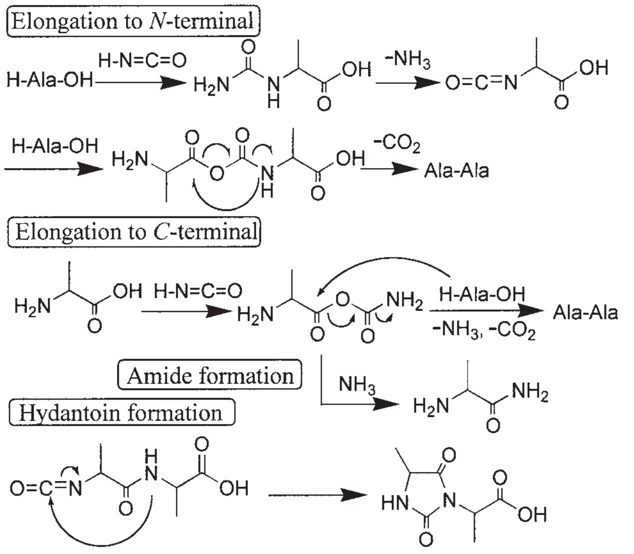


Figure 4. TG analysis of alanine, urea, and their mixture.

On the other hand, the presence of a hydantoin ring in the polymer indicates the contribution of isocyanic acid (decomposition product of urea) to the thermal polymerization as a condensing agent. Isocyanic acid can react with both an amino and a carboxyl group, resulting in peptide elongation toward both *N*- and *C*-terminals (Scheme 1). Formation of a *C*-terminal amide and a hydantoin ring may terminate the polymerization.



Scheme 1.

In conclusion, we have found a novel thermal polymerization of alanine, which would be likely applied to other amino acids, by taking advantage of a highly polar feature of molten urea as well as the dehydrating ability of urea. The polymer obtained by this method was revealed to have a special structure, bearing a hydantoin ring at the *N*-terminal. From the viewpoint of chemical evolution, this study offered a new candidate for a dehydrating agent or a polycondensation medium that can be present on the primitive Earth.

References and Notes

1 A part III of a series of studies entitled ‘on the role of urea in chemical evolution of biomolecules’.
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