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Urea as a Nitrogen Source in the Formation of Polyaspartic Acid from Malic, Maleic, and Fumaric Acids [1]

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We have demonstrated an excellent feature of urea in the molten state as a nitrogen source and a polar reaction medium in the nitrogen-incorporating thermal polycondensation of malic, maleic, and fumaric acids to produce polyaspartic acid.

Ammonium salts or amides of maleic acid and their homologs have been known to afford polymers of aspartic acid or its homologs upon heating in the solid state.[2] The thermal polymers were confirmed to be anhydropolyaspartic acids, which contained five-membered imide rings.[3] Those unsaturated dicarboxylic acids were likewise thermally condensed with glutamine, resulting in the formation of copolymers of aspartic and glutamic acid.[4] Ammonium salt of malic acid was also reported to afford aspartyl polymers under a similar condition through dehydration, giving maleic and fumaric acid.[5] These studies had suggested possible prebiotic formation of protein-like polymers from non-amino acid precursors, and focused mainly on elucidating the structural features of thermally produced polymers.

In the present study, we heated malic, maleic, and fumaric acids individually in the presence of a nitrogen source, such as urea or one of the ammonium salts of organic or inorganic acids, and evaluated their efficiencies of nitrogen introduction into these dicarboxylic acids in the formation of polyaspartic acid. Ammonium salts had been utilized as nitrogen sources in solution or in the molten state for conventional syntheses of monomeric aspartic acid from maleic and fumaric acid. These reactions required the salts to be in large excess. However, such an excess of ammonium ions or water prevents polymerization. From the viewpoint of chemical evolution as well as synthetic chemistry, we have come to pay attention to urea, which had probably been present on the primitive Earth[6] as a nitrogen source in the prebiotic formation of biomolecules. Nitrogen atoms in urea show rather high reactivity as a nucleophile, and urea is more resistant to hydrolysis by acids and bases, as compared to carboxylic acid amides including those in peptides. Urea melts at 135 °C and decomposes at a rate depending on the temperature to produce ammonia and isocyanic acid, which are also expected to react with other compounds as a nitrogen source and a carbamoylating or dehydrating agent, respectively. In the reported reaction of urea with maleic anhydrides under controlled conditions, urea introduces nitrogen into the anhydrides, and seems to undergo dehydration to give maleimides.[7] Molten urea dissolves various substances from polar organic compounds to inorganic ones, such as sodium chloride. It therefore provides a highly polar reaction medium, which may facilitate various reactions.

Our heating experiment was carried out with an equimolar mixture of malic, maleic, or fumaric acid and a nitrogen source in a degassed sealed tube for 12 h at 140–200 °C; the resulting polymeric product was hydrolyzed with 6 M HCl at 110 °C for 48 h. When the heating products were directly analyzed without being hydrolyzed, a negligible amount of aspartic acid and small amounts of aspartic acid amides were detected, indicating that the products were mainly composed of polyaspartic acid. Consequently, the efficiency of nitrogen introduction to the dicarboxylic acids was evaluated as a yield of aspartic acid that was determined by an amino acid analysis of the hydrolysate.

The results are summarized in Figs. 1a to 1c. The anhydrous thermal reactions of the dicarboxylic acids with the nitrogen sources were revealed to produce polyaspartic acid, as in the thermal polycondensation of ammonium salts or amides of the acids.[2–4] The reactions of malic and maleic acid with urea, and ammonium acetate (mp 114 °C), formate (mp 119 °C (dec)), and phosphate (mp 155 °C), all of which melt in the range of the reaction temperatures to become polar liquids, were similar in temperature-yield relationship, as shown in Figs. 1a and 1b. A large difference was not observed between the yields in the reactions of malic and maleic acid, which is reasonable because malic acid is transformed above 140 °C to maleic and fumaric acid via dehydration. The highest yield of aspartic acid determined after hydrolysis was observed in each reaction at 160 °C. The yields were slightly lower in reactions at 140 °C than at 160 °C, while they greatly diminished at a reaction temperature of over 160 °C. Urea proved to be best in introducing nitrogen into the acids at 140 and 160 °C, and showed a slower decrease in the efficiency at higher temperature, compared to ammonium salts of the weak acids.

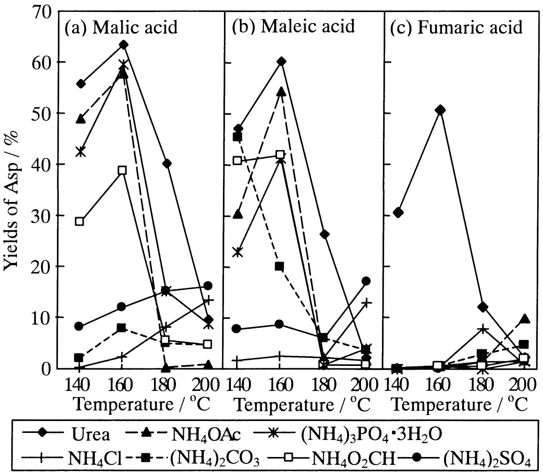


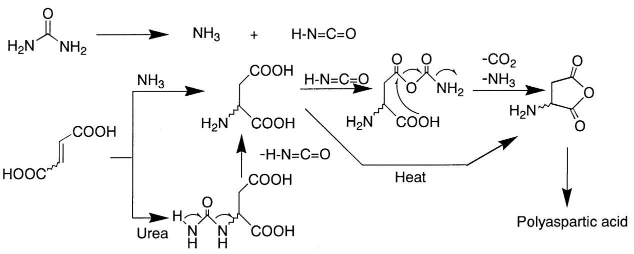
Fig. 1. Yields of thermal polycondensation. The yields are based on the dicarboxylic acids.

The yields in the reaction of ammonium carbonate with malic acid were very low at all reaction temperatures. However, this salt could introduce nitrogen to maleic acid in a yield comparable to that in the reaction of urea only at 140 °C, while the nitrogen-introducing ability of the carbonate into maleic acid decreased upon increasing the reaction temperature. Since this salt melts at 58 °C and decomposes to ammonia and carbon dioxide at higher temperatures, it is likely that it reacted with maleic acid only at an early stage of heating at a lower reaction temperature. If this is the case, the low yields in its reaction with malic acid may come from the fact that malic acid is required to be dehydrated at the initial stage of the reaction producing polyaspartic acid.

Ammonium salts of strong acids, namely ammonium sulfate (mp 280 °C) and chloride (mp 338 °C), generally afforded low yields of polyaspartic acid upon reaction with malic and maleic acid, although a higher temperature proved to favor their nitrogen introduction to these acids under the conditions employed. These salts do not melt under the reaction conditions, but the dicarboxylic acids in the molten state are considered to dissolve them, resulting in the formation of polyaspartic acid. The low nitrogen-introducing ability of these salts is naturally attributed to the low extent of liberation of ammonia from ammonium salts of strong acids.

The ammonium salts of strong acids practically failed to react with fumaric acid, as shown in Fig. 1c, probably due to the fact that both the salts and the acid did not melt at all at the reaction temperatures. Polyaspartic acid was only slightly produced from fumaric acid, even in reactions with ammonium salts of weak acids, such as ammonium carbonate, acetate, formate and phosphate, whose melting points are in the range or below the reaction temperatures. According to the conventional synthesis of monomeric aspartic acid, reactions of fumaric acid with ammonium salts required the presence of ammonia water or excess ammonium acetate in the molten state as a reaction medium. It is noteworthy that only urea reacted with fumaric acid in appreciable yields. The yield-temperature relationship in the reactions of urea with fumaric acid was similar to those with malic and maleic acid.

In order to characterize the obtained polymer, polyaspartic acid prepared from maleic acid and urea at 160 °C was dialyzed using 3 types of membranes, which are capable of removing polymers of molecular weight lower than 2,000, 5,000, or 8,000; the yields of polymers that remained after dialysis proved to be 27, 12, and 2.9%, respectively. Since the yield of aspartic acid in this reaction was about 60%, almost one half of the aspartic acid was shown to be incorporated in a polymer with molecular weight larger than 2,000 (degree of polymerization: ca. 24). The molecular-weight distribution of our polymer might be comparable to that of polyaspartic acid (the average degree of polymerization: 27) produced in the above-stated previous investigation,[5] although a detailed comparison is not possible based on the present data. Furthermore, as compared to the infrared spectrum of commercial poly(α,β)-DL-aspartic acid (Sigma Co., molecular weight: 7,000), that of the polymer obtained here showed characteristic bands at 1765 and 1720 cm^{-1}, indicating the presence of a 5-membered ring imide structure.



Scheme 1.

The results obtained in this study revealed a superior feature of urea in the molten state as a nitrogen source as well as a polar reaction medium in the thermal polycondensation of malic, maleic, and fumaric acids. The formation of polyaspartic acid may be started by the addition of urea or ammonia to a double bond of maleic and fumaric acids (Scheme 1). The double bond of fumaric acid should be saturated prior to the formation of the 5-membered ring anhydride, which is expected to undergo polymerization. The initiation is very likely to be effected by urea, as judged by the practical failure of a reaction of fumaric acid with other nitrogen sources of ammonium salt type. This thermally assisted addition of urea to the unsaturated dicarboxylic acids may provide another advantage of the present molten urea method over the conventional aspartic acid synthesis using molten ammonium salts. Furthermore, isocyanic acid, which is known to be formed from urea or a ureido group, can serve advantageously as a dehydrating agent for the formation of the cyclic anhydride.

Some workers have pointed out that gaseous ammonia could not have been present on the primitive Earth, which had been exposed to the solar wind and UV light before the formation of the ozone layer in the atmosphere,[8] while unsaturated dicarboxylic acids has been found in meteorites[9] as well as in the products of discharge experiments using methane or graphite[10] as carbon sources. In this article, we indicated the possibility that urea could have acted as an efficient nitrogen-introducing agent in fusion reaction with dicarboxylic acids on the primitive Earth. At the same time, we showed that the use of molten urea as a highly polar reaction medium could offer a potential new aspect in synthetic technology.

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1 A part I of a series of studies entitled "on the role of urea in chemical evolution of biomolecules".

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