

EFFECT OF L- AND D-PHENYLALANINE ON CRYSTALLIZATION AND TRANSFORMATION OF L-GLUTAMIC ACID POLYMORPHS

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Introduction

It is known that an additive (impurity) in other amino acids influences the crystallization behavior of the amino acid which is the main product, e.g. the crystal-size distribution and morphology are changed^{1, 2, 6}. In the crystallization of *L*-glutamic acid (*L*-Glu), the ratio of the two polymorphs of α and β (both are orthorhombic) in precipitates is influenced by such impurities that coexist in the fermentation broths. However, the mechanism of this effect is not clear. The additive effect on the crystallization is considered to comprise the effect on the nucleation and that on the growth process. Accordingly, both processes should be separately examined to understand the mechanism^{3, 4}. The additive effect is caused by molecular interaction between the bulk crystal and the additive molecule¹, and so the three-dimensional structure of the additive molecule is expected to be related to the effect.

In this work, the optical isomers *L*- and *D*-phenylalanine (*L*-Phen, *D*-Phen) are used as additives in the crystallization of *L*-Glu, and the additive effect on the ratio of the polymorphs, morphology of the crystals and the transformation rates are investigated.

1. Experimental Procedure

A 50-ml cylindrical jacketed glass vessel facilitated with a impeller was used as a crystallizer. The crystallization of *L*-glutamic acid (*L*-Glu, special grade, Ajinomoto Co.) was carried out in aqueous solutions by rapid cooling (differential crystallization³) and the crystals were precipitated at constant temperature of 318 K under stirring at 180 rpm because, as it was observed in the previous paper³, at 318 K both polymorphs tended to precipitate and the transformation rate was relatively high under these conditions. The initial concentration of *L*-Glu was kept constant at 0.29 mol/l and the concentrations of *L*- and *D*-Phen were changed between 0 and 0.0015 mol/l. The induction period of the nucleation was measured by observing the appearance of fine crystals in each run, and in the course of the crystallization the

changes in composition of precipitates and concentration of solutions with elapsed time were analysed using X-ray diffraction measurement ($\text{CuK}\alpha$ line) and UV spectroscopy³.

2. Results and Discussion

2.1 Precipitation ratio of polymorphs and transformation behavior

It appeared that at 318 K both polymorphs tend to precipitate in a similar manner to that in the previous paper³. However, the precipitation ratio dispersed more than did the results reported in the previous paper³. This may be due to a difference of a slight amount of impurity in the reagents used in the work reported in the previous paper³ and in this work. In **Fig. 1** typical results of the relationship between the composition of α in the precipitates, Y , and elapsed time after the nucleation occurred are shown. It was observed that the precipitation ratio of α in the initial stage of crystallization ($t = 0$), Y increases with *L*-Phen concentration and almost only α was obtained at a concentration of *L*-Phen larger than 2.6×10^{-3} mol/l. The change of Y with elapsed time indicates progress of the transformation from α to β , and it can be seen in **Fig. 1** that the rate decreases remarkably with a slight amount of *L*-Phen. When the concentration was larger than 5.2×10^{-3} mol/l, almost no transformation occurred.

In **Fig. 2** the concentration change in the course of crystallization (**Fig. 1**) is shown. In pure solution a small part flat portion appeared in the decreasing curve, whereas the concentration is very near the solubility of α , and the transformation by solution-mediated mechanism proceeded³. The transformation rate is controlled by the growth rate of β ³. When the concentration of *L*-Phen is 2.6×10^{-3} mol/l, *L*-Glu concentration decreases and attains a constant value at about the solubility of α , where the transformation can proceed at a very slow rate, as shown in **Fig. 1**. The result indicates that the growth rate of β is hindered remarkably by *L*-Phen. At 5.2 and 11.0×10^{-3} mol/l of *L*-Phen concentrations, where almost α alone precipitates (**Fig. 1**), the precipita-

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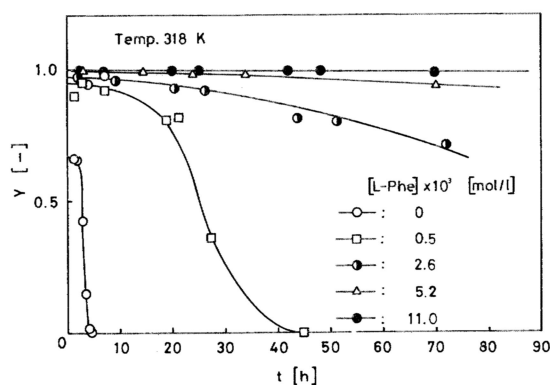


Fig. 1 Dependence of composition of α on elapsed time in crystallization

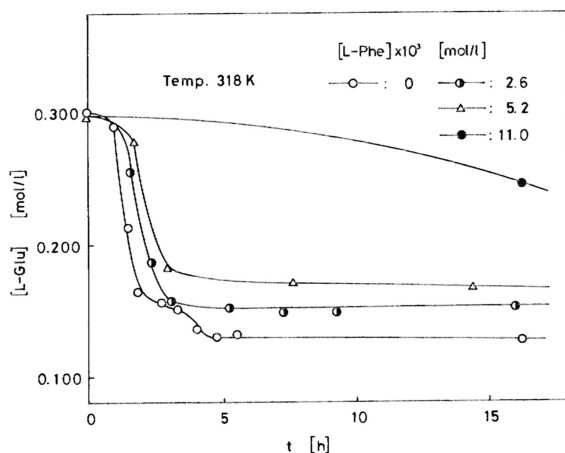


Fig. 2 Concentration change in the course of crystallization

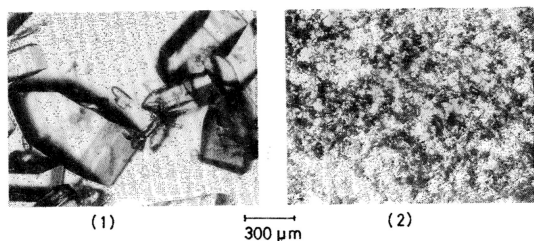


Fig. 3 Microscopic photographs at *L*-Phen concentrations of 5.2×10^{-3} mol/l (1) and 11.0×10^{-3} mol/l (2)

tion rates became much slower. This means that the growth rate of α is also retarded by *L*-Phen. At a concentration of 5.2×10^{-3} mol/l, crystals with different morphology from that observed in pure solution precipitated (Fig. 3 (1)). These facts indicate that the adsorption of *L*-Phen occurs on the specific surface of growing crystals of α . Furthermore, at a concentration of 11.0×10^{-3} mol/l it takes a very long time to precipitate, and fine crystals were finally obtained as shown in Fig. 3 (2). During the crystallization it was observed that a small amount of β of needle-like precipitates on the surface of the solution. Although the mechanism is yet unknown, it is supposed that the nucleation of β may occur advantageously at the surface of the solution.

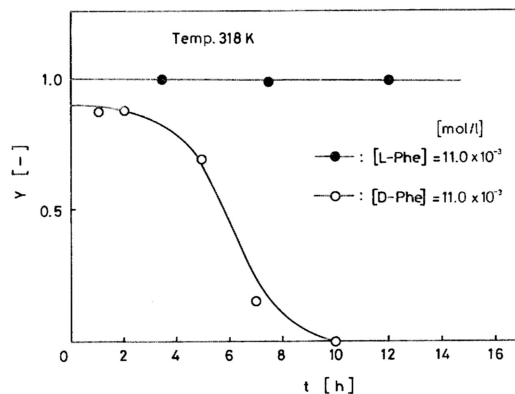


Fig. 4 Comparison of additive effect with *L*- and *D*-Phen on precipitation and transformation of *L*-Glu

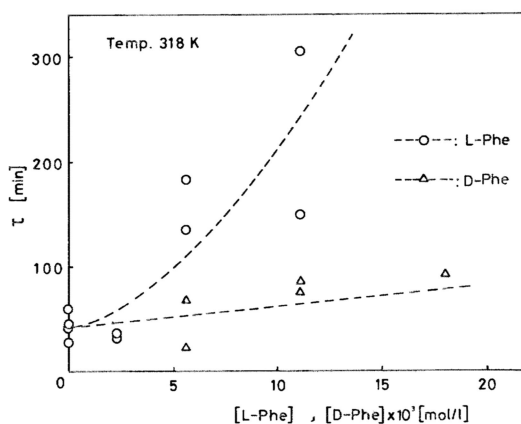


Fig. 5 Influence of *L*- and *D*-Phen on nucleation rates of *L*-Glu

On the other hand, when *D*-Phen was added to the solution up to a concentration of 11.0×10^{-3} mol/l, no influence on either the precipitation ratio of the polymorphs or the transformation behavior was observed, as shown in Fig. 4. In Fig. 4 the effect by the optical isomers is compared and it is shown that the effect is very highly stereoselective. These results may suggest that the *L*-isomer can adsorb on the crystal surface through the common part of amino acid molecules and can hinder the further accumulation of *L*-Glu with the phenyl group which cannot be included in the bulk crystals, however, for *D*-isomer adsorption is not possible because the configuration of the common group is different from that of bulk crystals and no effect on the growth of the bulk crystals is observed¹⁾.

2.2 Influence on nucleation rates

The nucleation rate is considered to be proportional to the reciprocal of the induction period for nucleation⁵⁾. It appeared that the induction period increase with the concentration of *L*-Phen at the constant concentration of *L*-Glu (0.29 mol/l) (Fig. 5). The result indicates that not only the nucleation of β is suppressed by *L*-Phen but also the rate of α is also retarded. On the other hand, in the presence of *D*-Phen almost no influence was observed on the nucleation rate or on the crystal growth of the polymorphs of *L*-Glu.

Conclusion

1. In the presence of *L*-Phen the precipitation of β of *L*-Glu is suppressed and only α tends to precipitate, and the transformation rate of *L*-Glu is much retarded by *L*-Phen. These results indicate that the nucleation and growth of β of *L*-Glu are much more hindered by a small amount of *L*-Phen than those of α of *L*-Glu.
2. On the other hand, *L*-Phen also retards the growth and nucleation rates of α crystal of *L*-Glu, and changes in morphology and crystal size were observed.
3. The additive effect is highly stereoselective, and *D*-Phen scarcely influences either nucleation or growth behavior of the polymorphs of *L*-Glu.

Acknowledgment

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Nomenclature

t	= time	[h]
Y	= mole fraction of α in precipitates	[-]
τ	= induction period of nucleation	[min]

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