Studies on the Polymorphism of L-Glutamic Acid

Part II. Measurement of Solubilities

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The solubilities of L-glutamic acid crystals in α -form and β -form in water were determined, and the results were formulated as follows.

α -form: $\text{Log } S = -0.377 + 0.0174 t$ (t is from 0° to 30°C)

$\text{Log } S = -0.328 + 0.0153 t$ (t is from 30° to 70°C)

β -form: $\text{Log } S = -0.461 + 0.0159 t$

The formula obtained for β -crystals showed a fair agreement with that reported by J. B. Dalton et al.

INTRODUCTION

As reported in the preceding paper¹⁾, α -crystal of L-glutamic acid changes gradually into its β -isomorph, when it stays in the mother liquor as the solid phase in the aqueous solution.

During the course of the author's investigation of the mechanism of the α - β transition, the need for measurement of the solubility-difference in water of the two alternative forms of L-glutamic acid became evident.

The results of solubility measurement are reported in this paper.

EXPERIMENTAL AND RESULTS

1. Materials.

L-Glutamic acid in α -form. To a solution of 400 g of commercial mono-sodium L-glutamate monohydrate in about 4 l of water (dissolved at room temperature), 2/3 equivalent of 35% hydrochloric acid was added promptly.

After stirring for some time, when nucleation of α -crystals took place, the rest of hydrochloric acid (1/3 equivalent) was added slowly under stirring. The deposited crystals were filtered as soon as the

addition of hydrochloric acid was finished. They were washed with water and dried in a vacuum desiccator with sulfuric acid.

N: 9.51%, $[\alpha]_D^{20}$: 31.93° (2N HCl)

β -form: 9.5% (by X-ray method²⁾)

L-Glutamic acid in β -form.

To 28% aqueous solution of mono-sodium L-glutamate monohydrate, 35% hydrochloric acid was added slowly under stirring at 70°C ; L-glutamic acid in β -form was crystallized. After the pH of the solution was adjusted to about 3.2 and cooled to room temperature under stirring, the crystals were filtered and washed with water and dried in a vacuum desiccator with sulfuric acid.

N: 9.51%, $[\alpha]_D^{20}$: 31.95 (2N HCl)

β -form: 100%

L-Phenylalanine. Commercial material of guaranteed grade.

2. Procedures.

The procedures for measurement of solubilities were as follows.

For the measurement near 0°C , Erlenmeyer flasks of 100 ml in an ice-water bath were used, stirring with a magnetic stirrer.

For the measurement at $15\sim 70^\circ\text{C}$, glass tubes of

1) Y. Sakata. This Journal, **25**, 829 (1961).

2) H. Takahashi et al. Presented at 14th Annual Meeting of Chemical Society of Japan held in Tokyo, 1961.

50 ml with a spiral stirrer and a water bath regulated at $15 \pm 0.05^\circ\text{C}$ were used.

After stirring for a definite time, an aliquot was taken with a cotton-stoppered pipett, and the concen-

tration of L-glutamic acid in the filtrate was determined by measuring the total nitrogen by the macro Kjeldahl method. As previously reported, α -crystals as the solid phase in the aqueous solution changed

TABLE I. SOLUBILITIES OF L-GLUTAMIC ACID IN α AND β -FORM

Temperature	Time of Stirring	Solubilities (S) ^{a,b)}			Remarks
		α -form	β -form	in the literature ^{c)}	
$0.5 \pm 0.5^\circ\text{C}$	2.0 hr	0.432*	0.353*		
	3.0	0.432*	0.352*		
15.0 ± 0.05		m. 0.432	m. 0.353	0.348	
	2.0 hr	0.766*			
	3.0	0.764*			
22.0 ± 0.05		m. 0.765			
	0.5 hr	1.00			
	1.0	1.016*			
	2.0	1.014*			
	3.0	1.015*	0.772		
	3.0	1.016			+L-Phe ^{d)}
30.0 ± 0.05		m. 1.015			
	0.5 hr	1.326			
	1.0	1.418*			
	2.0	1.378*			
	2.0	1.400*	1.040		+L-Phe
	3.0		1.042		
35.0 ± 0.05		m. 1.400		1.040	
	0.5 hr	1.620*			
	1.0	1.615*			
	1.5	1.563	1.247		
		m. 1.618		1.252	
40.0 ± 0.05	10 min	1.940*			
	20	1.900*			
	30	1.870			
	2.0 hr	1.933	1.488		+L-Phe
	5.0		1.485		
		m. 1.920		1.508	
50.0 ± 0.05	5 min	2.700			
	10	2.762*			
	20	2.681			
	30	2.552			
	2.0 hr	2.750*			+L-Phe
	4.0	2.250	2.170		
		m. 2.756		2.186	
60.0 ± 0.05	1.0 hr	3.880*			+L-Phe
	2.0	3.840*			+L-Phe
70.0 ± 0.05		m. 3.860		3.169	
	1.0 hr	5.515		4.594	+L-Phe

a) S: g of solute/100 g of water.

b) m: the mean of values with* mark.

c) J.B. Dalton and C.L.A. Schmidt, *J. Biol. Chem.*, **103**, 504 (1933).

d) 0.15% of L-phe. was contained in water.

into β -form gradually, microscopic examination on the crystal forms was necessary whenever the aliquot was taken for the measurement. Since the velocity of transition of α -crystals into β became very large at temperatures higher than 40°C, and the complete transition was recognized after stirring for a few minutes, an accurate measurement of the solubility of α -crystal was difficult at higher temperatures. To assure an enough time for stirring of α -crystals to reach the equilibrium, the addition of 0.15% of L-phenylalanine to water as a transition-inhibitor was tried in these cases.

Needless to say, the estimated amount of total nitrogen in the filtrate was corrected as to the added phenylalanine to calculate the concentration of L-glutamic acid. Furthermore, to check the influence of the presence of L-phenylalanine on the solubility of L-glutamic acid, the solubilities of β -crystals with and without addition of L-phenylalanine were compared.

3. Results.

The results of measurement are shown in the Table I.

DISCUSSIONS

It has been shown from the results obtained that L-glutamic acid in α -form has higher solubilities in water than its β -isomorph at all temperatures examined. As described previously, since α -crystals as the solid phase in the aqueous solution change into β -form rapidly at comparatively high temperatures, in the experiment for α -crystals, the concentration of glutamic acid in the filtrate reaches a maximum value after stirring for some time and decreases gradually, approaching asymptotically the value for β -crystals. For example at 50°C in Table I, the maximum value of the solubility of α -crystals was recorded after stirring for ten minutes, and the value showed a good agreement with the value in the presence of L-phenylalanine, stirring for two hours. At 60 and 70°C, the velocity of the transition

was too large to measure the solubility without an addition of L-phenylalanine. It was believed that the solubility obtained in the presence of L-phenylalanine was nearly valid, because unalternation of the crystal form was confirmed even after two hours' stirring, and the solubility of β -crystals was not influenced by the presence of L-phenylalanine as shown in Table I. The solubilities of L-glutamic acid in α - and β -forms are formulated respectively as follows.

$$\alpha\text{-form: } \log S = -0.377 + 0.0174 t$$

(0~30°C)

$$\log S = -0.328 + 0.0153 t$$

(30~70°C)

$$\beta\text{-form: } \log S = -0.461 + 0.0159 t$$

As seen in the equations for α -form, the solubility curve of α -crystal has a point of inflexion at about 30°C. The solubility of L-glutamic acid was measured previously by J. B. Dalton et al.³⁾, and formulated as follows.

$$\log S = -0.467 + 0.0161 t$$

The results obtained in this experiment as to β -crystals showed a fair agreement with the values obtained by J. B. Dalton.

It can be concluded from the measurement of solubility differences that β -form of L-glutamic acid is the more stable phase as a solid in the aqueous solution, and the mechanism of the transition should be a recrystallization of α -crystals into β -crystals, caused by the differences in the solubilities thereof.

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3) J.B. Dalton and C.L.A. Schmidt, *J. Biol. Chem.*, **103**, 504 (1933).