Studies on the Behaviors of Impurities on the Crystallization of L-Glutamic Acid

Part II. Structure of Crude Crystals of L-Glutamic Acid

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The structure of crude crystals of L-glutamic acid was conjectured from a phenomenon, the disintegration of the crude crystals when they were dipped in the saturated solution of L-glutamic acid of pH from 5 to 7, and from the measurement of the actual surface area on the crude crystals by means of determination of the adsorption equilibrium of humic acid; and it was considered that the crude crystals had a mosaic structure consisting of fine crystals, for example those of size of about $1\ \mu$ when the purity of crystals was about 97%.

INTRODUCTION

In the preceding paper¹⁾, it was reported that amino acids which co-existed in the supersaturated solution of L-glutamic acid were easily taken into the crystals of L-glutamic acid on the crystallization. During the course of the investigation of this phenomenon, it has been found that the crude crystals of L-glutamic acid which were crystallized from the hydrolyzates of vegetable proteins, and contained a few percent of impurities such as amino acids, humic acid etc., disintegrated into fine fragments spontaneously, when they were dipped in the saturated solution of L-glutamic acid (in α -form) of pH from 5 to 7. By a close pursuit of this phenomenon, it is expected to get a clue to clarify the mechanism of contamination of crystals on the crystallization of L-glutamic acid. Furthermore a measurement of the actual surface area on the crystals has been executed with application of the adsorption equilibrium of

humic acid between the crystals and the solution.

EXPERIMENTAL AND RESULTS

A. The Disintegration of the Crude Crystals in the Saturated Solution.

1. Materials.

i) The crude crystals of L-glutamic acid.

The hydrochloric acid hydrolyzate of wheat gluten was concentrated and saturated with hydrogen chloride, and after standing for two weeks, the crude crystals of L-glutamic acid hydrochloride were filtered, and dissolved in water. To this solution the aqueous solution of sodium hydroxide was added slowly until pH 3.2 was reached, and the crude crystals of L-glutamic acid in α -form were separated. The sizes of the crystals were from $30\ \mu$ to $200\ \mu$ and the crystals appeared to be single crystals in spindle shape.

The assay of the crude crystals is shown in Table I. The amino acids were determined by microbioassay

TABLE I. ANALYSIS OF CRUDE L-GLUTAMIC ACID

N	9.48%	L-Leu.	0.12%
$[\alpha]_D^{20}$	30.65 (2 N HCl)	L-Asp.	0.20
Chloride (Cl)	0.10%	L-Lys.	0.05
Humic acid	0.17	L-Met.	0.24
L-Ala.	0.09	L-Val.	0.14

1) Y. Sakata, T. Horikawa and K. Takenouchi, This Journal, **25**, 921 (1961).

using *Lactobacillus*, and the humic acid by colorimetry. Humic acid is a black-brown pigment which is produced at the time of acid hydrolysis of proteins. It was easily adsorbed by activated carbon when the solution of crude glutamic acid was treated with it. This was recovered from the adsorbent by eluting with diluted solution of sodium hydroxide and neutralizing the eluent with hydrochloric acid to about pH 5. The precipitated humic acid was washed completely with water, and dried in a vacuum desiccator with sulfuric acid. The material which was obtained here and contained 4.56% of nitrogen was used for preparation of the standard solution of humic acid for analysis.

ii) Mono-sodium L-glutamate monohydrate.

The commercial material was recrystallized from water by concentration under reduced pressure.

iii) L-Glutamic acid in α -form.

To an aqueous solution of mono-sodium L-glutamate (about 0.5 Mol/l), hydrochloric acid was added promptly and the solution was made to pH 3.2; L-glutamic acid crystals mainly in α -form were obtained.

N: 9.51% $[\alpha]_D^{20}$ 31.90 (2N HCl) β -form: 11.5%

2. Procedure.

Fifty grams of the crude crystal of L-glutamic acid was dipped in each of the saturated solutions of α -crystals which were prepared by the procedures shown in Table II, for one to seven hours under stirring from time to time. The treated crystals were filtered and washed with a small amount of water, and sieved in methanol with a net of 200 meshes (the opening of mesh is 74μ) and the percentage of the crystals which had passed through the net was measured. The contents of humic acid in the treated crystals were determined at the same time. The experiment was designed according to the two ways lay-out, where the factors were as follows. The pH of the saturated solution: from A₁ to A₆ in Table II.

TABLE II. PREPARATION OF SATURATED SOLUTIONS FOR DIPPING TREATMENT

No.	Procedure for preparation			pH
A ₁	100 ml of water	0 g	Saturated with α -Glu.	3.20
A ₂	"	5	"	5.05
A ₃	"	15	"	5.40
A ₄	"	30	"	5.85
A ₅	"	50	"	6.10
A ₆	"	70	"	6.30

* Mono-sodium L-glutamate monohydrate.

Time of dipping: B₁, B₂, B₃ and B₄ are 1, 3, 5 and 7 hours respectively.

FIG. 1. Disintegration of Crude Crystals.

—●— 1 hr. -○- 3 hr.
-□- 5 hr. -△- 7 hr.

FIG. 2. Humic Acid Content in the Treated Crystals vs pH^a.

a) The confidence interval illustrated is that of the level of 95%.

TABLE III. ANALYSIS OF VARIANCE FOR FINE FRAGMENT %

Factor	ϕ	SS	V	F ₀	F _(0.01)
A	5	2970	594	9.7**	4.56
B	3	1725	573	9.4**	5.42
E	15	914	61		

TABLE IV. ANALYSIS OF VARIANCE FOR HUMIC ACID CONTENT

Factor	ϕ	SS	V	F ₀	F _(0.01)
A	5	1216.7	243.3	13.5**	4.56
B	3	35.9	12		
E	15	271.0	18

FIG. 3. Absorption Spectrum of Humic Acid Soln.
Humic acid: 10 mg/l in 0.1 N NaOH aq. soln.
Cell: 10 mm width

FIG. 4. Relation Between A_s and pH.
Humic acid: 5.4 mg/l
Wavelength: 420 mμ
Cell: 10 mm width

3. Results.

The experimental results are shown in Figs. 1 and 2, and in Tables III and IV (variance analysis).

B. Measurement of the Surface Area on the Crude Crystals.

1. Materials.

i) The solution of crude L-glutamic acid hydrochloride.

The same solution as prepared in the preceding experiment (A) was used, and the assay is shown in Table V (The amino acids were determined by microbioassay). The solution was treated with activated carbon, and several solutions containing humic acid from 0.02 to 0.226 g/dl were prepared.

TABLE V. ANALYSIS OF CRUDE L-GLUTAMIC ACID HYDROCHLORIDE SOLUTION

Total-N	3.53 g/dl	L-Asp.	0.91 g/dl
NH ₃ -N	1.46	L-Lys.	0.51
L-Glu.	15.5	L-Met.	0.43
L-Ala.	0.40	L-Val.	1.09
L-Leu.	1.60	Humic acid	0.226

ii) L-Glutamic acid in α -form.

The aqueous solution of commercial mono-sodium L-glutamate monohydrate of about 0.5 Mol/l was neutralized rapidly to pH 3.2 with hydrochloric acid to crystallize L-glutamic acid. The crystals obtained were filtered, washed with water, dried in a vacuum desiccator containing sulfuric acid and pulverized in a mortar.

N: 9.51% $[\alpha]_D^{20}$ 31.92 (2N HCl) surface area: 10³cm²/g

The measurement of surface area was executed as follows. The powdered crystals were sieved into the next 8 fractions by means of standard sieves; +50, -50+100, -100+150, -150+200, -200+250, -250+325, -325+400, -400 meshes. And then the average size of powdered crystals in each fraction was measured under a microscope by means of micrometer, and the surface area was calculated approximately as cube. And the total surface area of the powdered crystals was obtained by summing up the surface area of them in each fraction.

2. Procedures.

(1) The crystallization of L-glutamic acid from each solution of crude L-glutamic acid hydrochloride.

The solutions of crude L-glutamic acid hydrochloride which were prepared by the procedure described above (B-1) and contained various quantities of humic acid were warmed at 60°C; then 20 percent sodium hydroxide aqueous solution was added rapidly to each of the solutions until pH 3.2 was reached under constant stirring to crystallize L-glutamic acid. The crude L-glutamic acid crystallized was filtered, and washed with 50 ml of water and 50 ml of ethanol, after the slurry was left to stand for one day at room temperature. The content of humic acid in the crude crystals was measured after the crystals were dissolved into a diluted solution of sodium hydroxide, and the concentration of humic acid in the mother liquor was determined at the same time.

(2) Measurement of the adsorption equilibrium of humic acid between the crystal surface and the solution.

The mother liquors obtained in the procedure (1), were used for the measurement. To 10 ml of each mother liquor, 5.0 g of the powdered α -crystals of L-glutamic acid was added, and the mixture was stirred for a few minutes, and the crystals were removed with a filter paper previously dried. The differences in transmissions at $460\text{m}\mu^*$ between the treated and the untreated mother liquors were measured directly without dilution, the untreated mother liquors being used as blanks of the measurement of transmissions. The quantity of humic acid adsorbed on the surface of crystals was calculated from the transmission difference determined above.

(3) Determination of humic acid.

The absorption spectrum of humic acid solution is shown in Fig. 3. The optical density of humic acid solution is effected by the change of its pH, i.e., the optical density decreases with the reduction of pH. The relations between the optical density and pH are shown in Fig. 4. It was recognized that the extinction of humic acid obeyed Beer's law at the wavelength from $380\text{m}\mu$ to $500\text{m}\mu$ as shown in Fig. 5.

FIG. 5. Relation Between A_s and pH.

Humic acid soln. in 0.1 N NaOH aq. soln.
Cell: 10 mm width

Because the absorption of the other colorless substances such as amino acids might overlap, the determination of humic acid in the region of the shorter wavelength is not proper. The measurement of transmission was carried out in principle after a sample was diluted so that the transmission became 20 to 70 percent when it was more concentrated.

Hitachi EPU-2 Photo-electric Spectrophotometer and

* Although the measurement of transmission can be executed at any wavelength from $380\text{m}\mu$ to $500\text{m}\mu$, a very wide opening of the slit became necessary at the shorter wavelength than $460\text{m}\mu$ when the measurement of the difference in transmission between two comparatively concentrated solutions of humic acid was made directly.

cells of 10 mm were used for the measurement of absorbance in this experiment. Though it became difficult to measure the extinction of the more concentrated solutions directly, it was assumed in order to estimate the quantity of humic acid adsorbed on the crystal surface from the measured difference in transmission that in the more concentrated solution of humic acid, Beer's law could be applied. Needless to say, the value of the extinction was corrected by the curve exemplified in Fig. 4 in accordance with pH of the solution to be measured. In other concentrations of humic acid, the same correction factor could be applied. The humic acid is not a unity substance, and considered to be a mixture of many analogous substances, because the IR spectrum of humic acid showed very diffused peaks, and chromatography on silica gel gave several bands. Therefore, the value thus obtained by colorimetry cannot have a very strict meaning as to the humic acid.

3. Results.

(1) The results of measurement of humic acid contents in the crystals and in the mother liquors which were obtained by the neutralization of the crude L-glutamic acid hydrochloride solution containing various quantities of humic acid are shown in Table VI.

The purities of the crude crystals obtained were from 96.5 to 97.5, when they were determined by enzymatic method using L-glutamic acid decarboxylase.

TABLE VI. HUMIC ACID CONTENTS IN THE CRYSTALS AND THE MOTHER LIQUORS

Content of humic acid	
in crude crystals	in mother liquor
0.235%	0.183 g/dl
0.206	0.163
0.181	0.148
0.156	0.138
0.139	0.118
0.112	0.098
0.082	0.087
0.066	0.072
0.032	0.058
0.027	0.040
0.015	0.021

(2) The adsorption of humic acid on the surface of powdered purified α -crystals of L-glutamic acid is shown in Table VII.

TABLE VII. ADSORPTION OF HUMIC ACID ON THE CRYSTAL SURFACE OF L-GLUTAMIC ACID

Humic acid concn. in the mother liquor before adsorption	Difference of humic acid concn. in the mother liquor after adsorption	Humic acid ^{a)} adsorbed on the crystals
0.183 g/dl	2.6 mg/dl	5.2 mg/100 g of crystals
0.163	1.8	3.6
0.118	1.4	2.8
0.072	0.54	1.1
0.021	0.23	0.46

a) Calcd. from the value in the left column.

DISCUSSIONS

1. It has been indicated from the experiment (A) that the crude crystals of L-glutamic acid disintegrated into very fine fragments spontaneously when they were dipped in the saturated solution of L-glutamic acid of pH from 5 to 7. This disintegration would not be caused by mechanical destruction by stirring, because the stirring was only at intervals and gentle. Concurrently with the disintegration of the crude crystals, the diffusion-out of humic acid from the crude crystals was observed, and a correlation between the degree of disintegration and the reduction of humic acid content in the treated crystals have been seen from the result of the experiment (A). Namely the diffusion-out of humic acid and the disintegrations of the crude crystals were not remarkable at pH from 3.2 to 5.0, but a very large amount of fine fragments were produced and the contents of humic acid in the treated crystals were reduced considerably at pH from 5 to 7. This phenomenon suggests that the crude crystal consists of many fine fragments with adsorbed impurities such as humic acid on their surfaces. A peak was observed in each of the curves shown in Fig. 1, at about pH 6, and it is considered that the formation of this peak would be concerned with viscosity of the solution prepared for the dipping treatment, but a more detailed investigation should be carried out

on this point. It cannot be considered that the disintegration was caused by recrystallization of the crude crystals in α -form into β -crystals because the transition of the crystals could not be recognized by the X-ray method.

2. The relations between the content of humic acid in the crude crystals of L-glutamic acid and the concentration of humic acid in the mother liquors which are shown in Table VIII show the adsorption equilibrium of humic acid, assuming that the contamination of crystals with humic acid was caused by adsorption on the crystal surfaces in the process of crystallization. As the adsorption equilibrium of humic acid on the crystal surface of a known area has been measured in the experiment (B) and shown in Table VII, the actual surface area on the crude crystals can be conjectured, and the calculated results are shown in Table VIII. The estimated values of the actual surface areas on the crude crystals which were calculated from the equilibrium values at various concentration of humic acid have shown a relatively good agreement and gave about $5 \times 10^4 \text{ cm}^2/\text{g}$ of crude crystals. In the method applied here, errors cannot be avoided to some extent because the adsorption on the crystallizing surface would be more dynamic, and moreover, the concentrations of humic acid and glutamic acid vary continuously in the process of crystallization.

TABLE VIII. ESTIMATION OF THE ACTUAL SURFACE AREA OF CRUDE CRYSTALS

Humic acid ^{a)} content in crude crystals	Humic acid ^{a)} concn. in mother liquor	Equilibrated ^{b)} value for the left	Estimated ^{c)} value of actual surface area
2.35 mg/g	0.183 g/dl	0.052 mg/ $10^3 \text{ cm}^2(\text{g})$	$4.5 \times 10^4 \text{ cm}^2/\text{g}$
2.06	0.163	0.036	5.7
1.39	0.118	0.028	5.0
0.66	0.072	0.011	6.0
0.15	0.021	0.0046	3.3

a) cf. Table VI.

b) cf. Table VII.

c) Calcd. from the values in the 1st and 4th columns.

As the adsorption equilibrium will be more dominant at the side of solution when L-glutamic acid in the mother liquor is more concentrated, the larger surface area than that obtained here may be expected. As the crude crystals obtained in the experiment (B), were spindle-shaped and of sizes from $50\ \mu$ to $100\ \mu$, the apparent surface area can be calculated to be about $10^3\ \text{cm}^2/\text{g}$, while the actual surface area is shown to be several tens times as large as the apparent one.

3. From the discussions described above, it can be concluded that a crude crystal of L-glutamic acid consists of many little crystalets, although it seems to be a single crystal, and the size of a crystalet must be about $1\ \mu$ to get the surface area of $5 \times 10^4\ \text{cm}^2/\text{g}$, and it will not be far smaller than this value, because the X-ray diffraction pattern of the crude crystals showed undiffused normal

peaks. It has been recognized by many investigators that a crystal often might have a mosaic structure caused by dislocations or adsorption of impurities or other factors. As for the contamination of the crystals of L-glutamic acid, the mechanism can be well explained by assuming the mosaic structure; i.e., when the content of impurities in the crude crystals is about 3% as in the case of the experiment (B) and it is assumed that the impurities are amino acids of molecular weight of 150, and of spacing area of $20\ \text{\AA}^2/\text{molecule}$; our results show the adsorption layers of the amino acids on the crystalet surface are most probably several-molecular.

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