Studies on the Polymorphism of L-Glutamic Acid

Part VII. Enthalpy Differences between the α - and β -Crystals

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The enthalpy differences between the α - and β -crystals of L-glutamic acid were measured and $\Delta H_{\alpha-\beta}$ of -75, -95, 8 and 27 ± 20 cal./mol. were obtained at temperatures of 5, 15, 30 and 40°C, respectively. From this result, it was found that the α -form is the more stable form in solid phase at temperatures lower than 30°C and *vice versa* when higher than 30°C, though the α -form had been found to be the less stable form as the saturating body in the aqueous solution in all range of temperature.

INTRODUCTION

As reported in the preceding papers^{1,2)}, the β -form of L-glutamic acid was found to be the more stable form as the saturating body in the aqueous solution, when judged from the facts that the solubility of the β -form in water is less than that of the α -form, and the α -form as the saturating body transforms into the β -form in the aqueous solution. In this experiment, the enthalpy differences between the dimorphs were measured at several points of temperature to know which is the more stable form in solid phase. Calorimeters such as a twin type³⁾, isothermal type⁴⁾ etc. were devised for the purpose of measurement of small energy difference between polymorphs or the others. After examination of these apparatus and procedures, it was decided here to apply a more simple apparatus instead of such elaborate and delicate ones.

EXPERIMENTAL AND RESULTS

1. Materials.

L-Glutamic acid in α -form. The aqueous solution

of L-glutamic acid, the concentration of about 2%, was left for standing in an open air, allowing natural evaporation. The α -crystals which contained about 4% of the β -form were separated and dried in a vacuum desiccator over silica gel. N 9.51%, $[\alpha]_D^{20} + 31.95$ (2 N HCl).

L-Glutamic acid in β -form. The aqueous solution of commercial monosodium L-glutamate monohydrate, the concentration of 20%, was warmed at 70°C. Then, 35% hydrochloric acid was added slowly with constant stirring until the pH of the solution reached to 3.2, the isoelectric point of glutamic acid. The β -crystals were crystallized and then filtered, washed with water and dried in an air bath at 90°C. No α -form was recognized. N 9.52%, $[\alpha]_D^{20} + 31.93$ (2 N HCl). Sodium hydroxide, commercial reagent of guaranteed grade.

2. Apparatus.

The calorimeter applied in this experiment is sketched in Fig. 1. Two thermometers, one for higher temperatures and the other for lower temperatures, were used to cover the temperature rising of ten and several degrees due to the heat of neutralization after dissolution of the crystals.

3. Procedure.

Measurements were carried out with one apparatus, applying alternately both the α and β -forms. Procedure was as following. Into the Dewar vessel, 300.0 g of 3 N caustic soda aqueous solution, which was previously held at 20.0°C in a thermostat, was weighed,

1) Y. Sakata, *This Journal*, **25**, 835 (1961).

2) Y. Sakata, *ibid.*, **26**, 355 (1962).

3) D.F. Evans and R.E. Richards, *J. Chem. Soc.*, **144**, 3932 (1952).

4) J.H. Beynon and A.R. Humphries, *Trans. Faraday Soc.*, **51**, 1065 (1955).

FIG. 1. Calorimeter.

- a, b. Beckmann thermometers (scale 1/100°C)
- c. Stirrer (glass)
- d. Dewar vessel (500 ml)
- e. Stopper (cork)
- f. Heater (manganine, filled with Diflo-oil)
- g. Seal (liquid paraffin)
- h. Wood container

and stirred until the temperature reached to a constant value. When an attainment of equilibrium was confirmed, 100.0 g of the crystals of L-glutamic acid in either α or β -form, whose temperature was previously controlled at a definite temperature, was thrown as quickly as possible into the vessel. After jar was stoppered, contents inside was stirred, and at the same time, a stopwatch was started to read the scale of thermometer at intervals of 15~30 seconds. The scale readings were plotted on a graph. The crystals to be treated were held in a thermostat, whose temperature was controlled selectively at 5°, 15°, 30° or 40° $\pm 0.1^\circ\text{C}$ and after their temperature attained to equilibrium, they were taken as quickly as possible out of the thermostat for measurement. The calorimeter was set in a wood vessel and put in an open atmosphere, the variation of whose temperature during the period of measurements was within $\pm 0.5^\circ\text{C}$.

4. Results.

The comparison of scale readings between the α

and β -crystals was exemplified in Fig. 2, when the temperature of the crystals was 5.0°C .

The enthalpy difference is calculated according to the following equation

$$\Delta H_{\alpha-\beta} = \frac{(C_1 + C_2)\Delta T_1 - (C_1 + C_3)\Delta T_2}{nP_\alpha}, \quad (1)$$

where

$\Delta H_{\alpha-\beta}$: enthalpy difference between the α and β -crystals, i.e., $H_\alpha - H_\beta$ (cal./mol.)

C_1 : heat capacity of the calorimeter (255 cal/°C)

C_2 : heat capacity of 300 g of 3 N caustic soda soln (268 cal/°C)

C_3 : heat capacity of the solution prepared by dissolving 100 g of glutamic acid into 300 g of 3 N caustic soda soln. (298 cal/°C)

ΔT_1 : temperature difference before addition of the crystal, ($T_\beta - T_\alpha$)

ΔT_2 : temperature difference after addition of the crystal, ($T_\beta - T_\alpha$)

n : mole number of L-glutamic acid applied

P_α : Content of α -form in the α -crystals applied (0.96)

Here, estimation will be tried as to the error in this measurement.

1) Error on weighing the crystals of L-glutamic acid: $\pm 0.2\%$, corresponding to $\pm 0.004^\circ\text{C}$ of ΔT_2 .

2) Error on weighing the caustic soda solution: $\pm 0.1\%$, corresponding to $\pm 0.008^\circ\text{C}$ of ΔT_2 .

3) Temperature difference between the α and β -crystals: $\pm 0.1^\circ\text{C}$, corresponding to $\pm 0.004^\circ\text{C}$ of ΔT_2 .

4) Difference in operating time for adding the crystals: even though care was taken so that the operation for adding the crystals is carried out as quickly as possible, difference of some extent in operating time is not avoidable. A large portion of the error in this measurement is caused by this, especially in estimating ΔT_2 , as understood from the exemplified result in Fig. 2.

When it is assumed that the time difference is 10 seconds at most, it corresponds to $\pm 0.015^\circ\text{C}$ of ΔT_2 .

5) Difference in the amount of air accompanied by the crystals: as the β -crystals are more bulky than the α -crystals, more quantity of air is added accompanying at the time of addition of the crystals. Although this effect becomes large as the temperature of crystals goes apart from 20°C , the error less than $\pm 0.002^\circ\text{C}$ in ΔT_2 is estimated even when the temperature difference is 20°C .

Summarizing the errors considered above, the au-

FIG. 2. Estimation of ΔT_1 and ΔT_2 .

thors found that all round error in this measurements would occupy $\pm 0.02^\circ\text{C}$ of ΔT_2 corresponding to ca. 20 cal/mol as the enthalpy difference.

The enthalpy differences calculated from the observed values are shown in Table I.

TABLE I. OBSERVED ENTHALPY DIFFERENCES

Temp.	ΔT_1	ΔT_2	$\Delta H_{\alpha-\beta}$
5°C	-0.03°C	0.06°C	$-75 \pm 20 \text{ cal./mol.}$
15	-0.01	0.10	-95
30	+0.03	0.02	8
40	-0.02	-0.05	27

DISCUSSIONS

The enthalpy difference between the α - and β -crystals can be obtained as the difference in heat of solution between these dimorphs, and the heat of solution can be calculated theoretically according to Brönsted equation⁵⁾ (Eq.-2) which was derived from van't Hoff

equation.

$$\Delta H_s = 2RT^2 \frac{\partial \ln m}{\partial T} \left(1 + \frac{\partial \ln \gamma}{\partial \ln m} \right) \quad (2)$$

where

ΔH_s : heat of solution

R : gas constant

T : absolute temperature

m : solubility mol./1000 g solvent

γ : activity coefficient; as for glutamic acid, following eq. was given by Hoskin et al⁶⁾.

$$\ln \gamma = -0.539 \ln m - 5.020. \quad (3)$$

As the solubilities of L-glutamic acid in α - and β -form are given by the following equations¹⁾;

α -form:

$$\text{Log } S = -0.377 + 0.0174 t \quad (t \text{ is from } 0^\circ \text{ to } 30^\circ\text{C})$$

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

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

the values of $\ln m/T$ are calculated as 0.04 (α -form t : $0^\circ \sim 30^\circ\text{C}$), 0.035 (α -form t : $30^\circ \sim$

5) J.N. Brönsted, *Z. physik. Chem.*, **100**, 139 (1922), J.B. Dalton and C.L.A. Schmidt, *J. Biol. Chem.*, **103**, 549 (1933).

6) W.M. Hoskin, M. Randall and C.L.A. Schmidt, *J. Biol. Chem.*, **88**, 215 (1930), J.B. Dalton and C.L.A. Schmidt, *ibid.*, **103**, 549 (1933).

70°C) and 0.037 (β -form). The heats of solution of the α - and β -crystals are estimated according to eq. (1), and the enthalpy differences are calculated. The results of calculation are shown in Table II.

TABLE II. CALCULATED VALUES OF HEAT OF SOLUTION AND ENTHALPY DIFFERENCE

Temp.	$\Delta H_{s(\alpha\text{-form})}$	$\Delta H_{s(\beta\text{-form})}$	$\Delta H_{\alpha-\beta}$
0°C	5550 cal/mol	5150 cal/mol	-400 cal/mol
10	5870	5450	-420
40	6340	6670	330
70	7610	8000	390

When the theoretically obtained values in Table II are compared with the observed values in Table I, it is known that the former values are larger than the latter, but an agreement is recognized in the respect that the signs of the values are inversed around 30°C in both cases. That is to say, the α -crystal has a lower energy than the β -crystal in the range of temperatures lower than 30°C. Hence, the α -crystal is the more stable form in solid phase, although it is apparently the less stable form as the saturating body in the aqueous solution as discussed in Part III²⁾. In the range of temperatures higher than 30°C, the observed enthalpy differences were very small, but it seemed that the β -crystal has a lower energy than the α -crystal. The densities of the α - and β -crystals are 1.535 and

1.570 (g/cm³) respectively. Therefore, the β -crystal is packed denser with the molecules of glutamic acid than the α -crystal, and so far as consideration is made on this point, the β -crystal is probably assumed as the more stable one in solid phase. The obtained result is contrary and this discrepancy will be caused by the other intermolecular affinities, e.g. electrostatic one due to zwitter ion formation, than van der Waals force. In any case, the observed enthalpy differences are so small that they can not be a driving force for the transformation in solid phase at any temperature.

As to the gap between the calculated and observed values and the inversion of the values, problems remain for closer pursuit. There may be some margin for investigation regarding the solubility measurement of the α -crystal in water because of its comparatively rapid transformation as mentioned in Part II¹⁾. Furthermore, a possibility is considered that L-glutamic acid has another unknown modification.

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