

# Enthalpy Change of Amylose Triiodide Complex Formation

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The enthalpy change ( $\Delta H$ ) of amylose-triiodide complex formation under the presence of excess KI was directly determined by calorimetric measurement at 25°C for amylose with a degree of polymerization of 50, 150 and 2500. The  $\Delta H$  depended of the degree of saturation for bound triiodide ions. The average number of created hydrogen bonds per glucose residue through the coloring process was estimated on the basis of the following consideration: The heat of evolution comes from excess energy as the difference between energy from the overdone conformational change of amylose lattice due to the cooperative formation of hydrogen bonds and the energy required for the intrinsic electronic change of bound iodide ions. As a result, the number of created hydrogen bonds increased with the DP.

**Enthalpie-Änderung der Amylose-Triiodidkomplex-Bildung.** Die Enthalpie-Änderung ( $\Delta H$ ) der Amylose-Triiodidkomplex-Bildung in Gegenwart eines Überschusses von KI wurde direkt durch kalorimetrische Messung an Amylosen mit Polymerisationsgraden von 50, 150 und 2500 bestimmt. Das  $\Delta H$  hing von dem Sättigungsgrad für gebundene Triiodidionen ab. Die durchschnittliche Anzahl der durch den Anfärbungsprozeß gebildeten Wasserstoffbindungen je Glucoserest wurde auf der Grundlage der folgenden Überlegung bestimmt: Die Bildungswärme kommt von der Überschussenergie der überlagernden Konformationsänderung des Amylosegitters als Folge der gleichzeitig stattfindenden Bildung von Wasserstoffbindungen und der für den inneren Elektronenwechsel an den gebundenen Iodidionen erforderlichen Energie. Insgesamt erhöhte sich die Zahl der gebildeten Wasserstoffbindungen mit dem Polymerisationsgrad.

## 1 Introduction

A number of studies have been made on amylose-iodine complex [1–7]. Handa and Yajima [8, 9] explained blueing of the complex as coming from the dimeric exciton-coupling of bent and twisted  $I_3^-$  ions among the adjacent ions in amylose lattice in the presence of excess KI. Therein, they proposed that the molecular orbitals of the ion are subjected to the perturbation through the mutual charge-transfer (CT) processes which occur from the ion to OH groups and compensatively from glycosidic oxygen atoms to the ion in the amylose lattice.

Enthalpy change ( $\Delta H$ ) of the complex formation has been estimated variously by several workers from temperature dependence of equilibrium constants obtained by spectrophotometric, potentiometric, or amperometric methods. Those given values of the  $\Delta H$  range from  $-11$  to  $-20$  kcal/mole of bound iodine [10–12]. Cronan and Schneider [13] expected that for a cooperative system the isosteric heat of binding was a function of the degree of saturation of bound iodine ( $q$ ) as well as from temperature and estimated the enthalpy of binding at  $q = 0.5$  as  $-16.6$  and  $-12.8$  kcal/mole for  $I_2$  and  $I_3^-$  as the free species, respectively. However, the actual value of the  $\Delta H$  from direct calorimetric measurement has been scarcely reported so far, except the one as reported by Takahashi and Ono [14]. They found that within the experimental error, no variation in heat data was obviously observed for the change in KI concentration and that the  $\Delta H$  was determined to be  $-16.8$  kcal/mole of  $I_2$ , on the average. Therefore, it seems likely that a clear-cut interpretation of the  $\Delta H$  has not yet been established. Hence, the objective of this study is to determine directly the  $\Delta H$  of amylose-triiodide complex formation in the presence of excess KI and also to probe the relationship between the  $\Delta H$  and the conformational change of amylose lattice through the coloring of bound triiodide ions ( $I_{3,b}^-$ ) in amylose.

## Experimental

### 2.1 Materials

Amyloses with average degree of polymerization (DP) of 50, 150 and 2500 were used. Amyloses with DP 50 and 150 were of

the commercial grade from Hayashibara Co., Ltd. Amylose with DP 2500 was isolated from potato starch by Schoch's method [15] and the molecular weight was determined by viscometry [16].

### 2.2 Methods

**Preparation of Amylose Solution:** Amylose 100 mg was dissolved into 3 ml of 1.0-N KOH solution. Then, the solution was neutralized through an Amberlite column and diluted to 0.1% solution with doubly distilled water.

**Apparatus:** The calorimeter used was either CM-204H twin type conduction microcalorimeter or RCM-1F automatic type rotary microcalorimeter from Applied Electric Lab., Ltd. The measurements of the absorption and circular dichroism (CD) spectra were made using Hitachi EPS-3T type spectrophotometer and Jasco J-20 type spectropolarimeter, respectively.

**Measurements:** The calorimetric measurements for DP 50 and 150 were made by use of the CM-204H type calorimeter at 25°C; 30 ml of amylose solution containing  $3.0 \times 10^{-2}$  g of amylose was put in the reaction cell (80 ml). An ampoule with 1 ml of KI- $I_2$  solution containing  $6.0 \times 10^{-2}$  g KI and various amounts of  $I_2$  ranging from 1.0 mg to 6.6 mg was attached to a break-ampoule type attachment. After confirming that the equilibrium state of the system was attained, mixing of KI- $I_2$  solution with amylose solution was performed by destructing the ampoule with the shockpole in the attachment. In order to cancel contributions of the heat of destruction of the ampoule and the heat of dilution of the KI- $I_2$  solution, 30 ml of water in the cell and 1 ml of KI- $I_2$  solution in an ampoule were in the reference side under the same conditions as those in the sample side.

The calorimetric measurement for DP 2500 was made by using the high precision RCM-1F type calorimeter at 25°C: 12 ml of amylose solution containing  $3.0 \times 10^{-3}$  g amylose and 12 ml of KI- $I_2$  solution containing 0.12 g KI and various amounts of  $I_2$  ranging from 0.13 mg to 0.72 mg were placed on both sides of partition-plate in an especially made cell (30 ml). After the system attained the thermal equilibrium, mixing of both solutions was performed by rotation of the cell. Contribution of the heat of dilution of the KI- $I_2$  solution was canceled by means of mixing of water and KI- $I_2$  solution in the

reference side under the conditions as those in the sample side. Preliminary measurement revealed that the heat of dilution of amylose solution was negligible. Absorption and CD spectra were measured for the reaction solutions at room temperature after the calorimetric measurements.

### 3 Results

The bound iodine-species is triiodide ion under the present concentration of KI [13]. Hence, the complex will be called "amylose-triiodide complex", hereafter.

Figure 1 shows the change of the calorific values for the binding of triiodide ions by DP 50 and 150 with the concentration of  $I_2$  in the system. The calorific value for each DP increased gradually with  $I_2$  concentration. Figure 2 also shows the similar relation for DP 2500. The calorific value increased stepwise with  $I_2$  concentration in the system. It should be noted that every calorific value was reproducible within about  $\pm 5\%$ .

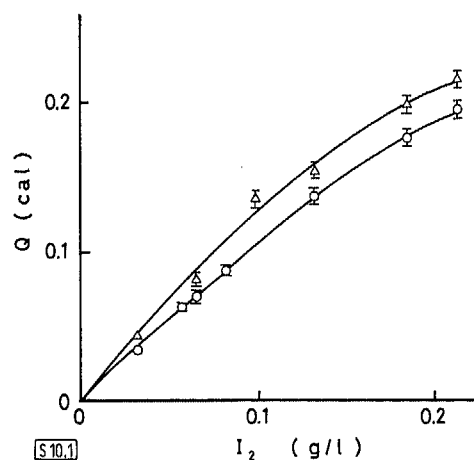


Figure 1. Change of the calorific value,  $Q$ , at 25°C with  $I_2$  concentration for amylose-triiodide complex formation in the reaction of  $3.0 \times 10^{-2}$  g amylose in 30 ml various amounts of  $I_2$  and  $6.0 \times 10^{-2}$  g KI in 1 ml. The concentration of  $I_2$  in the reaction solution of 31 ml is expressed by the unit of g/l. DP: ○, 50; △, 150.

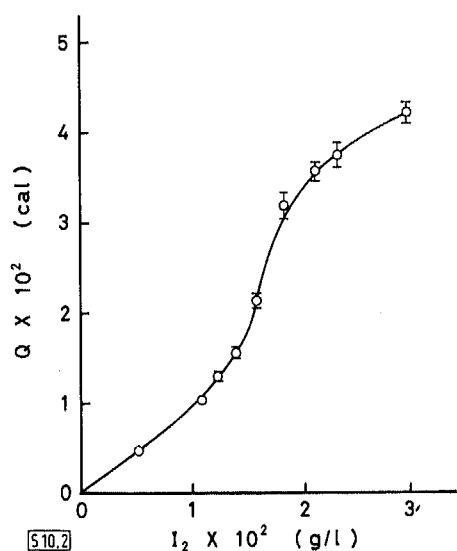


Figure 2. Change of the calorific value,  $Q$ , at 25°C with  $I_2$  concentration for amylose (DP 2500) - triiodide complex formation in the reaction of  $3.0 \times 10^{-3}$  g amylose in 12 ml with various amounts of  $I_2$  and 0.12 g KI in 12 ml. The concentration of  $I_2$  in the reaction solution of 24 ml is expressed by the unit of g/l.

Figure 3 shows the dependence of the  $\Delta H$  of amylose-triiodide complex formation for the DP 50 with a molarity of  $I_3^-$  ions determined by the dialysis method [9] or degree of saturation of the ions ( $q$ ) and also the corresponding change of molar ellipticity of  $I_3^-$  at 520 nm,  $[\theta]_{520}$ . Figure 4 also shows a similar

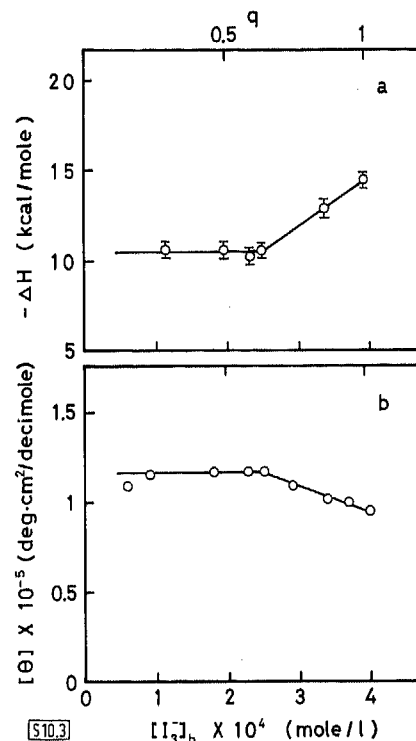


Figure 3. Dependence of (a) the enthalpy change,  $\Delta H$ , of the complex formation and (b) the molar ellipticity of  $I_3^-$  at 520 nm,  $[\theta]_{520}$ , for amylose (DP 50) - triiodide complex with the molarity of  $I_3^-$  ions,  $[I_3^-]_b$ , on the lower abscissa or the degree of saturation of  $I_3^-$  ions,  $q$ , on the upper abscissa.

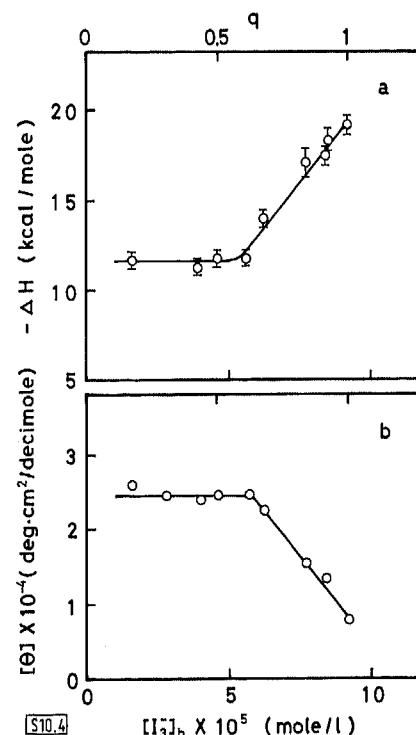


Figure 4. Dependence of (a) the  $\Delta H$  and (b) the molar ellipticity of  $I_3^-$  at 550 nm,  $[\theta]_{550}$ , for amylose (DP 2500) - triiodide complex with  $[I_3^-]_b$  (lower abscissa) or  $q$  (upper abscissa).

relationship for the DP 2500. For each DP, the  $\Delta H$  and  $[\theta]$  remained almost unchanged up to  $q \approx 0.6$ . The  $\Delta H$ 's were found to be equally ca.  $-11$  kcal/mole of  $I_{3,b}^-$ , regardless of the DP. Above  $q \approx 0.6$ , more or less depending on the DP, the  $\Delta H$  increased and the  $[\theta]$  decreased with the  $q$ . The  $\Delta H$ 's at  $q = 1$  were  $-14.6 \pm 0.4$ ,  $-15.0 \pm 0.4$  and  $19.2 \pm 0.5$  kcal/mole of  $I_{3,b}^-$  for the DP 50, 150 and 2500, respectively. In particular, for the DP 2500 the  $[\theta]$  decreased remarkably above  $q \approx 0.6$  and the signs of the CD bands in the blue band changed from (+, -) to (+, +) [9].

## 4 Discussion

Handa and Yajima [9] presented an interpretation of the bathochromic shift of the blue band with DP in that it can be explained as coming from the increase in the dipole moments of the long-axis transitions of monomeric  $I_{3,b}^-$  in the exciton-coupled dimer, which is subjected to the appearance of the shoulder band around 480 nm belonging to the short-axis transitions. This change in the dipole moments is accompanied with the change in the spatial configuration of the dipoles on the dimer coordinate system. Two species of the long-axis (L) transitions and one species of the short-axis (S) transitions are concerned with coloring of triiodide ions in amylose. The one of the L-transition species consists of the transition of the antibonding in-plane pi orbital ( $a_1^*$ ) to the vacant antibonding sigma orbital ( $b_1^*$ ) perturbed through the CT process from the nonbonding 2p orbitals of the surrounding glycosidic oxygen atoms to the  $b_1^*$  orbital and the transition of the bonding inplane orbital ( $a_1$ ) to the perturbed  $b_1^*$  orbital ( $b_1^*$ ). Another species consists of the antibonding out-of-plane pi  $b_2^* \rightarrow b_1^*$  and bonding out-of-plane pi  $b_2 \rightarrow b_1^*$  transitions. The former species depends mainly on bending of the ion, while the latter species depends on perturbation of the  $b_1^*$  orbital through the CT process due to twisting of the ion. The species of the S-transitions consists of the transitions of either  $t'$  or  $t''$  orbital, which is created by the perturbation of the nonbonding in-plane pi orbital ( $b_1$ ) or the nonbonding out-of-plane pi orbital ( $a_2$ ) due to the CT from the  $b_1$  or  $a_2$  orbital to the antibonding orbitals of the surrounding OH groups, to the  $b_1^*$  orbital. These transition species depend on promotion of the energy levels from  $b_1$  and  $a_2$  to  $t'$  and  $t''$  owing to the CT perturbation.

Thereby, energies required intrinsically for the electronic change in coloring of the ions through the CT process and bending of the ions were estimated to be ca. 45, 47 and 50 kcal/mole of  $I_{3,b}^-$  for DP 50, 100 and 1000, respectively.

We evaluated the energy for the conformational change in amylose lattice through the coloring process, on referring to these energy values for the electronic change and in considering that the heat of evolution in the reaction comes from excess energy as the difference between the energy from the overdone conformational change of the amylose lattice due to the cooperative hydrogen bonding [17] and the energy required to make up for the intrinsic electronic change of  $I_{3,b}^-$ . Therefore, the energies were as follows: at  $q = 0.5$ , ca. 56, 58 and 61 kcal/mole of  $I_{3,b}^-$  and at  $q = 1.0$ , ca. 60, 62 and 69 kcal/mole of  $I_{3,b}^-$  for the DP 50, 150 and 2500, respectively.

On the other hand, the average number of glucose residues occupied by one  $I_{3,b}^-$  ion was follows: at  $q = 0.5$ , ca. 36, 26 and 16 glucose unit (GU) and at  $q = 1.0$ , ca. 18, 13 and 8 GU for the DP 50, 150 and 2500, respectively. Then, using these data and taking the value of 2.5–3.0 kcal/mole as an average energy for the formation of one hydrogen bond in compounds similar to polysaccharides [18, 19], the average number of created hydrogen bonds per one residue through the coloring

process was estimated as follows: at  $q = 0.5$ , ca. 0.5, 1 and 1.5 hydrogen bonds and at  $q = 1.0$ , ca. 1, 2 and 3 bonds for the DP 50, 150 and 2500, respectively. Thus, the number of created hydrogen bond increased with the DP. It can be seen that this increase is closely involved with the bathochromic shift with DP, which results from the change in configuration and electronic state of the monomeric  $I_{3,b}^-$  in the exciton-coupled dimer constituting the polyiodide chain.

The result that the value of  $\Delta H$  was given as ca.  $-11$  kcal/mole of  $I_{3,b}^-$  within the range of  $q$  below ca. 0.6 regardless of the DP, is interpreted in terms of the concept that change in conformational energy of the amylose lattice is compensated by that in the electronic energy of triiodide ion in complex formation. This concept is based on our foregoing criteria on the character of the  $\Delta H$  which must be the sum of [H(amylose in the complex)-H(original amylose in aqueous solution)] and [H( $I_{3,b}^-$ )-H(free  $I_{3,b}^-$ )]. With this understanding, increase in the number of the created hydrogen bonds with the DP can be explained as coming from the degree of cooperativity with the DP for the formation of hydrogen bonds per one  $I_{3,b}^-$  in the coloring process, in presuming that the degree of cooperativity is governed by the original conformation of amylose in aqueous solution.

Though the conformational characteristics of amylose in solid state have been well established, conformation of amylose in aqueous solution is still a matter of discussion [20–24]. Goebel et al. [18] made the model calculation on total conformation energy and the formation energy of each hydrogen bond composing amylose helix. For hydration of the stable V-amylose with left chirality, they predicted that three types of hydrogen bonds,  $O(6)_i \rightarrow H_2O \rightarrow O(2)_{i-6}$ ,  $O(3)_i \leftarrow O(2)_{i-1}$  and  $O(6)_i \leftarrow O(3)_{i-5}$ , were given (the direction of arrow is from hydrogen donor toward hydrogen acceptor).

On the other hand, from the effect of amylose/iodine complexation on the hydrodynamic properties of amylose, Senior and Hamori [25] proposed a loose and extended helix model for the solution conformation of amylose. Jordan et al. [26], on the basis of statistical studies of the amylose chain, presented a random coil model for dissolved amylose that is not at all unlike Senior and Hamori's proposal.

The following were presumed for the explanation of the coloring mechanism of  $I_{3,b}^-$  ions in terms of the exciton-coupled dimer model [8, 9]: three glycosidic oxygen atoms and four OH groups must be associated with a triiodide ion in amylose; the double pairs of OH groups are possibly a pair of residual OH groups from the structural water and  $O(2)H$  in hydrogen bond  $O(6)_i \rightarrow H_2O \rightarrow O(2)_{i-6}$  and the corresponding pair in the next turn; the formation of a pair of the  $O(6)_i \rightarrow H_2O \rightarrow O(2)_{i-6}$  type bonds combined with a pair of the terminal iodine atoms of each ion is first achieved in the dimeric unit through the coloring process, being favored by the cooperative formation of  $O(3)_i \leftarrow O(2)_{i-1}$  bonds. Furthermore, on the basis of this consideration, new interpretation was given for the experimental results in the kinetic study of amylose-iodine reaction by Hiromi et al. [27]. Therein, it was expected that the amylose chain in the sense of Jordan et al. creates stepwise hydrogen bonds of  $O(6)_i \rightarrow H_2O \rightarrow O(2)_{i-6}$ ,  $O(3)_i \leftarrow O(2)_{i-1}$  and  $O(6)_i \leftarrow O(3)_{i-5}$  through the coloring process of the  $I_{3,b}^-$  ions and has finally the helical structure with a perturbed V-helix of Goebel et al. In addition, the half-time of the reaction  $t_{1/2}$  for the growth of the blue band, which shortened strikingly with DP up to ca. 140 and levelled off above there, was suggested to be closely related to the partition probability of the  $O(3)_i \leftarrow O(2)_{i-1}$  bonds in a statistical coil of the original amylose.

From these views and the estimation on the average number of the created hydrogen bonds, we may well assert that no other hydrogen bonds except  $O(3)_i \leftarrow O(2)_{i-1}$  bonds can exist originally and the partition of the latter bonds increased with DP up to 100–150, whereas for the complexes, the formation of the  $O(6)_i \rightarrow H_2O \rightarrow O(2)_{i-6}$  and  $O(6)_i \leftarrow O(3)_{i-5}$  bonds occurs with DP besides the additional formation of  $O(3)_i \leftarrow O(2)_{i-1}$  bonds.

On the other hand, the value of the  $\Delta H$  increased above  $q \approx 0.6$  being associated with the decrease in the  $[\theta]$ , which depended more or less on the DP. From the explanation of the cause for decrease in the  $[\theta]$  [9], it is suggested that this increase in the  $\Delta H$  must be ascribed to the interhelical association due to formation of new hydrogen bonds from some combinations among  $O(6)H$ ,  $O(3)H$ ,  $O(2)H$  and  $O(5)$ . Particularly, the high polymer with DP 2500 is inferred to form a rod-like structure of Bittiger et al. [28] through side-by-side intramolecular association due to the folding of amylose chain involved with end-to-end intermolecular association. Existence of this kind of structure in aqueous solution has been expected by resonance polarized light scattering (He-Ne laser) and viscosity studies [29].

## Summary

The enthalpy change ( $\Delta H$ ) of amylose-triiodide complex formation in the presence of excess KI was directly determined by calorimetric measurement at 25°C for amyloses with a degree of polymerization (DP) of 50, 150 and 2500. The  $\Delta H$  for each DP depended on the degree of saturation ( $q$ ) for bound triiodide ions ( $I_{3,b}^-$ ) in amylose. The  $\Delta H$  was ca.  $-11$  kcal/mole of  $I_{3,b}^-$  at  $q = 0.5$  regardless of the DP, while at  $q = 1.0$  the  $\Delta H$ 's were ca.  $-15$ ,  $-15$  and  $-19$  kcal/mole of  $I_{3,b}^-$  for the DP 50, 150 and 2500, respectively.

The average number of created hydrogen bonds per one glucose through the coloring process was estimated on the basis of the following consideration with respect to the heat of evolution in the reaction: this heat comes from excess energy as the difference between energy from the overdone conformational change of amylose lattice due to the cooperative formation of hydrogen bonds and the energy required to make up for the intrinsic electronic change of  $I_{3,b}^-$ . As a result, the number of created hydrogen bonds increased with the DP, which was associated with the bathochromic shift of the blue band in the absorption spectra. Thereby, the change in skeletal structure of the amylose lattice in complex formation was discussed.

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