Carbohydrate Research, 163 (1987) 155–167
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

EFFECT OF CONCENTRATION OF IODIDE ON THE BOUND SPECIES OF I_2/I_3^- IN THE AMYLOSE–IODINE COMPLEX

HIROFUMI YAJIMA, TAKESHI NISHIMURA, TADAHIRO ISHII, AND TAKASHI HANDA
Department of Applied Chemistry, Faculty of Science, Science University of Tokyo, 1-3, Kagurazaka, Shinjuku-ku, Tokyo 162 (Japan)

(Received September 30th, 1986; accepted for publication in revised form, December 15th, 1986)

ABSTRACT

A liquid–liquid distribution method, with heptane as the organic solvent, involving evaluation of the concentration of free I^- by magnetic circular dichroism, has been developed for determining the bound amounts of I_2/I_3^- in the amylose–iodine complex in unbuffered aqueous solutions. The effect of I_2 and I^- concentrations on the bound species of iodine in the complex was investigated by using this method. We found that the stoichiometric bound species of I_2/I_3^- is independent of the concentration of I_2 at a given I^- concentration. However, the bound species strongly depends on I^- concentration, and varies from I_3^- at 10mM KI to I_{15}^- at 0M KI. Moreover, the number of D-glucosyl residues required for including one iodine atom is within the range of 2.7 to 3.0, regardless of I^- concentration. It was concluded that the bound species are governed by the distribution of the actual species $I_2 \cdot I_2$ (I_4), $I_2 \cdot I_3^-$ (I_5^-), and $I_3^- \cdot I_3^-$ (I_6^{2-}), which are responsible for the blue color of the complex.

INTRODUCTION

A number of extensive studies have been made by various investigators[1–11] on the iodine-binding capacity of amylose, or binding fraction or both, of I_2/I_3^- in amylose–iodine–iodide complexes under a wide range of experimental conditions. Recently, Knutson *et al.*[12] showed, by a combination of potentiometric titration and spectrophotometric titration methods, that the bound species (the number of iodine atoms per charge) varies from I_3^- at 100mM I^- to I_{11}^- at 0.5mM I^-. A similar dependence of I^- concentration on the bound species in the I^- range of 0.0107–25mM was presented by Cronan and Schneider[6]. In conventional spectrophotometric methods for the determination of the bound species, a constant molar-extinction coefficient (ϵ_C) of amylose–iodine complex has usually been employed, regardless of the bound-species dependence on I^- concentration. However, the accuracy for this treatment has not been verified experimentally. Consequently, we have developed a new method for stoichiometric determination of the bound species without ϵ_C, on the basis of a liquid–liquid distribution method with heptane as the

0008-6215/87/$ 03.50

organic solvent, involving determination of the free I^- concentration by magnetic circular dichroism (m.c.d.).

We now report the advantages of using our method for the determination of the bound species of I_2/I_3^- in the complex, compared to using conventional methods, and discuss the effect of the concentration of I^- on the bound species.

EXPERIMENTAL

Materials. — A sample of amylose of high molecular weight was obtained from Wako Pure Industries Co., Ltd. Amylose having an average degree of polymerization (d.p.) of 100 was prepared by enzymic degradation of the long polymer, followed by fractionation in a column of Sephadex gel (G-75). Both the KI and the I_2 were of the purest grade commercially available. The I_2 was purified by double sublimation. Heptane of spectroscopic grade, obtained from Dohjin Kagaku Research Co, Ltd., and distilled water were used as solvents.

Methods

Preparation of the solutions for liquid–liquid distibution. — All of the procedures were carried out at 25°. A control (blank) solution was prepared by mixing 10 mL of heptane containing I_2 (0.445 to 40.2mM) with an equal volume of aqueous KI solution (0 to 40mM) in a stoppered glass tube. A corresponding solution of the complex was prepared by mixing 10 mL of heptane-I_2 solution with an equal volume of aqueous KI solution containing amylose of d.p. 100 (0.25 g/L). These solutions were stored for >12 h after stirring, in order to attain equilibrium distribution of I_2 between the organic and aqueous phases. In this study, buffers and oxidizing agents were not used, because we wanted to omit their effects on the formation and stability of the complex[13].

Measurements. — Absorption and m.c.d. spectra were respectively measured by using a Hitachi 320 spectrophotometer and a Jasco J-20 spectropolarimeter.

Determination of the bound amounts of I_2/I_3^- in the complex. — The concentration of total bound iodine ($[I]_{B,T}$) in the complex is given as

$$[I]_{B,T} = [I_2]^w_{c,B} + [I_3^-]^w_{c,B}$$
$$= (1 + D + \gamma D)\{[I_2]^o_b - [I_2]^o_c\} + KD\{[I_2]^o_b \cdot [I^-]^w_b - [I_2]^o_c \cdot [I^-]^w_{c,F}\}, \quad (1)$$

using the following parameters,

$$D = [I_2]^w_b/[I_2]^o_b = [I_2]^w_{c,F}/[I_2]^o_c, \quad (2)$$
$$\gamma = [I^-]^w_{b,h}/[I_2]^w_b = [I^-]^w_{c,h}/[I_2]^w_{c,F}, \text{ and} \quad (3)$$
$$K = [I_3^-]^w_b/[I_2]^w_b \cdot [I^-]^w_b = [I_3^-]^w_{c,F}/[I_2]^w_{c,F} \cdot [I^-]^w_{c,F}, \quad (4)$$

where the superscripts o and w refer to the organic and aqueous phases, respectively, and subscripts b, h, c, T, F, and B refer to blank system, hydrolysis, complex system, and total, free, and bound species, respectively; D and γ respectively stand

for the distribution ratio of I_2 in the two phases and the parameter for the hydrolysis of I_2; and K is the equilibrium constant for I_3^- formation, and is[14] 768 (at 25°). In addition, the concentration of bound I_3^- is expressed by

$$[I_3^-]^w_{c,B} = [I^-]^w_{c,i} + (\gamma - K\cdot[I^-]^w_{c,F})\cdot D[I_2]^\circ_c - [I^-]^w_{c,F}, \quad (5)$$

where the subscript i refers to initial dosage. The detailed derivations of Eqs. *1* and *5* are described in the Appendix. The parameters indispensable for the evaluation of $[I_2]_B$ and $[I_3^-]_B$ are D, γ, $[I_2]^\circ$, and $[I^-]_F$. Therefore, the methods for the determination of these parameters are described.

(*1*) Determination of $[I_2]^\circ$. The concentration of I_2 in heptane was determined by measuring the absorbance (A) of I_2 in heptane solution at 522 nm (A_{522}) using the relationship

$$[I_2]^\circ = A^{522}/\epsilon_{522,I_2}, \quad (6)$$

where $\epsilon_{522,I_2} = 897$ (I_2 in heptane)[15].

(*2*) Determination of the distribution ratio of D. The concentration of I_2 in aqueous solution in the blank system was evaluated by measuring the absorbance at 400 and 460 nm, using the following two equations.

$$A_{400} = \epsilon_{400,I_2}\cdot[I_2]^w_b + \epsilon_{400,I_3^-}\cdot[I_3^-]^w_b, \quad (7)$$

and

$$A_{460} = \epsilon_{460,I_2}\cdot[I_2]^w_b + \epsilon_{460,I_3^-}\cdot[I_3^-]^w_b, \quad (8)$$

where ϵ_{400,I_2}, ϵ_{460,I_2}, ϵ_{400,I_3^-}, and ϵ_{460,I_3^-} in H_2O are 195, 746, 5700, and 975, respectively[16]. The D value was obtained by dividing $[I_2]^w_b$ by $[I_2]^\circ_b$ determined by Eq. *6*, and was a constant of $(2.63 \pm 0.07) \times 10^{-2}$ for varying $[I_2]^\circ_i$(0.445 to 40.2mM). The resulting D value was used in Eqs. *1* and *5* for the determination of $[I_2]_B$ and $[I_3^-]_B$.

(*3*) Determination of $[I^-]_F$. The absorption spectrum of free I^- in aqueous solution consists of two bands, having peaks at 194 and 226 nm, as shown in Fig. 1 (a). Correspondingly, the m.c.d. spectrum exhibits two mutual split bands with opposite signs (+ and −), as shown in Fig. 1 (b). For the determination of $[I^-]_F$, we took advantage of the magnetic ellipticity Θ_M at 233 nm, where the absorbance of I_2 in H_2O is strikingly lessened[17] and also the m.c.d. spectrum of I_2 in heptane was not observed in the present experiments. Fig. 2 shows the calibration curve of Θ_M^{233} (measured at 14 kG) *vs.* I^- concentration in KI aqueous solution. It should be noted that, in the blank system, $[I^-]$ determined from this calibration curve coincided, whithin experimental accuracy, with the one calculated by K (Eq. *4*) using $[I_2]^w_b$ and $[I_3^-]^w_b$ obtained from Eqs. *7* and *8*. On the other hand, $[I^-]^w_{c,F}$ in the complex solution was determined by deducting the ellipticity of the solution of the complex,

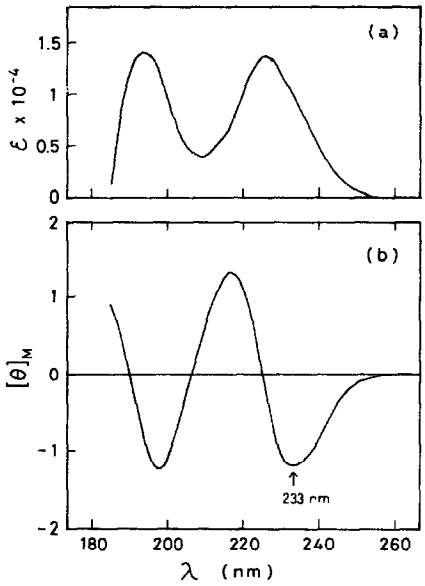

Fig. 1. Absorption (a) and m.c.d. (b) spectra of free I^- ions in aqueous solution.

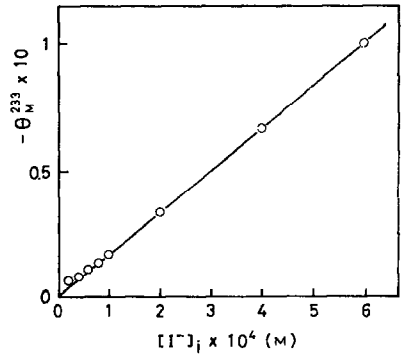

Fig. 2. Calibration curve of the magnetic ellipticity at 233 nm, Θ_M^{233}, measured at 14 kG *vs.* KI concentration in aqueous solution.

at 233 nm at nonmagnetic field (Θ_c^{233}), from the ellipticity measured at 233 nm under a magnetic field at 233 nm ($\Theta^{233}{}_{M,c}$), and using the following relationship with the assumption that only I^- contributes to the magnetic ellipticity at 233 nm.

$$[I^-]_{c,F}^{w} = (\Theta_{M,c}^{233} - \Theta_c^{233})/[\Theta]_M^{233} \times 1/H \times 100, \tag{9}$$

where $[\Theta]_M^{233}$ is the molar ellipticity per unit magnetic field at 233 nm for free I^- in aqueous solution, and H is the magnitude of the magnetic field. $[\Theta]_M^{233}$ and H are -1.2 deg.cm^2/G.dmol and 14 kG, respectively.

(*4*) Determination of the γ value. There exist the following equilibria with respect to I_2 in aqueous solution in the blank system[18].

$$I_2 + H_2O \rightleftarrows H_2O{\cdot}I_2 \overset{aq}{\rightleftarrows} [H_2OI]^+_{aq} + I^-_{aq} \overset{H_2O}{\rightleftarrows} HOI + H_3O^+_{aq} + I^-_{aq} \qquad (10)$$

$$I_2 + I^-_{aq} \rightleftarrows I^-_{3\,aq} \qquad (11)$$

In the absence of KI, the concentration of I^- ($[I^-]^w_{b,h}$) produced by hydrolysis of I_2, which is in equilibrium with I_2, was merely given by measuring Θ^{233}_M for I_2 aqueous solution using the calibration curve in Fig. 2. In addition, the concentration of I_2 ($[I_2]^w_b$) was calculated by using Eqs. *7* and *8*. Then, we obtained the γ value by dividing $[I^-]^w_{b,h}$ by $[I_2]^w_b$.

In the presence of KI in the blank system, using Eq. *A–2*, $[I^-]^w_{b,h}$ was determined by deducting an initial dosage of I^- ($[I^-]^w_{b,i}$) from the total iodide concentration ($[I^-]^w_{b,T}$), which was calculated by adding $[I^-]^w_b$, determined from the Θ^{233}_M value, to $[I^-_3]^w_b$, obtained by using Eqs. *7* and *8*. Also, the γ value in the presence of KI system was given in the same way as in the absence of KI.

The resulting γ value was then applied to Eqs. *1* and *5* for the determination of $[I_2]_B$ and $[I^-_3]_B$.

RESULTS AND DISCUSSION

Contribution of the hydrolysis of I_2 to $[I^-]$. — As seen in reaction *10*, the acidity in the aqueous solution, in the absence of initial KI in the blank system, increases as the I_2 concentration increases. In fact, the pH changed from 5.6 to 4.0 when $[I_2]^o_i$ increased to 40.2mM. However, the γ ($=[I^-]^w_{b,h}/[I_2]^w_b$) value remained constant for varying $[I_2]^o_i$, and was equal to 0.1. This implies that the increases in $[I_2]$ and $[I^-]$ are completely compensated by the formation of I^-_3, as shown in reaction *11*. Furthermore, the γ value was also kept constant for changing $[I^-]_i$, regardless of $[I_2]^o_i$.

Binding isotherm of I_2/I^-_3 in amylose. — The concentrations of the free and bound species in the complex-system were evaluated from Eqs. *1–5* by using the resulting values of D, γ, $[I_2]^o_i$ and $[I^-]_F$. Fig. 3 (a–c) shows the changes in $[I_2]_F$, $[I_2]_B$, $[I^-_3]_B$, and $[I]_{B,T}$ with increasing $[I^-_3]_F$ for several initial concentrations of KI. As shown in Fig. 3, at a given $[I^-]_i$, $[I^-_3]_B$ increases nearly proportionally to $[I_2]_B$, and $[I^-_3]_B$ and $[I_2]_B$ almost simultaneously level off at a constant $[I^-_3]_F$. At very low $[I^-]_i$, the predominant bound species is I_2, whereas, at high $[I^-]_i$, the bound species is I^-_3. This conclusion is reinforced by the results found in Fig. 4, which shows the $[I^-]_F$ dependence on the apparent binding-constant ($1/m_{0.5}$). Cronan and Schneider[6] interpreted similar results on the basis of limiting binding models for either of the two binding species, I_2 and I^-_3.

Dependence of the bound species of I_2/I^-_3 on $[I^-]_i$. — The stoichiometry of the complex is expressed in terms of the ratio (R) of bound I^-_3 to total bound

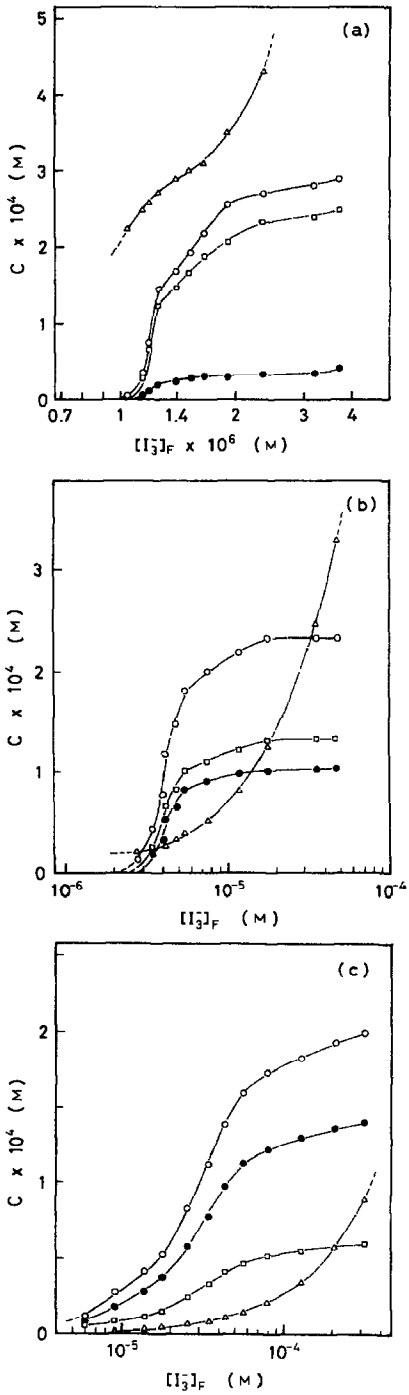

Fig. 3. Variation in C (M); $[I_2]_F$ (Δ), $[I]_{B,T}$ (O), $[I_2]_B$ (□), and $[I_3^-]_B$ (●) with respect to $[I_3^-]_F$ at various KI concentrations (M): a, 0; b, 2.15×10^{-4}; c, 4.08×10^{-3}. Amylose (d.p. 100), 0.25 g/L.

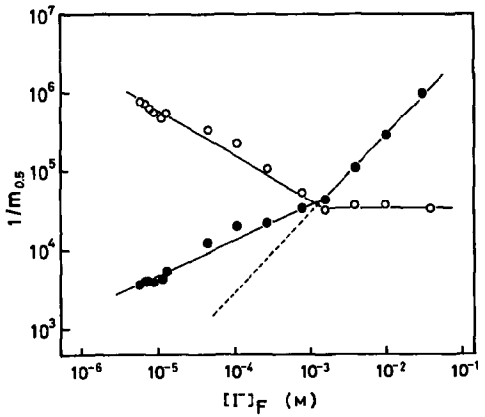

Fig. 4. Apparent binding constant, $1/m_{0.5}$, *vs.* $[I^-]_F$ at half saturation, where m refers to $[I_2]_F$ (●) and $[I_3^-]_F$ (○), respectively.

iodine (see Eq. *12*).

$$R = [I_3^-]_B/[I]_{B,T} = [I_3^-]_B/\{[I_2]_B + [I_3^-]_B\} \quad (12)$$

Table I summarizes the characteristics of the saturated complexes at various $[I^-]_i$, including: the ratio of the number of iodine atoms per charge (N), the wavelength of maximum absorption (λ_{max}), the molar extinction coefficient at λ_{max} (ϵ_{max}), and the ratio of D-glucosyl residues per bound iodine atom (Q). For comparison, a list of the R values which are dependent upon experimental conditions, reported by other

TABLE I

CHARACTERISTICS OF THE SATURATED COMPLEXES AT VARIOUS $[I^-]_i$

$[I^-]_i$(M)	R	N[a]	λ_{max} (nm)	ϵ_{max}[b]	Q[c]
0.00	0.14	15.3	596	13,600	2.67
1.26×10^{-6}	0.16	13.5	596	14,700	2.78
4.66×10^{-6}	0.19	11.5	595	14,400	2.82
1.11×10^{-5}	0.17	12.8	594	14,000	2.68
2.23×10^{-5}	0.20	11.0	592	15,000	2.72
4.45×10^{-5}	0.29	7.90	591	16,900	2.86
1.09×10^{-4}	0.34	6.88	589	19,600	2.81
2.15×10^{-4}	0.44	5.55	588	22,400	2.73
4.30×10^{-4}	0.46	5.35	586	23,500	2.67
1.02×10^{-3}	0.49	5.08	584	24,300	2.66
2.04×10^{-3}	0.60	4.33	583	29,900	2.84
4.08×10^{-3}	0.70	3.86	582	31,100	2.96
1.00×10^{-2}	~1	3.0	580	35,200	2.93
4.00×10^{-2}	~1	3.0	576	34,500	2.96

[a]Number of iodine atoms per charge in bound species. [b] ϵ_{max} (at λ_{max}) = $A_{max}/[I]_{B,T}$ (at saturation). [c]Number of D-glucosyl residues required for including one iodine atom.

TABLE II

STOICHIOMETRY OF THE AMYLOSE–IODINE–IODIDE COMPLEX IN AQUEOUS SOLUTION

Investigator	*KI (M)*	*R*	*Method*
Gilbert and Marriott[1]	10^{-4}–10^{-3}	0.67	Potentiometric
Banks and Greenwood[19]	2×10^{-3} -5×10^{-2}	0.39–0.81	Potentiometric
Knutson *et al.*[12]	5.32×10^{-4} -2.03×10^{-1}	0.2–1.0	Potentiometric and spectrophotometric
Watanabe *et al.*[5]	2×10^{-4} -1.8×10^{-3}	0.18	Spectrophotometric
Cronan and Schneider[6]	1.07×10^{-5} -2.5×10^{-2}	0.1–1.0	Spectrophotometric
Cesàro *et al.*[10]	10^{-5}–10^{-4}	0.3 ±0.11	Spectrophotometric

investigators, is given in Table II. In the earlier reports, we could not find any R values for an $[I^-]_i$ below 0.01mM, where free I_2 predominantly prevails (rather than free I_3^-). This may be due to the fact that conventional methods are unable to reproduce their findings experimentally. This shows that our method is therefore superior to the conventional method for evaluation of the R value, irrespective of experimental conditions.

As shown in Table I, the R (or N) value depends strongly on $[I^-]_i$ and tends to increase (or decrease) with increasing $[I^-]_i$. A similar R dependence on I^- has been reported by many researchers[1,6,12,19]. In the present study, however, the R value for each $[I^-]_i$ remained approximately constant for a varied concentration of total I_2 in aqueous solution. On the other hand, Schneider *et al.*[6,20] analyzed the binding isotherms of I_2/I_3^- in amylose, by the implicit assumption that the R value was constant for $[I_3^-]_F$ at a given $[I^-]_i$, using one-dimensional Ising models involving noncompetitive and competitive binding with first nearest-neighbor interactions. However, we obtained a constancy of the R value for $[I_3^-]_F$ at a given $[I^-]_i$ without making any such assumption.

It was found that the complex is in the R range of 0.14–0.20, even at an $[I^-]_i$ of <0.01mM. This result suggests that I_3^- ions are mandatory for the stabilization of the complex. Cesàro *et al.*[21] presented a model for amylose–iodine binding in which the sequence of bound iodine was initiated by I_3^- and propagated by I_2, in view of the fact that I_3^- has a considerably higher affinity[20,22–24] for the amylose lattice than I_2 has.

With respect to the R value in a concentration range of $[I^-]_i$ >0.01mM, our values deviate from those of past researchers. In particular, compared to the R value obtained by the spectrophotometric method, our values tend to be higher than those

of Watanabe *et al.*[5] and Knutson *et al.*[12], wheras our values tend to be lower than those of Cronan and Schneider[6] and Cesàro *et al.*[10]. This discrepancy is, in part, attributed to the differences of various experimental conditions, such as differing concentrations of the reagents and the molecular weight of the amyloses employed. However, more importantly, this discrepancy is related to the fact that, in the conventional method, the determination of the bound species of iodine was made by using a constant ϵ_{max} of the complex, regardless of the bound species of iodine. As given in Table I, we have obtained the finding that the ϵ_{max} value decreases with decreasing $[I^-]_i$, accompanied by a shift of λ_{max} to a longer wavelength. We thus conclude that the *R* value determined by using our method, without assuming a constant ϵ_{max}, gives the most reasonable value for the stoichiometry of the complex.

A more detailed interpretation of the relationship between the spectroscopic properties of the complex and the bound species of iodine has not yet been established by electronic theory, except for that [25–28] for the amylose–I_3^- complex in the presence of an excess of $[I^-]_i$.

As for the iodine-binding capacity of amylose, the *Q* value is within the range of 2.7–3.0, regardless of $[I^-]_i$, or the bound species of iodine. Similar *Q* values were obtained by Cronan and Schneider[6]. These values are in good agreement with those determined by X-ray analyses[29,30]. On the other hand, Knutson *et al.*[12] reported, in a potentiometric excess-iodine titration study, that the complex has an appreciably low *Q* value, in the range of 1.9–2.2; they presented the concept that the complexation of iodine occurs outside the helical cavity of amylose.

Existence of polyiodine chain. — With respect to the wide variation of the *R* value as a function of $[I^-]_i$, Rawlings and Schneider[20] carried out a statistical analysis of the binding isotherms of I_2/I_3^-, using a one-dimensional method involving competitive binding with first-nearest-neighbor interaction, as previously described. Therein, they obtained the results that the intrinsic binding constant of I_3^- was significantly larger than that of I_2, and that the attractive interaction energy between mixed species I_2–I_3^- surpassed those between like species I_2–I_2 and I_3^-–I_3^-. Table III shows the actual species of iodine in various complexes. Extensive measurements, such as resonance Raman, ^{129}I Mössbauer spectroscopy[31–33], and X-ray diffraction[34–36], revealed that I_5^- (or $I_2 \cdot I_3^-$) is the species involved in the complexes. Furthermore, for iodine in aqueous solution, it was proposed from equilibrium studies that I_5^- exists as a free species and, when the concentrations of I_2 and I^- are very high, the other complex ions, such as I_4^{2-} and I_6^{2-} are formed[37–40]. In the present study, it seems possible that I_5^- (or $I_2 \cdot I_3^-$) species in the amylose–iodine complex is easily formed at $[I^-]_i \simeq$ mM.

Along with many other researchers, we have concluded that the bound species of iodine in the amylose–iodine complex in the presence of an excess of I^- is[1,6,12] I_3^-. Moreover, in X-ray studies, Reddy *et al.*[41] found linear chains of polymerized $(I_3^-)_n$ units in the (benzamide)$_2 \cdot HI_3$ complex. In the terms of the exciton-coupled I_3^- dimer model, Handa and Yajima[25–28] interpreted the spectroscopic properties, such as absorption and c.d. spectra, of the amylose–I_3^- complex in the presence of an

TABLE III

EXISTENCE OF POLYIODIDES IN SEVERAL COMPLEXES

Polyiodide	*Complex*	*Method*	*References*
$(I_3^-)_n$	(benzamide)$_2$·HI_3	X-ray resonance Raman	41 31,32
$(I_5^-)_n$	(trimesic acid·H_2O)$_{10}$·HI_5	resonance Raman ^{129}I Mössbauer	31,32
$(I_2 \cdot I_3^-)_n$	(α-CD)$_2$·LiI_3 ·I_2·8H_2O	resonance Raman X-ray	31,32,33 36
$(I_5^-)_n$	(α-CD)$_2$·$Cd_{0.5}$·I_5·27H_2O	resonance Raman X-ray	33 36
I_5^-	$N(CH_3)_4$·I_5	X-ray	34
I_5^-	phenacetin·HI_5	X-ray	35
I_7^-	$N(C_2H_5)_4$·I_7	X-ray	42
I_8^{2-}	$[(CH_2)_6N_4CH_3]_2I_8$	X-ray	43

excess of I^-; they concluded that the unit responsible for the blue color of the complex at excess I is I_6^{2-} ($I_3^- \cdot I_3^-$).

On the other hand, concerning the mean stoichiometry of the bound species (the number of iodine atoms per charge) in a considerably low $[I^-]_f$, Cesàro *et al.*[10,23] presented $I_7^- - I_9^-$, Knutson *et al.*[12] I_{11}^-, and our group I_{15}^-. However, the existence of polyiodide units in complexes with $N > 7$ have not been experimentally verified, except for the I_7^- unit in[42] $N(C_2H_5)_4I_7$. In view of this situation and our results showing that significant changes in such spectral characteristics as λ_{max}, ϵ_{max}, and c.d. spectral patterns of the complex do not appear for appreciably differing bound species, from I_7^- to I_{15} (see Table I), it is hard to accept that the polyiodide units for $N \geqq 7$ are actual bound-species in the amylose–iodine complex.

Cronan and Schneider[6] proposed that, at very low $[I^-]_f$, the limiting bound species was I_2. However, the fact that I^- or I_3^- is mandatory for stabilization of the amylose–iodine complex[20,22–24] invalidates the possibility of existing polyiodine chains completely without charge, that is, $(I_2)_n$, in the complex. On the other hand, it has been found that, in a nonpolar solvent, a self-intermolecular charge-transfer complex with the formula I_4 is observably formed when the concentration of I_2 is very high[44–46]. In view of this matter and the facts that I_4^{2-} in (α-CD)$_2$$BaI_2$·$I_2$·12 H_2O is somewhat correlated with the bronze color[36] and that amylose has, in part, a hydrophobic environment inside a helical cavity[47], we propose that $I_2 \cdot I_2$ species bound to amylose is converted into $I_2^- \cdot I_2$ (I_4^{2-}) through charge-transfer interaction with the amylose lattice, and contributes to the blue color of the complex at a very

low $[I^-]_i$.

Consequently, the bound species are governed by the distribution of the inherent species $I_2 \cdot I_2$ (I_4), $I_2 \cdot I_3^-$ (I_5^-), and $I_3^- \cdot I_3^-$ (I_6^{2-}), which are responsible for the blue color of the complex.

CONCLUSION

It was found that the liquid–liquid distribution method, with heptane as the organic solvent, involving the evaluation of free I^- concentration by means of m.c.d. is efficient for determining the bound amounts of I_2/I_3^- in the amylose-iodine complex. The bound species of iodine varies from I_3^- at 10mM KI to I_{15}^- at 0 M KI. Moreover, the number of D-glucosyl residues required for including one iodine atom is in the range of 2.7–3.0, regardless of the I^- concentration. Finally, the bound species are governed by the distribution of the inherent species $I_2 \cdot I_2$ (I_4), $I_2 \cdot I_3^-$ (I_5^-), and $I_3^- \cdot I_3^-$ (I_6^{2-}) which are responsible for the blue color of the complex.

ACKNOWLEDGMENTS

We are indebted to Dr. Tatsuya Sekine, Professor at the Science University of Tokyo, for his valuable comments, and also to Mr. Mitsuyoshi Iwai and Miss Tomoko Takagi, for their cooperation in the present study.

APPENDIX

Calculation of the bound amounts of I_2/I_3^- *in the complex*

In view of the formation of I_3^- in the reaction of I_2 with I^-, the formation of I^- by hydrolysis of I_2 in aqueous solution[18] and complexation of amylose with I_2/I_3^- but not I^-, the mass conservation relations for I_2, I^-, and I_3^-, in distribution equilibrium between the aqueous and organic phases in the blank and complex systems, are expressed by the following.

In the blank system,

$$[I_2]^o_{b,i} = [I_2]^o_b + \{ [I_2] + [I_3^-] + [I^-]_h \}^w_b \qquad (A\text{-}1)$$

$$[I^-]^w_{b,T} = \{ [I^-]_i + [I^-]_h \}^w_b = \{ [I^-] + [I_3^-] \}^w_b \qquad (A\text{-}2)$$

In the complex system,

$$[I_2]^o_{c,i} = [I_2]^o_c + \{ [I_2]_F + [I_3^-]_F + [I^-]_{F,h} \}^w_c + \{ [I_2]_B + [I_3^-]_B \}^w_c \qquad (A\text{-}3)$$

$$[I^-]^w_{c,T} = \{ [I^-]_i + [I^-]_h \}^w_c = \{ [I^-]_F + [I_3^-]_F + [I_3^-]_B \}^w_c \qquad (A\text{-}4)$$

where the superscripts and subscripts are the same as described in Eqs. *1–5*. These relations have the following three restrictions: (*1*) amylose is insoluble in heptane[48]; (*2*) the formation of other complex ions, such as I_4^{2-}, I_5^-, and I_6^{2-}, as free species in aqueous solution, which was proposed by Haddock *et al.* and others[37–40], is precluded, because of the extremely low association constants for these three ions[37–40]; (*3*) I^- ions are not bound to amylose alone[49], a fact that we have verified with the finding that the m.c.d. spectrum of the amylose–KI system is the same as that of free KI, regardless of the amylose concentration.

The concentration of total bound iodine ($[I]_{B,T}$) in the complex is given as Eq. *1* from the relation of $[I_2]^o_{b,i} = [I_2]^o_{c,i}$ by Eqs. *A-1* and *A-3*, using the parameters D, γ, and K expressed by Eqs. *2*, *3*, and *4*, respectively. In addition, the concentration of bound I_3^- is given as Eq. *5* from Eq. *A-4*, in the same way, using D, γ, and K.

REFERENCES

1 G. A. Gilbert and J. V. R. Marriott, *Trans. Faraday Soc.*, 44 (1948) 84–93.
2 E. F. Paschall and J. F. Foster, *J. Am. Chem. Soc.*, 75 (1953) 1177–1180.
3 D. L. Mould, *Biochem. J.*, 58 (1954) 593–600.
4 L. Coton, L. H. Lampitt, and C. H. F. Fuller, *J. Sci. Food Agric.*, 6 (1955) 660–664.
5 T. Watanabe, K. Ogawa, and S. Ono, *Bull. Chem. Soc. Jpn.*, 43 (1970) 950–952.
6 C. L. Cronan and F. W. Schneider, *J. Phys. Chem.*, 73 (1969) 3990–4004.
7 W. Banks, C. T. Greenwood, and D. D. Muir, *Staerke*, 23 (1971) 118–124.
8 S. V. Bhide and N. R. Kalb, *Indian J. Biochem. Biophys.*, 15 (1978) 227–228.
9 W. Banks, C. T. Greenwood, and D. D. Muir, *Staerke*, 26 (1974) 73–78.
10 A. Cesàro, E. Jerian, and S. Saule, *Biopolymers*, 19 (1980) 1491–1506.
11 M. Yamamoto, T. Sano, and T. Yasunaga, *Bull. Chem. Soc. Jpn.*, 55 (1982) 1886–1889.
12 C. A. Knutson, J. E. Cluskey, and F. R. Dintzis, *Carbohydr. Res.*, 101 (1982) 117–128.
13 T. Kuge and S. Ono, *Bull. Chem. Soc. Jpn.*, 33 (1960) 1269–1272.
14 M. Davies and E. Gwynne, *J. Am. Chem. Soc.*, 74 (1952) 2748–2752.
15 H. Tsubomura and R. Lang, *J. Am. Chem. Soc.*, 83 (1961) 2085–2092.
16 A. D. Awtrey and R. E. Connick, *J. Am. Chem. Soc.*, 73 (1951) 1842–1843.
17 B. B. Bhowmik and S. P. Chattophadhyay, *Indian J. Chem.*, 17A (1979) 85–87.
18 R. S. Mulliken, *J. Phys. Chem.*, 56 (1952) 801–822.
19 W. Banks and C. T. Greenwood, *Starch and Its Components*, Edinburgh University Press, Edinburgh, 1975.
20 P. K. Rawlings and F. W. Schneider, *J. Chem. Phys.*, 52 (1970) 946–952.
21 A. Cesàro, W. Konic, and D. A. Brant, in D. A. Brant (Ed.), *Solution Properties of Polysaccharides, ACS Symp. Ser.*, 150 (1980) 477–490.
22 J. A. Thoma and D. French., *J. Am. Chem. Soc.*, 82 (1960) 4144–4147.
23 A. Cesàro and D. A. Brant, *Biopolymers*, 16 (1976) 983–1006.
24 J. A. Thoma and L. Stewart, in R. L. Whistler and E. F. Paschall (Eds.). *Starch Chemistry and Technology*, Vol. 1, Academic Press, New York, 1965, Chap. 9.
25 T. Handa and H. Yajima, *Biopolymers*, 18 (1979) 873–886.
26 T. Handa and H. Yajima, *Biopolymers*, 19 (1980) 723–740.
27 T. Handa and H. Yajima, *Biopolymers*, 19 (1980) 1723–1741.
28 T. Handa, H. Yajima, T. Ishii, and T. Nishimura, in D. A. Brant (Ed.), *Solution Properties of Polysaccharides, ACS Symp. Ser.*, 150 (1980) 455–475.
29 R. E. Rundle and F. C. Edwards, *J. Am. Chem. Soc.*, 65 (1943) 2200–2203.
30 T. L. Bluhm and P. Zugenmaier, *Carbohydr. Res.*, 89 (1981) 1–10.
31 R. C. Teitelbaum, S. L. Ruby, and T. J. Marks, *J. Am. Chem. Soc.*, 100 (1978) 3215–3217.
32 R. C. Teitelbaum, S. L. Ruby, and T. J. Marks, *J. Am. Chem. Soc.*, 102 (1980) 3322–3328.

33 M. MIZUNO, J. TANAKA, AND I. HARADA, *J. Phys. Chem.*, 85 (1981) 1789–1794.
34 R. J. HACH AND R. E. RUNDLE, *J. Am. Chem. Soc.*, 73 (1951) 4321–4324.
35 F. H. HERBSTEIN AND M. KAPON, *Nature (London), Phys. Sci.*, 239 (1972) 153–154.
36 M. NOLTEMEYER AND W. SAENGER, *J. Am. Chem. Soc.*, 102 (1980) 2710–2722.
37 A. HADDOCK, JR, M. STEIDEMANN, AND M. READNOUR, *Synth. React. Inorg. Met.-Org. Chem.*, 9 (1979) 39–56.
38 R. W. RAMETTE AND R. W. SANDFORD, JR., *J. Am. Chem. Soc.*, 87 (1965) 5001–5005.
39 T. SEKINE, *Nippon Kagaku Zasshi*, 90 (1969) 951–983.
40 F. A. COTTON AND G. WILKINSON, *Basic Inorganic Chemistry*, Wiley, New York, 1976, p. 328.
41 J. M. REDDY, K. KNOX, AND M. B. ROBIN, *J. Chem. Phys.*, 40 (1964) 1082–1089.
42 E. E. HAVINGA AND E. H. WIEBENGA, *Acta Crystallogr.*, 11 (1958) 733–737.
43 P. K. HON, T. C. W. MAK, AND J. TROTTER, *Inorg. Chem.*, 18 (1979) 2916–2918.
44 R. S. MULLIKEN AND W. B. PERSON, *Molecular Complexes*, Wiley, New York, 1969, Chap. 10.
45 P. A. D. DEMAINE, *J. Chem. Phys.*, 24 (1956) 1091–1093.
46 R. M. KEEFER AND T. L. ALLEN, *J. Chem. Phys.*, 25 (1956) 1059–1061.
47 P. C. MANOR AND W. SAENGER, *Nature (London)*, 237 (1972) 392–393.
48 T. KUGE AND K. TAKEO, *Agric. Biol. Chem.*, 32 (1968) 1232–1238.
49 J. F. FOSTER, IN R. L. WHISTLER AND E. F. PASCHALL (Eds.), *Starch Chemistry and Technology*, Vol. 1, Academic Press, New York, 1965, Chap. 15.