Deep Blueing Mechanism of Triiodide Ions in Amylose Being Associated with Its Conformation

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It is generally accepted that the increase in the degree of polymerization (DP) of amylose is accompanied with the bathochromic shift of the blue band (1, 2, 3, 4, 5) and the bluing of iodine/iodide in amylose depends on their concentrations (6) and the mixing rate of iodine/iodide with amylose (7, 8). The bluing mechanism of triiodide ions in starch was first explained by Robin (9) using electronic theory. As an extension of his work, the change of circular dichroism (CD) spectra with the bathochromic shift of the blue band was studied by Handa and Yajima (10, 11) in correlation with the conformation of the amylose-triiodide complex in aqueous solution. However, the characteristics of CD bands correlated with the nature of the deep bluing band peculiar to high DP amylose have not yet been satisfactorily interpreted in accordance with the conformational change of amylose in aqueous solution.

The object of this paper is first to correlate the deep bluing of iodine/iodide with the characteristics peculiar to the conformation of high DP amylose on the basis of the changes in the CD spectra and secondarily to clarify the underlying mechanism governing the phenomena.

Experimental

Materials. Amyloses with average DP 10-500 were prepared by enzymatic degradation of the long polymer. Those samples with DP 10, 20, and 30 were obtained by fractionation through Sephadex-Gel columns. Amylose of DP 1000 was isolated from potato starch by Schoch's method (12). Amyloses with DP 2500 and 4500 were of commercial grade from Wako Pure Industries and Nagase Co., Ltd., respectively. The molecular weights of the amyloses were determined by reducing-end measurement (13) and viscometry (14).

Preparation of Amylose Solutions. Amylose (100 mg) was dissolved into 3 ml of 1 N KOH solution. Then, the solution was

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neutralized through an Amberlite column and diluted to 0.1 %.

Preparation of Amylose-Iodine-Iodide Complex Solutions. The complex solution was parepared at room temparature by mixing of an amylose solution with an equal volume of KI-I_2 solution using different mixing times.

Measurements. Measurements of absorption and CD spectra were made using a Hitachi EPS-3T spectrophotometer and Jasco J-20 spectropolarimeter, respectively. The spectra were always measured 24 hrs after preparation.

Results and Discussion

KI-Effect. It is known that an increase in KI concentration brings about a blue shift of the blue band at a relative low concentration of I_2. Cronan and Schneider (6) pointed out on the basis of a stoichiometric study that the composition of the bound species of iodine/iodide ($I_2.I^-_b$) varied from I_2 to I^-_3 ($b = 0 \rightarrow 1$) with increase of the KI concentration. Handa and Yajima (10) explained the bluing mechanism of triiodide ions bound cooperatively by amylose in the presence of excess KI as coming from the exciton-coupling in a dimeric unit, using electronic theory. They (15) also proposed the lengthening of the coloring unit constituting a segment of polyiodide chain from I^{2-}_6 ($I^-_3.I^-_3$) to I^{2-}_{10} ($I^-_3.I_2.I^-_3.I_2.I^-_3$, $I_2.I^-_3$, $I_2.I^-_3.I^-_3.I_2$ or $I_2.I^-.I_2.I_2.I^-.I_2$) through I^{2-}_8 ($I^-_3.I_2.I^-_3$) corresponding to decrease in the b value, on the basis of the assignment of the four fundamental resonance Raman lines which obviously appear at 159, 111, 55, and 27 cm^{-1}. Likewise, it should be mentioned that the coloring is strongly involved with the multiple charge transfer processes which combine the aforesaid species with the amylose lattice.

Figure 1 shows the change in the CD spectra with an increase of KI concentration at high I_2 concentration for high polymer DP 1000 in the rapidly mixed system. Therein, the composition of the bound species varies depending on the change of b-value in $I_2.I^-_b$ from 0.5, 0.7, 0.9 through 1 correspodning to the increase of KI concentration from 1.2×10^{-4}, 6.0×10^{-4}, 4.8×10^{-3}, 8.4×10^{-3}, 1.2×10^{-2}, and 2.4×10^{-2} M, respectively, based on the stoichiometric determination (6). The mutually split CD bands with opposite signs and symmetrical intensity in the blue band region at low iodide concentration give way to a mild asymmetry above 6.0×10^{-4} M of KI. At high iodide concentration above 8.4×10^{-3} M, the CD bands have the same positive sign. On the other hand, for low DP below 100, the change in intensities of the absorption and CD spectra with iodide concentration was insignificant, although the spectra did show a blue shift depending on the change of bound species. Therefore, it can be said that the behavior of the CD spectra in Fig.1 is peculiar to high DP amylose.

On the electronic aspect of the phenomena, the lowering of

the positive peak associated with the asymmetric shallowing of the negative valley can be ascribed first to the increase in the contributions from the interaction of in-plane oriented dipoles belonging to a pair or double pairs of ions in side-by-side aggregated helices and secondly from a skewed pair of ions at the intermolecular junctions of amylose chains (11). To conform the electronic interpretation of the phenomena at a submolecular level to the understanding from the light scattering study at a molecular level as described in later section, we had to consider that uncomplexed amylose chain in aqueous solution may possess intrisically the parts for two kinds of junctions, i.e., intra- and intermolecular junctions. In this respect, the side-by-side association of partially or completely filled helices originates probably from the rearrangement of hydrogen bonds by iodine/iodide at the intramolecular junctions, whereas the occurrence of the binding of ions in a skewed pair is presumably specific to the rearrangement of hydrogen bonds at the intermolecular junctions.

From another aspect based on the kinetic study of the complex reaction (11, 16), it is believed that the binding of most of iodine/iodide with the lattice of a long polymer is achieved within 1 msec and the configuration of iodine/iodide in the single helices is almost attained close to that for DP 100 within 200 msec. The completion of the configuration of the bound iodine/iodide at the intra- and intermolecular junctions is conceivably achieved with a longer relaxation time. Hence, the extent to which the binding of iodine/iodide occurs in side-by-side associated helices and at intermolecular junctions in the aggregates must depend on the composition of the bound species. We suggest that with increasing KI concentration, the extent of the compact aggregation of helices and the intermolecular entanglement are pronounced not only due to the shielding of electrostatic repulsion but also due to the decrease in the flexibility of the polyiodide chain conforming to the unit segment length which depends on the nature of the bound species. The consideration that the intermolecular junctions increases with KI concentration is supported by the result of KI dependence of sedimentation coefficient of the complex by Dintzis et al (17).

DP-Effect. Figure 2 shows the DP dependence of the absorption and CD spectra for the complex in excess KI at the rapid mixed rate. The spectra are represented by displaying the molar extinction coefficient (ε) and molar ellipticity ([θ]) of bound triiodide ions ($I^-_{3,b}$) in amylose. Those spectra were obtained from the observed ones as reported previously (11). The molarity of the $I^-_{3,b}$ ions was determined using dialysis. The I_2 concentration (5.1×10^{-2} mM) of the system corresponds to a degree of saturation of the $I^-_{3,b}$ ions (q) from 0.9 to 1.0 on the basis of the guide-line method (6) for the plot of the molarity of the $I^-_{3,b}$ ions vs. the dosage of I_2. As shown in the absorption spectra, the bathochromic shift of the colored band from 440 to 800 nm was

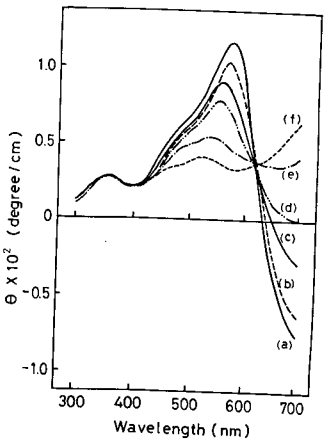

Figure 1. Effect of KI concentration on the CD spectra in the rapidly mixed system KI (in moles): (a) 1.2 × 10⁻⁴; (b) 6 × 10⁻⁴; (c) 4.8 × 10⁻³; (d) 8.4 × 10⁻³; (e) 1.2 × 10⁻²; (f) 2.4 × 10⁻². Amylose (DP 1000), 0.05 g/L; I_2, 5.1 × 10⁻²mM.

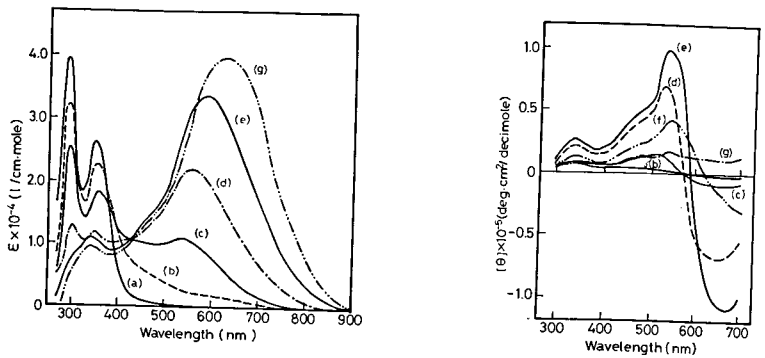

Figure 2. Effect of DP on the absorption and CD spectra of $I^-_{3,b}$ in amyloses in the rapidly mixed system DP: (a) 10; (b) 20; (c) 30; (d) 42; (e) 100; (f) 190; (g) 1000. Concentrations: amylose, 0.05 g/L; KI, 1.2 × 10⁻²M; I_2, 5.1 × 10⁻²mM.

observed with the increase of DP above 10 which was taken as the apparent achromic point. A similar hyperchromic effect was observed in CD bands up to DP 100. The intensities of CD bands decreased significantly with the increase of DP above 100, associated with the gradual bathochromic shift of the blue band. CD bands for DP 1000 did not form a normal pattern. These results suggest that the high polymer complex possesses the deep bluing species in the aggregate of the amylose helices. The asymmetric shallowing of the CD bands can be ascribed to the increase of the foregoing exciton-coupled interactions as discussed in the previous section.

I_2-Effect. Figure 3 shows the effect of iodine concentration or q value on the CD spectra for high polymer DP 1000 in the rapidly mixed system. Up to $q \simeq 0.8$, the mutually split CD bands with opposite signs remained almost unchanged in the pattern and intensities, but above $q \simeq 0.8$, the intensities of the CD bands decreased asymmetrically and very pronouncedly. However, it should be noted that a symtom for the latter change was indicated at a q of 0.6. Then, the CD bands had the same signs around a q of 1, where the wavelength of maximum absorption shows a continuous red shift.

Normally, the intensities of CD bands (+,-) appear much weaker for long polymers than that for the DP 100 even at a q of 0.2. This implies that the bluing of the ions in side-by-side associated helices and in portions at intermolecular junctions occurs even at a low q. The anomalous change of CD pattern above $q \simeq 0.8$ can be ascribed to an initiation of specific aggregation as affected by the extra-ions belonging to the second adsorption (6). The results of component analysis by the decomposition of each spectrum in Fig.3 reveal that above $q \simeq 0.8$, a considerable part of the dipole interaction tends to occur in-plane with scanty rotatory strengths due to the side-by-side aggregation of filled helices through folding with a turn of ca. DP 100.

From the calorimetric data (18), an extra-change of the lattice is expected from the structural energy being involved with the packing of the ions above $q \simeq 0.6$, despite of the fact that the energy of the cooperative adsorption in dimeric units did not vary below $q \simeq 0.6$. Figure 3 shows the corresponding symtom at a q of 0.6 with regard to the change of CD spectral pattern for the rapidly mixed system.

Conformational Change with q at a Molecular Level. The resonance light scattering study with polarized lights from Ne-He laser provided information at a molecular level on the conformational change of the complex with q (19). The highlights of the study can be characterized first by a priority of rod character which suggests a specific extension of the conformation toward one direction at the binding of iodine/iodide as being manifested by an anomalously high value of the depolarization ratio ρ_u and its

pronounced increase with q. Secondly, the highlights can also be characterized by an outstanding contraction of the molecular volume even in a lower range of q in accordance with a decrease in the dissymmetry value Z being endorsed by a pronounced increase in ρ_u. Thirdly, the structure of the complex must preserve some coil nature intermixed with rod character as being suggested by a quasi-constancy of the ρ_h value which stayed always close to 1, irrespective of q.

To characterize the conformational change with q, the fitting of the results was pursued on computer with regard to ρ_u and Z on the basis of the model in a combination of coil and rod besides that in the worm-like chain. In the former model, the conformational change was characterized by the change in the rod factor, contour length and anisotropic factor at a given segmental number, whereas the conformational change was characterized by the change in the contour length, persistent length and anisotropic factor in the latter model. Both results conformed well to each other except some inconsistency on the estimates of the contour length at a low q below 0.2. However, the benefit in employing the latter model exists in that the simulated result can uniquely depict the process schematically in the q-dependent conformational change of the complex. The complex solutions contained in the rapidly mixed system: 0.01 g/l of amylose (DP 2000), 0.24-2.4 mM of KI, and $1.6 \times 10^{-3} - 2.4 \times 10^{-2}$ mM of I_2.

As a result, the following are surmised:

(1) Uncomplexed amylose in aqueous solution must exist as an aggregate on average in tetramer-pentamer which was estimated from the best fit of the observed Z value (1.3) with the theoretical one using the persistent length of 13.6 Å (20).

(2) In so far as the rapidly mixed systems were concerned, the extreme shortening of the contour length even at a q within the range of 0.1-0.2 was uniquely confirmed by the fitting either in the former or in the latter model. Being based on an obvious increase in ρ_u or ρ_v, it was predicted that the anisotropy of the effective segment increases eminently and the persistent length off the complex chain elongates appreciably. The latter result relates to a serious decrease in the number of the effective segments. Accordingly, we have to admit the occurrence of some critical change similar to the phase transition in the conformation of the amylose matrix due to the binding of iodine/iodide even at a low dose. Hence, it seems likely that there is no other alternative explanation on these results except the following: First, a remakable contraction occurs in an amylose molecule itself through some transition of coil to helix by a cooperative action in the propagation and rearrangement of hydrogen bonds resulting in the unravelling of intermolecular junctions. The unravelling must bring about an extra-contribution to the shortening of the contour length in an extent to which the dissociated molecules indicated a persistent length of ca. 300 Å at q = 0.1-0.22. This value was significantly longer than that of deformed helix

(ca. 40-70 Å) (21, 22). Therefore, it may be considered that the surplus in the elongation of the persistent length comes from the side-by-side association of partially filled helices. In respect of an pronounced increase in the anisotropy, the occurrence of a further aggregation of the side-by-side associated helices onto their traverse direction can hardly be considered in this range of q. The vertical progression in the side-by-side association of helices will result in the shortening of the contour length on one hand and on the other hand, in the elongating of the persistent length effectively even at a low dose of iodine/iodide with q below 0.2.

(3) In accordance with a pronounced increase in ρ_u with q in the range of 0.2-0.7 and the corresponding decrease in Z, the increase in the persistent length and anisotropy still continued despite of a slight difference in the shortening of the contour length with q in this range as compared to that in the former range of q. Taking account of a slight decrement in the contour length and a significant increment in the anisotropy with q, the remakable increase in the persistent length can be understood as coming from the tightening of helices through the progression of the complexation. This stimulates the orientation of thin-rod segments toward the vertical direction. Therefore, the incidence of the aggregation to pile up the side-by-side associated helices toward their traverse direction must be yet rare in the q range of 0.2-0.7. Provided that the creation of skewed pairs of ions at the intermolecular junctions can be regarded as a difference between the incidence of the authentic unravelling and that of the ravelling by the intermolecular entanglement, the number of unravelled intermolecular junctions by the binding of iodine/iodide is presumably fewer than that in the former range of q.

(4) In accordance with a specific decrease in ρ_u and Z in the higher range of q above 0.7, a decrease in the anisotropy was predicted despite of a still further elongation of the persistent length associated with the levelling off in the decrease of the contour length. The simulated result predicts that a considerable aggregation of filled helices occurs toward their traverse direction through a propagation of folding action with a turn of ca. DP 100 in accordance with a promotion in the extent of aggregation toward the vertical direction. Thereby, the vertical growth may be promoted being associated with a coagulation probably end-to-end by the intermolecular entanglement in taking account of the balance between an anomalous elongation of the peristent length and an inferior thickening toward the traverse direction through folding.

At q = 1, the persistent length reached finally ca. 1000 Å which was approximately equivalent to the contour length. Similar value of persistent length was found for DNA in an estimate by the worm-like model (23). The result seems somewhat unreal. Nevertheless, it looks adequate in representing the authentic character of the complexation leading to the formation of the rod-like

structure of Bittiger et al (24). It should be noted herewith that the results differed in the slowly mixed system and the final conformation of the complex must remain in a zig-zag rod structure. The conceptualized scheme on the process of the conformational change leading to the rod-like structure of Bittiger et al. will be illustrated later in Fig.14.

Amylose-Effect. Figure 4 shows the effect of amylose concentration on the CD spectra at a weight ratio of iodine to amylose, 0.26, which corresponds to $q \simeq 1$. Up to 0.01 g/l of amylose, the split CD bands in the blue band region had opposite signs and the intensities were almost symmetrical. Above 0.01 g/l, the intensities of the CD bands decreased remarkably and above 0.025 g/l, the split CD bands had the same signs. However, at $q \simeq 0.5$, the change in the spectral pattern with amylose concentration did not occur so susceptibly as described above.

The intensities of the mutually split CD bands (+,-) with a symmetric pattern at the low concentration (0.005 g/l) of amylose with DP 1000 was found to be still considerably smaller than those for DP 100 at $q \simeq 1$. This is primarily ascribed to the side-by-side association of helices at the intramolecular junctions. An obvious increase in the asymmetry of the CD bands (+,-) with increasing amylose concentration can be explained by an increase in the contribution from a skewed pair of the ions at the intermolecular junctions, corresponding to the increase in the number of the junctions in uncomplexed amylose with amylose concentration.

Solvent-Effect. The effect of the intermolecular junctions in the uncomplexed conformation of amylose on the aggregation of helices was examined using the amylose complex solution in H_2O and in 95 % H_2O-5 % DMSO, as shown in Fig.5. Thus, for the H_2O-DMSO system, 10 mg of amylose (DP 1000) was dissolved in 10 ml of DMSO by heating the mixture at 70°C for 2 hrs, and then the solution was stored at room temperature for a day. Subsequently, the stock solution was diluted to 0.1 g/l with H_2O and then equal volumes of the solution and KI-I_2 solution were rapidly mixed.

A similarity is observed in the spectral patterns between the complex solution in the H_2O-DMSO system and the complex solution in H_2O at a low amylose concentration. However, the complex in 99.7 % H_2O-0.3 % DMSO solution with the ratio of DMSO to H_2O close to that in the system of Dintzis et al. (17) gave only a positive CD pattern similar to that in the H_2O system (b) in Fig.5. The phenomena can be interpreted by assuming that the perturbation occurs for hydrogen bonding contributing to the structure at the intermolecular junctions due to the complexation with DMSO. This will exclude the formation of tight bonding between the ions and the lattice occupied with DMSO in the junction portions.

It should be noted that the complex solution containing 5.8×10^{-3} N NaCl as a product of neutralization of alkaline amylose solution gave a symmetric pair of CD bands with a normal pattern

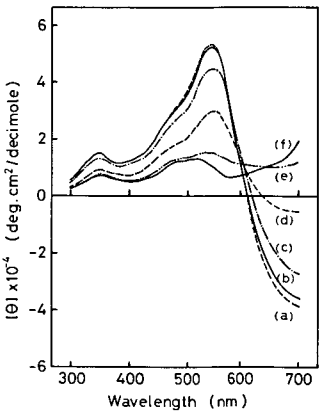

Figure 3. Effect of I_2 concentration on the CD spectra of $I^-_{3,b}$ in amylose (DP 1000) in the rapidly mixed system I_2 (in millimoles): (a) 7.9×10^{-3} (q = 0.2); (b) 2.4×10^{-2} (q = 0.6); (c) 3.1×10^{-2} (q = 0.8); (d) 3.9×10^{-2} (q = 0.95); (e) 5.1×10^{-2} (q = 1); (f) 1.2×10^{-1}. Amylose, 0.05 g/L; KI, 1.2×10^{-2}M.

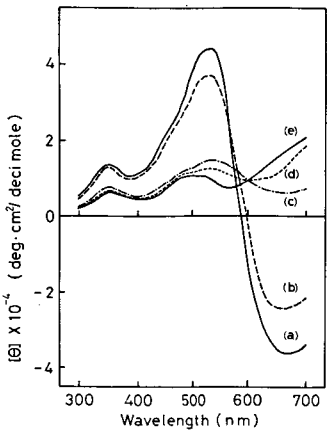

Figure 4. Effect of amylose concentration on the CD spectra of $I^-_{3,b}$ in amylose (DP 1000) at a weight ratio $(I_2)/(amylose) = 0.26$ and KI 1.2×10^{-2}M in the rapidly mixed system amylose (in grams/Liter): (a) 0.005; (b) 0.01; (c) 0.025; (d) 0.1; (e) 0.5.

(+,-) presenting high intensities close to those for DP 100 (a) in Fig.5. Therein, the complex solution was prepared by mixing the $KI-I_2$ solution with the neutralized solution of amylose which was prepared by intermixing the twentyfold diluted solution of 10 g/l amylose in 1 N NaOH with HCl. However, the mere addition of an equivalent amount of NaCl to the present amylose solution was not effective in giving symmetric CD bands with a normal pattern (+,-) at the complexation with $KI-I_2$ solution. It only produced an aymmetric pattern close to that in H_2O system (b) in Fig.5. Therein, the amylose-NaCl solution was prepared by adding 1.2 x 10^{-3} mol NaCl to 100 ml of 0.1 g/l amylose solution which was prepared by the present method through an Amberlite column.

Consequently, the conformations of the complex in aqueous solution must be seriously affected by the in-situ conformation of uncomplexed amylose depending strongly on the method and cation-effect in the neutralization of alkaline amylose solution.

Mixing-Rate Effect. The effect of the rate of mixing iodine/iodide solution with amylose on CD spectra was examined to pursue the function and performance of hydrogen bonding at the intra- and intermolecular junctions in producing different conformations by the binding of iodine/iodide. Figure 6 shows the CD spectra for the complex using varying mixing rates. Slow mixing at 0.08 ml/min and rapid mixing at 300 ml/min is abbreviated by SM and RM, respectively. The spectra all had the same pattern under the mixing rate below 0.08 ml/min. As the mixing rate decreased, the CD bands for the complex reverted into the usual normal pattern, being associated with the blue shift of the band. This blue shift can be recognized as the extinction of deep bluing bands owing to the unravelling of a specific configuration of dipoles at the intermolecular junctions accompanied by the decline in the incidence of the side-by-side association of helices. However, the positive peak of the symmetric CD bands with a normal pattern (+,-) is still located in the longer wavelength side than that for DP 100, indicating a somewhat lower intensity than that for the latter. As for low polymers with DP below 1000, symmetric CD bands with a normal pattern appeared at the SM rate even for a considerably higher amylose concentration (0.1 g/l) and an I_2 concentration more than that corresposning to q = 1. As for the long polymers with DP above 1000, the shallowing of the negative CD band tended to be magnified with DP at the SM rate, indicating an asymmetry for the CD bands (+,-). It is likely that the hydrogen bonding at the intermolecular junctions is comparatively stable in the long polymers (DP 2500 and 4500) and some parts of them still survive even at the SM rate, indicating an equivalent occupied ratio to that at the RM rate as a result of the component analysis by the resolution of spectra. Therein, the occupied ratio refers to the contribution ratio of each species in a mole of the $I_{3,b}^-$ ions involved with the bluing.

The mixing rate dependence of CD spectra can be interpreted

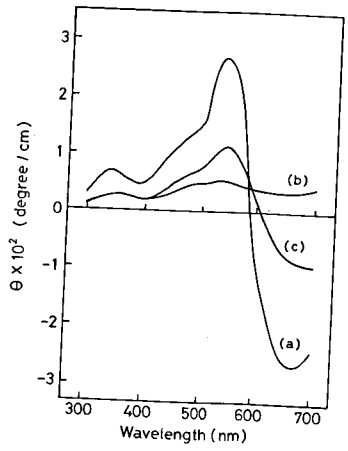

Figure 5. Effect of solvent on the CD spectra in the rapidly mixed system: (a) DP 100 in H_2O; (b) DP 1000 in H_2O; (c) DP 1000 in 95% H_2O–5% DMSO. Concentrations: amylose, 0.05 g/L; KI, 1.2×10^{-2}M; I_2, 5.1×10^{-2}mM.

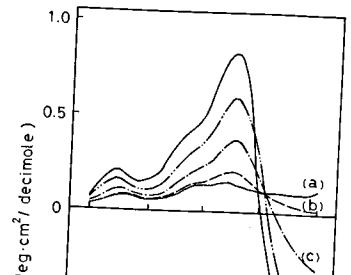

Figure 6. Effect of the mixing rate (in milliliters/min) of KI–I_2 solution with amylose (DP 1000) solution on the CD spectra of $I^-_{3,b}$: (a) 300 (RM); (b) 0.7; (c) 0.25; (d) 0.13; (e) 0.08 (SM). Concentrations: amylose, 0.05 g/L; KI, 1.2×10^{-2}M; I_2, 5.1×10^{-2}mM.

in terms of the difference in a mode of the relaxation between the rearrangement of hyrogen bonding in the labile intramolecular junctions and that in the comparatively stable intermolecular ones. Therefore, the mode of relaxation must be governed by a time difference to attain a given value of q between two extremes (SM and RM). Taking account of these results, we propose that at rapid-mixing rate, the rearrangement of hydrogen bonds at the intra- and intermolecular junctions causes the propagation of side-by-side association of helices, whereas at slow-mixing rate, the rearrangement of the bonds particularly at the intramolecular junctions issues presumably another type of association, i.e., a braided double helix.

Decomposition and Assignment of Spectra. Following the method as applied to the decomposition of absorption and CD spectra for the DP 100 in the previous papers (10, 11), eight bands were isolated from both spectra of $I_{3,b}$ in amylose with DP below 1000 in the slowly mixed system, regardless of q. Those bands were named S_1, S_2, S_3, ..., S_8 going from the long to the short wavelength. The isolated bands S_1 and S_2 in the blue band region were further decomposed into a pair of isolated bands, respectively. The species having the structure which produces the normal blue band equivalent to that of the DP 100 was named the A species whereas that producing another isolated band peculiar to long polymer was named the A´ species. For the long polymer in the rapidly mixed system, the blue band composed of S_1 and S_2 bands plus a deep blue band S_0 around 700 nm was decomposed into three pairs of isolated bands: a pair of isolated bands around 570 and 700 nm with asymmetric rotatory strengths was named the C species, and the other two pairs of isolated bands with symmetric rotatory strengths were named as the A and B species, respectively.

Therefore, the conformation of the complex in the rapidly mixed system is presumed to consist of three structural parts: the normal single helix (A), the side-by-side associated helices (B) and the entangled packet around intermolecular junction (C). Thereby, the C part includes the subsidiarily created junction by intermolecular entanglement in the complexation besides the authentic junction existing in uncomplexed amylose. The complex in the slowly mixed system is composed of two structural parts for amylose with DP less than 1000 and three structural parts for higher DP amylose: the normal single helix (A), a braided double helix (A´) and the entangled packet around intermolecular junction (C).

Figure 7 shows the characteristic spectral patterns of the A, A´, B, and C species. Here, S_1^A, $S_1^{A'}$, S_2^A, and $S_2^{A'}$ are called as A_1, A_1', A_2, and A_2', respectively, and also S_1^B, S_2^B, S_1^C, and S_2^C as B_1, B_2, C_1, and C_2, respectively. The doubly split bands of the A´ and B species are located close to those of the A species. The doubly split bands of each species have signs opposite to each other in the CD band. The dipole strengths of the twin bands (D_1, D_2) were estimated for each species in unit of 10^{-35} cgs:

4.9, 4.9; 9.9, 5.7; 9.6, 5.3, 9.5, 4.7 for the A, A´, B, and C species, respectively. The rotatory strengths of the twin bands (R_1, R_2) were estimated in unit of 10^{-38} cgs: -3.8, 3.8; -2.0, 2.0; -0.4, 0.4; 2.1, -0.4 for the A, A´, B, and C species, respectively. The ratios of the dipole strengths D_1 to D_2 for the A, A´, B, and C species are about 1.0, 1.7, 1.8, and 2.0, respectively. The absolute values of ratios of the rotatory strengths R_1 to R_2 are approximately equal to 1 for the A, A´, and B species, while the ratio is about 6 for the C species.

In order to characterize the isolated bands, a simulation was carried out by using dimer as a coloring unit in the refinement of the exciton-coupled model proposed by Robin (9). As reported previously (10, 11), the characterization of the isolated bands for the A, A´, and C species was made on the basis of a 12-th order secular equation. As for the B species, the simulation was made by using a tetramer model. As shown in Fig.8, the A´ species is characterized by a configuration with a larger angle as compared with the A species. The B species is characterized by an approximately coplanar configuration with a wide fanning-out angle for each pair of dipoles in the side-by-side associated helices. The C species is characterized by the strong interaction between the long-axis transition dipoles of the ion and the short-axis transition dipoles of the other ion under asymmetric perturbation. Therein, the configuration of the short-axis transition in the skewed dimers was abbreviated for simplicity's sake. The C species is probably enclosed in a packet at the intermolecular junction portions or between the helices in the aggregate structure of side-by-side associated ones.

Conformation of the Bluing Species at a Submolecular Level. Figure 9 shows the schematic diagram of the proposed amylose conformation at a submolecular level governing the characteritics of the bluing species. The conformation of the A species is represented by a normal helix which takes a rather looser conformation in solution than in solid. The conformation of the A´ species is represented by a braided double helix which are more restricted in freedom of segmental motion than that of helix for the A species, being mutually tied together by hydrogen bonds at the braiding portions. As mentioned earlier, the A´ species is suggested to originate from the transient binding at the intramolecular junctions which are also expected to take part in the formation of the B species in the rapidly mixed system. The conformation of the B species is represented by side-by-side associated helices, being considerably fixed by tight hydrogen bonds. The conformation of the C species is represented by a packet of the entangled helices among intermolecular chains.

Several Factors on the Occupied Ratios of the Bluing Species. The factors which govern the formation of the A, A´, B, and C species are examined in correlation with the solution characteris-

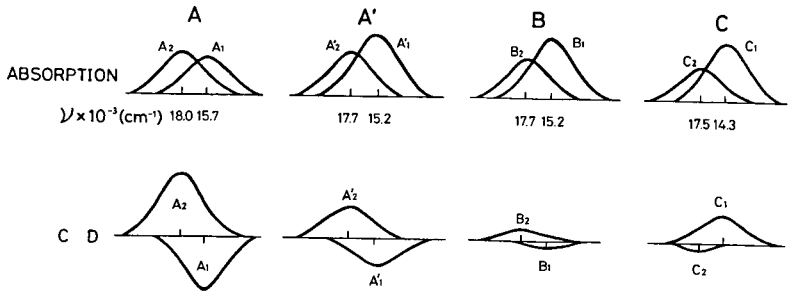

Figure 7. Spectral feature of the blueing species A, A', B, and C

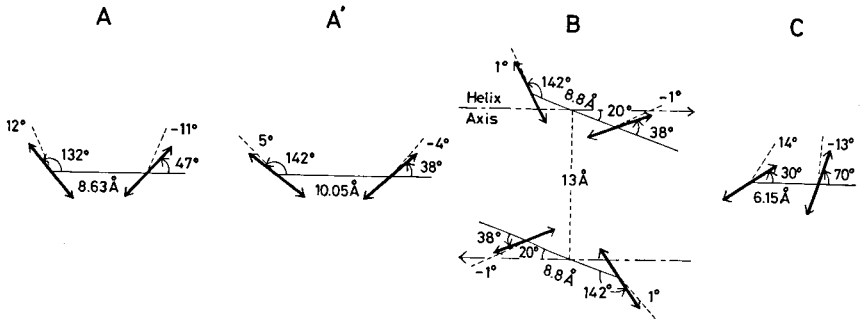

Figure 8. Configuration of the long-axis transition dipoles for the blueing species

tics of amylose in terms of the isolation of bands by spectral resolution and the results of component analysis. The dependence of the occupied ratios of these species on DP above 100 at $q = 0.5$ and 1.0, in the SM ans RM systems is shown in Fig.10. A similar dependence of the occupied ratios on the concentration of amylose for DP 1000 is shown in Fig.11.

The presumption that the C species originates primarily from the authentic intermolecular junctions peculiar to high polymer can be justified except in a low range of q: (1) the q-independence of the formation of the C species in the relation of the occupied ratio to DP and approximately the same level of the occupied ratio for the C species covering both SM and RM systems for high polymers; (2) the q-independence of the increase in the occupied ratio of the C species with amylose concentration.

The presumption that high DP amylose tends to be associated in aqueous solution is also supported by the level of threshold concentration of amylose for the onset of the formation of the C species, which occurs at a low concentration of amylose around 5×10^{-3} g/l even for the DP 1000. For higher polymers, the levels of the threshold concentration are expected to shift to a very low range around 10^{-3} g/l or less, in so far as the present method for the preparation of amylose aqueous solution is concerned.

From the q-independence of the occupied ratio for the C species in Fig.12, it is also revealed that the C species originates primarily from the intermolecular junctions existing intrinsically in uncomplexed amylose and is not created so much during complexation.

The threshold concentration of amylose for the formation of the B species is located considerably below that for the formation of the C species. as shown in Fig.11. This implies that the creation of the transient binding at the intramolecular junctions capable of yielding either B or A´ species must occur independent of the creation of the binding at the intermolecular junctions. However, it is not too much exaggeration to say that the aggregation of helices through folding leading to the rod-like structure of Bittiger et al. can occur first in the concentration range of amylose above 0.005 g/l where the aggregation of uncomplexed amylose can be recognized correspodning to the revelation of the C species. It is expected that in the concentration range below 0.005 g/l, the conformation of the complex at $q = 1$ will stay in a zig-zag rod structure which consists of single helices and side-by-side associated helices even in the rapidly mixed system. Further, it is suggested that the conceptualized rod-structure coagulated end-to-end may be achieved first by a long time crystalzation of these zig-zag rods in dilute solution through a quasi-static coagulation process associated with folding action.

The presumption for a correlation between the formation of the B species and that of the A´ species is based on the approximate coincidence of the threshold time-interval in the mixing for the initiation of the decrease of the B species with that for the

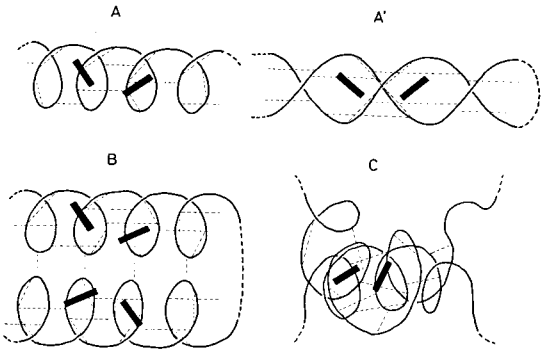

Figure 9. Schematic of the conformation of the blueing species at a submolecular level

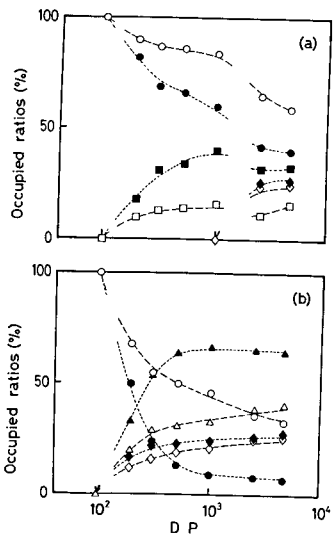

Figure 10. Change in the occupied ratios (percentages) of the blueing species with the DP above 100 at q = 0.5 (○, □, △, ◇) and q = 1 (●, ■, ▲, ◆) in the SM (a) and RM (b) systems: (○, ●) A; (□, ■) A'; (△, ▲) B; (◇, ◆) C. Amylose, 0.05 g/L; KI, 1.2 × 10^{-2}M. The occupied ratio refers to the contribution ratio of each species in a mole of the $I^-_{3,5}$ ion involved with the blueing.

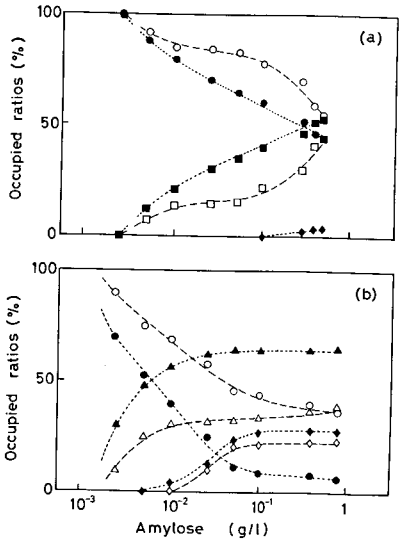

Figure 11. Change in the occupied ratios (percentages) of the blueing species with amylose (DP 1000) concentration at q = *0.5* (○, □, △, ◇) *and q = 1* (●, ■, ▲, ◆) *in the SM (a) and RM (b) systems:* (○, ●) *A;* (□, ■) *A';* (△, ▲) *B;* (◇, ◆) *C. KI, 1.2* × 10^{-2}M.

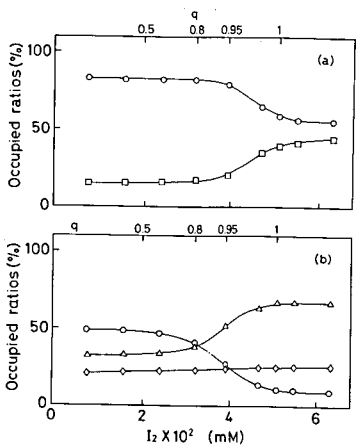

Figure 12. Change in the occupied ratios (percentages) of the blueing species with I_2 *concentration or* q *in the SM (a) and RM (b) systems:* (○) *A;* (□) *A';* (△) *B;* (◇) *C. Amylose (DP 1000), 0.05 g/L; KI, 1.2* × 10^{-2}M.

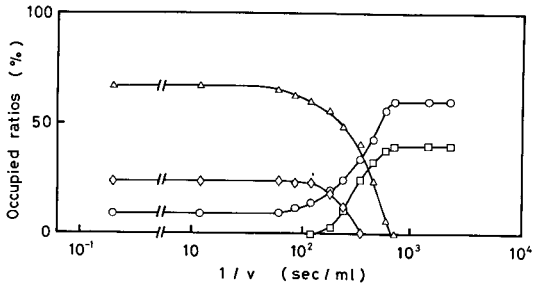

Figure 13. Change in the occupied ratios (percentages) of the blueing species with the reciprocal of the mixing rate (in milliliters per second) at q = *1: (○) A; (□) A'; (△) B; (◇) C. Amylose, 0.05 g/L; KI, 1.2 × 10^{-2}*M.

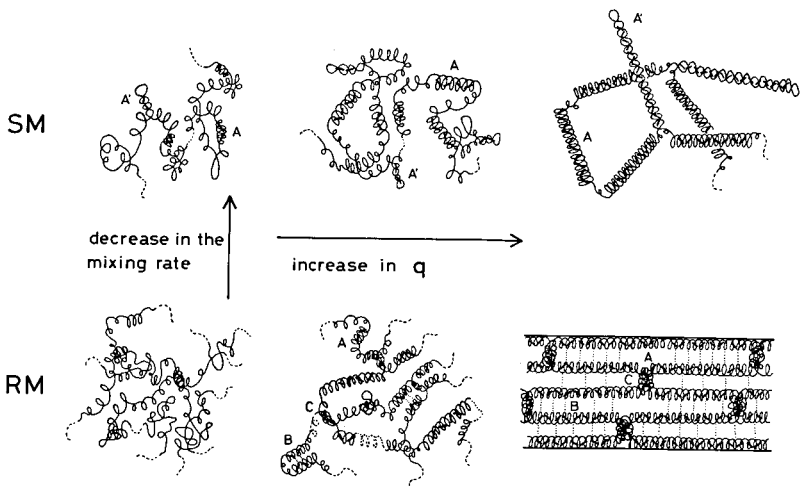

Figure 14. Schematic of the change of the complex conformation with q *in aqueous solution for the slowly and rapidly mixed systems*

initiation of the increase of the A species associated with that of the A species as shown in Fig.13.

Conformational Change of the Complex with q in Aqueous Solution. On the basis of the above results at a submolecular level, a schematic for the conformational change of the complex in aqueous solution with q is proposed in Fig.14 with regard to the slowly- and rapidly mixed systems and the correlation of the changes between both systems, being endorsed by the previous results at a molecular level.

In the slowly mixed system, the complex conformation is characterized by a worm-like helical chain or a randomly oriented zig-zag rod accompanied by the formation of braided double helices. The intramolecular side-by-side association of helices and intermolecular entanglement are scarely developed due to the fractional mixing with a long interval periods exceeding the relaxation time for the rearrangement of hydrogen bonds. This may result in the exclusive formation of the A and A´ species by suppressing the formation of the B species due to extinction of the C species as an anchor.

In the rapidly mixed system, the complex conformation is characterized by a rod-like structure with the intramolecular aggregation of side-by-side associated helices through folding of the chain involving intermolecular association, as presented by Bittiger et al (24).

Abstract

The deep bluing of iodine/iodide peculiar to high DP amylose was studied and a correlation of the abnormal CD spectral patterns with the conformational of the amylose-iodine complex is proposed. The conformational changes in excess KI were characterized by the characteristics of isolated bands (A, A´, B, and C) from spectral decomposition of the observed blue band. Bands A, A´, B, and C come from the exciton-coupling of the basic units of I_3^- ions in a normal helix, braided double helix, side-by-side associated helices, and an entangled packet of intermolecular junctions, respectively. The triiodide complex with high DP amylose can be characterized by the formation of stabilized binding of the ions with the lattice at intermolecular junctions and transient binding at intramolecular junctions. The former binding induces the aggregation of side-by-side associated helices through folding at the rapidly mixed system whereas the latter binding induces the the braiding of double helices as well as the releasing helices singly in a zig-zag rod structure at the slowly mixed rate. These may intrinsically originate from the multiple characters of high DP amylose chain, which is prone to coagulate end-to-end,

fold in side-by-side helices and sometimes braid in double helices, depending on the conditions.

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