Physicochemical Properties of Dilute Aqueous Solutions of Amylose. Optical Rotatory, Viscometric and Osmometric Studies

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Amylose is metastable in a neutral aqueous solution, and tends to retrograde. In the retrograded precipitates, amylose molecules take linear extended configurations and form well-ordered crystallites[1]. It has also been well known that amylose can be precipitated as complexes having a helical configuration from the aqueous solutions by the addition of various complexing agents[1]. Hence, it is natural to suppose that the configuration as well as the degree of aggregation of amylose molecules in solution can be changed, depending on the condition under which they are dissolved[2]. Accordingly, it may be inferred that a dilute aqueous solution of amylose has characteristic properties due to the high tendency to associate and the possibility of the transition among various probable configurations of amylose molecules. However, there have been very few experiments[3] concerning the physicochemical properties of the solution. Therefore, in the present experiments, in order to obtain further knowledge of the properties of dilute aqueous solutions of amylose, optical rotatory power, viscosity and osmotic pressure of the solution at various temperatures have been investigated.

Experimental

Materials. — Amylose was obtained from potato strach by a selective precipitation method with *n*-butyl alcohol and isoamyl alcohol[4]. This amylose was recrystallized twice from boiling water in the presence of excess butanol and the resulting pure amylose was used in this investigation unless otherwise indicated. The β-limit dextrin used was prepared from glutinous rice starch by digestion with soyabean β-amylose which was given by Dr. Yamamoto of Osaka City University. The method of digestion was essentially similar to that described

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1) For a review, see H. Fuwa, "Starch Chemistry" (Denpun Kagaku), Ed. by Z. Nikuni, Asakura Syoten, Tokyo (1951), Chapter III.

2) C. T. Greenwood, *Adv. Carbohydrate Chem.*, **11**, 359 (1956).

3) a) E. F. Paschall and J. F. Foster, *J. Polymer Sci.*, **9**, 73, 85 (1952); b) *J. Am. Chem. Soc.*, **75**, 1177 (1953); c) J. F. Foster and E. F. Paschall, ibid., **75**, 1181 (1953); d) W. W. Everett and J. F. Foster, ibid., **81**, 3459, 3464 (1959).

4) E. J. Wilson, T J. Schoch and C. S. Hadson, ibid., **65**, 1380 (1943).

by Meyer and Bernfeld[5]. The β-limit dextrin obtained was found to be a 41% degraded product of the parent material by measuring the reducing power of the digested mixture.

Optical Rotatory Power. — A Rudolph photoelectric polarimeter model 200 was used for the measurements of the dispersion of rotatory power. A Hitachi polarimeter having a vernier scale of one handredth degree, was used for the measurements of the temperature dependence of rotatory power with a jacketed polarimeter tube which enables thermoregulated water to circulate. All measurements were made with two-decimeter tubes.

About 500 mg. of the amylose was dispersed in 70 ml. of 0.5 M aqueous potassium hydroxide and allowed to stand overnight to complete the dispersion and then filled up to 100 ml. with the same solvent. The solution thus obtained was diluted 2.5-fold either with distilled water, after neutralization (pH 5.4~6.0) with hydrochloric acid, or with the 0.5 M potassium hydroxide. The solutions were filtered and the filtrates were used for measurements. Likewise, the β-limit dextrin was also used.

For the measurements of the variations of rotatory power with temperature, the temperature of the circulating water was changed at an average rate of 3 degrees per 10 min. The temperature of the solution investigated was read from a thermometer immersed in the circulating water. The temperature seemed to be controlled to ±1.0°C. At a given constant temperature six readings were made and the average was taken.

Viscosity.—A usual Ostwald type viscometer was used throughout this investigation. For the solutions investigated the viscometer had flow times of 123~271 sec. at the experimental temperatures. Temperature control within ±0.01°C could be achieved by keeping a thermostated water-bath in an outer larger thermostat.

The procedure used for determining the viscosities was as follows: The amylose solution prepared as described in the above section was filtered through a glass filter. Ten milliliters of the filtrate was pipetted into the viscometer which was placed in the thermoregulated water-bath set at 25.0°C and allowed to stand for 20 min. The flow time was then determined three times and the average was taken. Thereafter the temperature of the water-bath was raised to another given temperature and after a further 20 min. the flow time was determined. This process was repeated at 5 degree intervals, until 50.0°C. The temperature was then decreased and the flow time was redetermined in the same way. This required approximately seven hours. Another aliquot of the solution was allowed to stand at 25.0°C during the period of the above experiment and thereafter its viscosity was also measured.

Osmotic Pressure. — Amylose obtained by a method similar to that described by Azumi and Nakajima[6] was recrystallized three times from water by the addition of *n*-butanol and the resulting pure potato amylose was used in this experiment.

The amylose was first dissolved in 0.5 M aqueous potassium hydroxide solution by stirring under nitrogen atmosphere for about two hours in a flask having a glass stopper which was then sealed and stored at 5.0°C for a night. This stored solution was then neutralized with hydrochloric acid and diluted with distilled water to yield 0.2 M solution with respect to potassium chloride containing 0.1~0.5% amylose which was used for the measurements. The concentration of amylose was calculated from the dry weight of the sample dissolved.

The osmometer employed was a modified Zimm-Myerson type osmometer[7,8]. The inner diameter of the measuring capillary was about 0.8 mm. The amount of the solution required to fill the osmometer was about 9 ml. Toluene was used as a pressure indicator, for the solutions examined tended to stick in the measuring capillary. Collodion membranes prepared in the manner described by Fuoss[9] could be useful in this study.

Setting the meniscus in an appropriate position, the measuring apparatus was allowed to stand at a given temperature controlled within ±0.05°C. The approach of the meniscus to equilibrium was followed by a cathetometer. The equilibrium value was attained within six hours in this case. The experimental error with regard to the equilibrium value was within ±0.1 cm.

Results and Discussion

Specific rotations obtained at room temperature at different wavelengths using solutions of 0.2% concentration are given in Table I. The rotatory dispersions of the amylose and the β-limit dextrin were normal. That is, when the reciprocal of the specific rotation was plotted against the square of the wavelength, the points lay on a straight line (Fig.

Table I. Specific rotations of amylose and β-limit dextrin at various wavelengths

Wavelength mμ	Specific rotation			
	Amylose		β-Limit dextrin	
	0.2 M KCl	0.5 M KOH	0.2 M KCl	0.5 M KOH
589.3	—	150.0	190.6	165.0
577.0	196.2	156.2	197.3	175.3
546.1	217.8	175.5	224.6	197.5
491.6	274.9	215.6	259.4	232.5
435.8	363.0	290.0	369.9	326.6
404.7	428.9	343.8	430.0	381.5
365.0	549.6	437.1	545.1	481.8
312.6	809.9	633.4	—	—

5) K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **23**, 875 (1940).
6) H. Azumi and T. Nakajima, *Sci. Rep. Tohoku Univ., Series I*, **36**, 278 (1952).
7) T. Kawai, "Zikken Kagaku Kōza", Vol. 8, Maruzen Co., Tokyo (1956), Chapter 3.
8) T. Matsuo, *High Polymers Chem.* (*Kōbunshi Kagaku*), **15**, 640 (1958).
9) R. M. Fuoss and D. J. Mead, *J. Phys. Chem.*, **47**, 59 (1943).

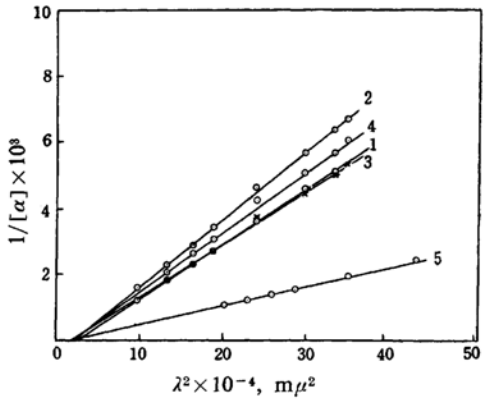


Fig. 1. The rotatory dispersions of amylose, β-limit dextrin and D-glucose. The date for D-glucose are taken from International Critical Table. Only for glucose, the ordinate scale should be read in one tenth of the presented. 1, amylose in 0.2 M KCl; 2, amylose in 0.5 M KOH; 3, β-limit dextrin in 0.2 M KCl; 4, β-limit dextrin in 0.2 M KOH; 5, D-glucose in H_2O.

1). Hence, the variation in the rotation measured can be expressed by a single term Drude equation

$$[\alpha]_\lambda = A/(\lambda^2-\lambda_c^2) \qquad (1)$$

where $[\alpha]_\lambda$ is the specific rotation at a given wavelength, λ, and A and λ_c are the rotation constant and the critical wavelength, respectively. A and λ_c can be obtained from the slope and the intercept of the straight line with the abscissa, respectively. Their values calculated by the least square method are given in Table II. Already determined correlations between rotatory properties and molecular structure make it apparent that optical rotatory dispersion is one of the best qualitative methods for detecting changes in configuration of very large optically active molecules[10]. Particularly, the helical configuration of protein and polypeptide could be well reflected in λ_c. Murakami[11] pointed out from a theoretical viewpoint that the helical structure in which many identical groups are asymmetrically arranged shifts apparently an original λ_c corresponding to random structure towards longer wavelengths. Hence, it may be considered that amylose taking a helical configuration should possess a distinctively different λ_c from that of amylose which has no helical configuration. All of the λ_c found for the amylose and the β-limit dextrin in the two solvents are in close agreement with each other. It seems that the β-limit dextrin never takes a helical configuration because of its highly branched structure[1]. Moreover, D-glucose has also a nearly equal λ_c, as can be seen in Fig. 1 and Table II. Wolff et al.[12] also gave 147 mμ for λ_c of corn amylose in 1 M potassium hydroxide, though they obtained 103 mμ for corn amylopectin. Hence, it can be stated that the wavelength near 140 mμ is an intrinsic λ_c for D-glucopyranose residue, and the amylose in the two solvents at room temperature does not take a well-oriented helical configuration. Although there are some possibilities that right handed and left handed helices exist in the same amount, this seems to be rather rare.

TABLE II. CRITICAL WAVELENGTH AND ROTATION CONSTANT

Substance	Solvent	λ_c, mμ	A
Amylose	0.2 M KCl	151	60.2
	0.5 M KOH	145	48.8
β-Limit dextrin	0.2 M KCl	134	62.1
	0.5 M KOH	141	54.4
D-Glucose	H_2O	141[a]	

a) The value was calculated from the data given in International Critical Table.

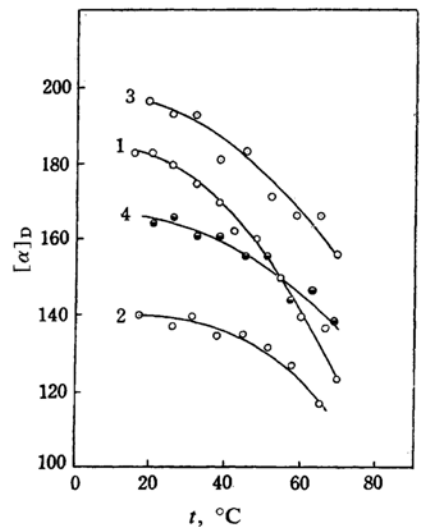


Fig. 2. The temperature dependences of specific rotations: 1, 0.2% amylose in 0.2 M KCl; 2, 0.2% amylose in 0.5 M KOH; 3, 0.2% β-limit dextrin in 0.2 M KCl; 4, 0.2% β-limit dextrin in 0.5 M KOH.

Figure 2 shows temperature dependence of the specific rotations of the amylose and β-limit dextrin in the two solvents at a wavelength 589 mμ (Na-D line). Although the rotation of the amylose in 0.2 M potassium

10) For a review, see C. Djerassi, "Optical Rotatory Dispersion, Applications to Organic Chemistry", McGraw Hill Book Co., Inc., New York (1960), Chapter 17.
11) H. Murakami, *J. Chem. Phys.*, **27**, 1231 (1957).
12) I. A. Wolff, P R. Watson and C. E. Rist, *J. Am. Chem. Soc.*, **74**, 3064 (1952).

chloride solution is considerably larger than that of the amylose in 0.5 M potassium hydroxide at 25°C, the former approaches the latter with increasing temperature, having a large temperature coefficient of negative sign. The rate of the change of the specific rotation is $-1.4°/°C$ in the region of the most rapid change, which is unusually large compared with those of carbohydrates of low molecular weight; for example, sucrose has the rate $\sim 0.01°/°C$[13].

Changing temperature can affect the optical rotation in four possible ways: 1) Configuration of the chain of amylose might change, namely, the helix-coil transition might occur. This was seen in cases of proteins and polypeptides[10]. 2) Ring conformation of glucopyranose residue might differ. Reeves[14] considered that the different specific rotations of corn amyloses in neutral aqueous solution and alkaline solution may be ascribed to different conformations of their glucopyranose rings, that is, B1 may be the conformation in neutral solution and 3B in alkaline solution (the symbols follow Reeves' convention). 3) The vicinal action of solvent might change[15]. This generally decreases with increasing temperature. 4) Hindrance to free rotation around bond axis might change. This would change axial symmetry.

Among these possibilities, the first is unlikely, since the β-limit dextrin also shows qualitatively the same behavior as to temperature dependence as the amylose does (Fig. 2). In addition, the specific rotation at the higher temperature is close to that of α-D-glucose, which may suggest no contribution due to the helical structure to the rotation at the higher temperature. The second and the third possibilities are not clear at present. However, judging from the small temperature coefficients of the rotations of the carbohydrates having low molecular weights, the possibilities appear to be unimportant. Even if the transition between the two ring conformations mentioned above occurs, that would not explain the whole change of the rotation without other causes, since the rotation in neutral solution (corresponding to B1) becomes smaller than that in alkaline solution at room temperature (corresponding to 3B) with increasing temperature. The fourth seems to be most important in the present case. According to Kauzmann and Eyring[15], those influences which tend to restrict freedom of orientation about bonds connecting groups to the asymmetric carbon atom will tend to increase the order of magnitude of the optical activity, causing the first order contribution defined by them. At the lower temperature, the ability of free rotation around bonds such as C–O–C, C–OH and $C-CH_2-OH$ in amylose molecule seems to be strongly hindered owing to hydrogen bonds occurring between hydroxyl groups which come close to each other. This will lead to the higher rotation due to the first order contribution. With increase in temperature, the hydrogen bonds will be more or less ruptured and the free rotation will become easier. This process must also be taken into consideration in order to explain the anomalous behavior of viscosity of amylose, as can be seen later. Consequently, the lower rotation of amylose at the higher temperature may be ascribed to the vanishing of the first order contribution.

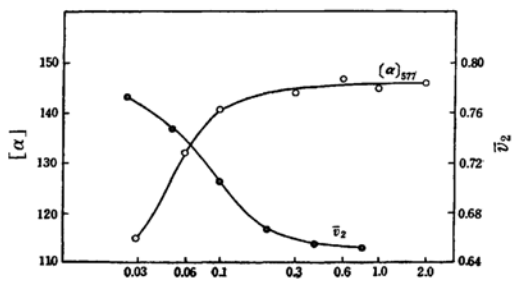


Fig. 3. The variations of specific rotation, $[\alpha]_{577}$, and partial specific volume, $\bar{v}_2$, of amylose in 0.5 M KOH with change in the concentration of amylose. $[\alpha]_{577}$ was measured by the Rudolph photoelectric polarimeter.

In Fig. 3 the variation of the specific rotation with that of partial specific volume which was found by Yoshikawa[16] by measuring density of amylose solution, is shown. Although the experimental error increases as the concentration becomes lower, it may be said that both the specific rotation and the partial specific volume sharply alter with decreasing concentration in the region of low concentration below 0.1%. These facts seem to be explained by the decrease of segment-segment interaction with decreasing concentration, and support the authors' opinion concerning the temperature dependence of the rotatory power.

From the point of view given above, it may be suggested that the large rotation of the β-limit dextrin compared with that of amylose would arise from the inability of free rotation around the bonds between carbon 5 and carbon 6 at branch points in the β-limit dextrin; besides, the highly branching structure would

13) T. R. P. Gibb, Jr., "Optical Methods of Chemical Analysis", McGraw Hill Book Co., Inc., New York (1942), Chapter 8.
14) R. E. Reeves, *J. Am. Chem. Soc.*, **76**, 4595 (1954).
15) W. Kauzmann and H. Eyring, *J. Chem. Phys.*, **9**, 41 (1941).
16) Y. Yoshikawa, Dissertation for Degree of Master, College of Agriculture, Univ. of Osaka Prefecture (1958).

increase segment-segment interaction occurring inside the molecule. On the contrary, in amylose which has no branch point, all of the bonds between carbon 5 and carbon 6 can rotate freely unless the hydrogen bond at carbon 6 is formed with another segment which comes into contact with it. The well known fact that amylopectin shows a greater rotation than that of amylose may be explained by the same consideration.

As for the viscosity, its change may be qualitatively parallel with that of the rotation. Figures 4 and 5 show the variations of the reduced viscosities of 0.2% amylose in 0.2 M potassium chloride and 0.5 M potassium hydroxide, respectively. Arrows marked on the curves in these figures indicate directions of the temperature change. Cross marks in the figures represent viscosities of the samples allowed to stand at 25.0°C for seven hours. It can be seen in these figures that the reduced viscosities in the two solvents decrease with increase in temperature, independent of the

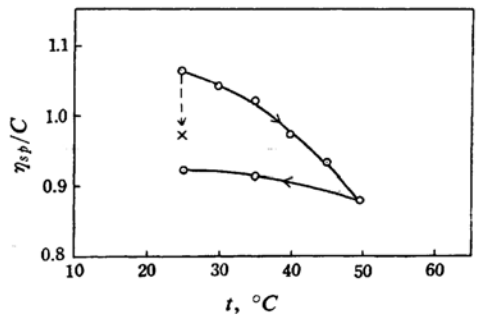


Fig. 4. The variation of reduced viscosity of 0.2% amylose in 0.2 M KCl with temperature.

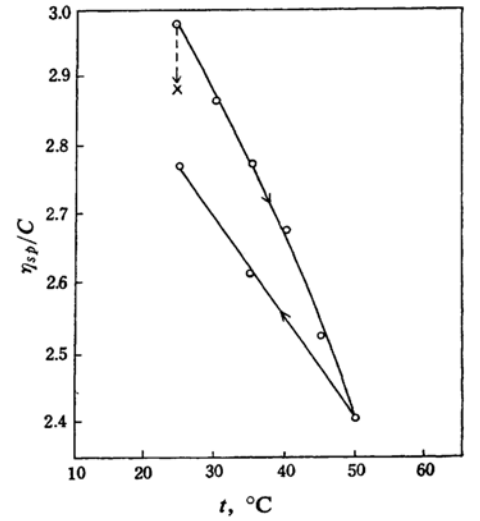


Fig. 5. The variation of reduced viscosity of 0.2% amylose in 0.5 M KOH with temperature.

nature of the solvents used. In a poor solvent, the intrinsic viscosity should increase with increasing temperature, according to the theory of viscosity. Flory[17] gave the following expression for intrinsic viscosity of polymer, assuming a random coil configuration.

$$[\eta] = \Phi(\bar{r}_0^2/M)^{3/2} M^{1/2} \alpha^3 \quad (2)$$

$$\alpha^5 - \alpha^3 = 2C_M \psi_1 (1-\theta/T) M^{1/2} \quad (3)$$

$$\theta = \kappa_1 T/\psi_1 \quad (4)$$

where meanings of the letters are the same as those given by Flory. Although a change in temperature may affect the intrinsic viscosity through alteration of both $(\bar{r}_0^2/M)^{3/2}$ and α^3 occurring in Eq. 2, the change of the latter of these factors is likely to be dominant in a usual case. In a poor solvent, where both κ_1 and ψ_1 generally are positive, θ also will be positive, while negative κ_1 and positive ψ_1 in a good solvent will result in a negative θ. Consequently, in general, the intrinsic viscosity of polymer in a poor solvent should increase with increasing temperature, since α should increase with increasing temperature according to Eq. 3. The increase of α should be most rapid in the immediate vicinity of $T=\theta$.

The viscosity of the amylose even in the aqueous potassium chloride solution, which is a poor solvent having a θ-temperature of 25°C[3d], decreases with increase in temperature, though the reduced viscosity instead of the intrinsic viscosity is dealt with. The anomalous behavior of the viscosity should be accounted for by assuming that $(\bar{r}_0^2/M)$ of the amylose displays an unusually rapid alteration with temperature. $\bar{r}_0^2$ is modified by hindrance to free rotation around bond linking monomers, being greater with increase in the restriction. It is probable that the free rotation around glycosidic bond is strongly hindered by hydrogen bondings between segments through many hydroxyl groups of amylose. With increasing temperature, these hydrogen bonds will be ruptured and the restriction will decrease, which in turn will result in the decrease of $\bar{r}_0^2$. That is, the flexibility of the amylose chain seems to be considerably increased with increasing temperature through the decrease of the degree of the hydrogen bonding.

In the potassium hydroxide solution the possibility of the degradation of the amylose due to oxidation might be considered; however, in the potassium chloride solution the possibility can be ruled out[18]. Figure 4 shows that the solution recooled to 25.0°C does not completely recover its viscosity, and the viscosity

17) P. J. Flory, "Principles of Polymer Chemistry", Cornell Univ. Press, Ithaca, New York (1953), Chapter 14.
18) J. F. Foster and E. F. Paschall, *J. Am. Chem. Soc.*, 74, 2105 (1952).

of the solution after aging at 25°C is lower than that of the solution before aging. These facts may probably arise from the metastability of the amylose solutions having a tendency to retrograde. Possibly the change of the state of the amylose including hydrogen bonding cannot be completely reversible.

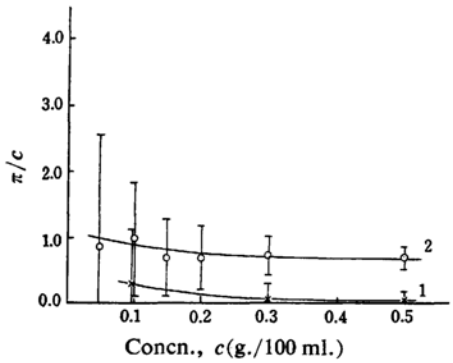


Fig. 6. π/c ratio plotted against concentration of amylose in 0.2 M KCl: 1, at 25°C; 2, at 35°C. The vertical lines drawn represent the ranges of experimental error.

In Fig. 6, the ratio of the osmotic pressure to the concentration of the amylose, π/c, is plotted against the concentration, c. The vertical lines drawn in the figure represent the ranges of experimental error. The results of the osmotic measurements are not satisfactory because the experimental error is relatively great compared with the observed osmotic pressure. It may be, however, stated from Fig. 6 that osmotic pressure at 35°C is greater than that at 25°C, and the initial slopes of the curves of $\pi/c \sim c$ plot are negative. These facts seem to indicate that a significant amount of association of amylose occurs in the solutions examined. The amount may decrease with increase in temperature, and also decrease with decrease in concentration. It should be considered that the solutions used by Everett and Foster[3d] (solutions of amylose in aqueous potassium chloride, aqueous potassium hydroxide and dimethyl sulfoxide) to investigate molecular configuration of amylose in solution, are by no means molecularly dispersed solutions, judging from the above results. Therefore, their conclusion concerning the molecular configuration should not be accepted without modification.

Thus it may be said that all of the anomalous phenomena observed, indicate that hydrogen bonding plays a significant role in the properties of the dilute aqueous amylose solutions used in these experiments just as in those of more concentrated solutions or pastes[19]. In other words, any interpretation of the behavior of amylose aqueous solutions may be impossible without postulating hydrogen bondings, even in the case of such dilute solutions as 0.1% or so. At present, however, it is unfortunate that intermolecular and intramolecular hydrogen bondings can not be distinguished with respect to their effects acting on the described properties of the solution except in the case of osmotic pressure. The osmotic measurements may show the existence of the intermolecular hydrogen bondings. However, it seems natural to suppose that the intramolecular hydrogen bondings also exist and have great influence on the properties of the solution together with intermolecular hydrogen bondings.

As already stated above, the well-oriented helix of the amylose in the neutral aqueous solution can be excluded. However, the possibility of the existence of the deformed helix of amylose suggested by Holló and Szejtli[20] remains uncertain, and it seems worth while to reconsider this problem. According to them, the viscosity of acid solution of amylose is unchanged by the addition of iodine, as far as the added iodine is taken up to form the complex. Only when excess iodine is added, is the viscosity changed. If the configuration were substantially altered by the addition of iodine, it would affect the viscosity of the solution. Therefore keeping in mind the helical configuration in amylose-iodine complexes, the configuration of amylose in the solution should not be supposed to be substantially different from the helical configuration. The amylose in the neutral aqueous solutions may be permitted to take the deformed helical configuration, if it is assumed that the deformed helix has no contribution to the optical rotation. In the previous paper[21], the present authors suggested that the vanishing of the blue color of amylose-iodine complex at high temperature (70°C or so) appears to be ascribed to the random configuration of the amylose molecules. Considering these facts with the ease of the amylose-iodine reaction at room temperature, it seems probable that the amylose in neutral solution at room temperature may take the configuration of the deformed helix, which may easily be transformed into the well-oriented helix, rather than take a quite disordered random configuration. The deformed helical molecules appear to be more disordered with increasing temperature. Accordingly, the helical configuration of amylose in the solution at higher temperature may be

19) G. V. Caeser, "Chemistry and Industry of Starch", Ed. by R. W. Kerr, Academic Press, Inc., New York (1950), Chapter 9.

20) J. Holló and J. Szejtli, *Periodica Polytechnica*, **1**, 223 (1957); **2**, 25 (1958); *Die Stärke*, **10**, 49 (1958).

21) T. Kuge and S. Ono, This Bulletin, **33**, 1273 (1960).

more unreasonable than that at lower temperature.

Although Everett and Foster[3d] suggested a random coil configuration of amylose in aqueous neutral solutions from the studies of viscosity and light scattering, their results should be reconsidered, taking the above stated high degree of molecular association of amylose into account. If we assume the high degree of association of molecules having some flexibility, it may be reasonably supposed that the molecules must behave as a random coil in appearance, since a real polymer chain that is restricted in free rotation may be approximated by an equivalent freely jointed chain[22]. Associated amylose molecules, as a whole, may be statistically equivalent to a random coil polymer having great molecular weight.

As a consequence, the most probable configuration of amylose molecules dissolved in neutral aqueous solutions at room temperature, seems to be associated deformed helix which is tentatively drawn in Fig. 7.

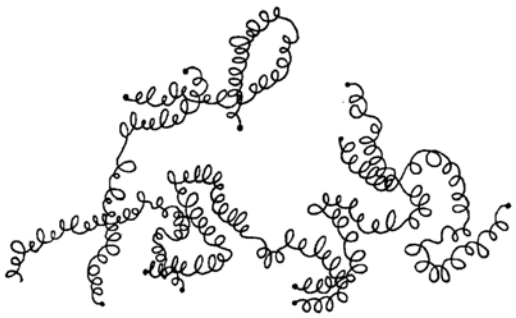
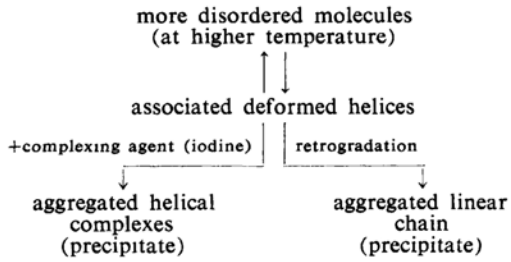


Fig. 7. Supposed configuration of amylose molecules dissolved in aqueous solutions. Dot drawn represents the end of molecule. The length of chain corresponding to each molecule is conveniently drawn to be shorter than that of real molecule.

It should be noted that amylose is metastable in neutral aqueous solutions. Hence, the supposed configuration drawn in Fig. 7, which seems to correspond to "native aggregate" proposed by Paschall and Foster[3a], is a metastable form and then may be stabilized by retrogradation (phase separation). Paschall and Foster also found that the native aggregate can not retrograde at room temperature without first undergoing at least partial disaggregation, and the disaggregation continues along with retrogradation. Therefore, the linear extended configuration of amylose seems to be formed only when retrogradation (crystallization) takes place. Consequently, the following scheme for molecular configuration change of amylose in aqueous solution may be proposed;



22) P. J. Flory, "Principles of Polymer Chemistry", Cornell Univ. Press, Ithaca, New York (1953), p. 411.

Summary

Optical rotatory power, viscosity and osmotic pressure of dilute aqueous solutions of amylose have been studied. Optical rotatory dispersions of amylose and β-limit dextrin in 0.2 M potassium chloride and 0.5 M potassium hydroxide are normal and can be well expressed by a single term Drude equation. All of the λ_c obtained from the analysis of the dispersion curves are close to 140 mμ which may be considered as a characteristic value of glucopyranose residue. This seems to indicate that there is no well-oriented helix of amylose in the solutions. With increasing temperature, the optical rotations of amylose and β-limit dextrin decrease. Amylose, especially in a neutral aqueous solution, has an unusually great temperature coefficient of specific rotation amounting to $-1.4°/°C$ as the largest value. Parallel with this change, the viscosities of the amylose solutions decrease with increasing temperature. These results seem to be well explained by assuming that the flexibility of amylose molecules is considerably influenced by temperature through the change of the degree of hydrogen bonding between segments of the molecules. Osmotic measurements have given evidence to prove the occurrance of the association of amylose molecules in the solution examined. The degree of the association has been found to decrease with increasing temperature. These facts indicate that hydrogen bonding plays a significant role in the properties of the dilute aqueous solution.

The molecular configuration of amylose in aqueous solutions has been discussed. It seems to be most probable that amylose molecules in the solutions take the configuration of deformed helix, and associate highly with each other. No linear extended configuration is possible. The figure of the supposed configuration is drawn.

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