

The Transition from Helix to Coil at pH 12 for Amylose, Amylopectin, and Glycogen¹

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ABSTRACT

Previous studies have indicated that amylose undergoes a transition from the helix to the random coil conformation at pH 12. Such a transition produces a drop in intrinsic viscosity which corresponds to approximately 40% of the initial viscosity. To examine this phenomenon more fully, the viscosities of amylopectin, glycogen, and dextran as well as that of amylose were obtained at pH 7 and 12. The results show that when sufficient salt is present, there is a drop of approximately 40% in intrinsic viscosity for amylopectin, glycogen, and amylose but not for the α -1,6-linked dextran. The helix therefore must also exist in amylopectin and glycogen, but not in dextran. It is concluded from this and other data that the stabilizing force for the helix must be hydrogen bonds between the C-2 and C'-3 hydroxyl groups of adjacent glucose units. In the absence of added salt, the value $[\eta]$ increases in going from pH 7 to pH 12. This increase is caused by a stiffening of the chain due to electrostatic repulsion of the ionized hydroxyl groups of amylose. Addition of 32% methanol (theta solvent) to a 0.5N NaOH solution reduces the value of $[\eta]$ for amylose to that obtained at pH 12. Hence, the reduction in $[\eta]$ at pH 12 in the presence of salt is not due to ion-binding or similar phenomena. Rather, this reduction in $[\eta]$ for both linear and branched α -1,4-linked polyglucosides is caused by elimination of electrostatic repulsion forces and by destruction of the helix. It is concluded that the helix of different molecules in the same medium may have a variable number of glucose units per helical turn (most likely seven to eight glucose units in water). This number may be fractional rather than integral, because the stability of the helix does not depend on hydrogen-bonding between helical rings.

There is now much evidence that amylose exists as a helix at neutral pH in aqueous solutions. Foster and Zucker (1) and Hollo and Szejtli (2) were among the first to propose that amylose exists as a helix in aqueous solutions. Their conclusions were based on streaming dichroism and on the viscosity studies of amylose. These studies showed that the amylose did not undergo any conformational change upon addition of I_3 to the amylose solution, and it is known that this complexing agent is associated with the helical structure of amylose (3). On the basis of other data, Kuge (4) in 1961 also proposed that amylose exists as a helix at neutral pH. The most substantial proof concerning the existence of helices in amylose is in the publication by Rao and Foster (5): in their studies an abrupt drop in viscosity occurred at pH 12 and they therefore concluded from this and from other data that the amylose helix is transformed into a random coil. This drop in viscosity was confirmed by Doppert and Staverman (6). In later work, Rao and Foster (7) did a similar study using carboxymethyl amylose; it underwent the same type of changes as those for the helix-to-coil transformation. However, because the carboxymethyl amylose has electrostatically charged carboxyl groups attached to it, the destruction of the helix in this amylose occurred at a lower pH. The recent work of Szejtli *et al.* (8) in studying

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the amylose- I_3^- complex at low pH has shown that such helices exist in segments. Erlander and Tobin (9) have proposed that such helical segments are undergoing rapid formation and are caused by the stress imposed on the amylose helix by the thermal motion of the molecule. Such a model accounts for the observation that the amylose helix behaves as a continuous helix when subjected to periodate oxidation (10). The physical properties of amylose therefore show that it behaves as a continuous helix undergoing rapid reversible segmentation in neutral aqueous solutions.

But how is this helix destroyed at pH 12? By observing that amylose migrates in an electrostatic field at around pH 12, Doppert and Staverman (6,11) have now shown definitely that amylose becomes negatively charged at around pH 11. This negative charge is due to the ionization of the hydroxyl groups of starch rather than to the association of OH^- groups to the starch (11). Moreover, these authors observed that 0.003M $BaCl_2$ or higher concentrations will cause amylose to retrograde from a 0.05M KOH (pH 12.7) solution. This experiment again indicates that any agent which can neutralize the charge will also precipitate the amylose. They estimated that the pK of amylose must be approximately pK = 12.6 or 12.7 at 25°C. Consequently, there is conclusive evidence that ionization of the hydroxyl groups of amylose destroys the amylose helix at pH 12.

Application of the studies made by Rao and Foster (5) will now be made to the branched polyglucosides amylopectin, glycogen, and dextran. It will be shown that amylopectin and glycogen (but not dextran) undergo the same helix-to-coil transition at pH 12 as in the case of amylose.

EXPERIMENTAL

Preparation of Samples

The 148,000-molecular weight (MW) amylose was prepared by extracting the amylose from a laboratory-prepared, SO_2 -steeped corn starch by autoclaving under 15 p.s.i. (120°C.) for 2 hr. in water saturated with butanol. The amylose-butanol complex was recrystallized five times by cooling a hot solution saturated with butanol according to the method of Schoch (12). The 287,000- and 316,000-MW corn amyloses were prepared by first grinding the kernels with water in a Waring Blendor. The protein was removed by shaking the centrifuged starch with 20% Pentasol. The starch was then dispersed in 8M LiBr at 114°C. for 4 hr. under a helium atmosphere as described by Erlander and French (13). The LiBr was removed by precipitating the starch in 95% ethanol and washing the precipitate several times with 95% ethanol. Amylose was separated by cooling a hot aqueous solution of the starch saturated with Pentasol. The resulting amylose was dispersed under helium atmosphere in a hot aqueous solution saturated with butanol. The solution was buffered at pH 6.3 with 0.5M phosphate. The hot solution was cooled slowly to room temperature and then slowly to 2°C. in a cold room. Throughout the cooling, the solution was stirred briskly. The precipitate was removed by centrifugation and washed with butanol-saturated water. This procedure was repeated two more times. The potato amylose was isolated in a similar manner. The amyloses were washed with methanol, air-dried at room temperature, and then stored in a closed container.

The rabbit-liver glycogen was purchased from Pfanstiehl Laboratories Inc., lot No. 6290. The sweet-corn glycogen was prepared and purified in the following manner (14): The kernels of sweet corn were ground in a Waring Blendor for 2 min. in the presence of water (100 ml. of kernels/500 ml. water). The slurry was centrifuged in an International centrifuge to sediment the ground kernels. This water-extraction process was again repeated on the ground kernels to ensure complete extraction of the glycogen. The supernatant material was added to methanol plus a pinch of salt, the final percentage of methanol being approximately 80%. The precipitated soluble glycogen was separated from the supernatant material by centrifugation in 250-ml. glass bottles in a swinging buckethead of the International centrifuge and then redissolved in water. The solutions were boiled for 1 or 2 min. to get rid of most of the methanol in aqueous solution. Solid guanidinium chloride (GCl) was then added to make a 4M solution. The purpose of the 4M GCl is to aid in the separation of the glycogen from the protein. The glycogen was precipitated from this solution by addition of 20% potassium iodide plus 12% iodine according to a modification of the method of Pucker *et al.* (15) and Kunetz (16). The isolated glycogen was dispersed in water and lyophilized.

The dextran used in these studies was a fraction of acid-hydrolyzed NRRL B-512 dextran obtained by the method of Senti *et al.* (17).

The amylopectin was obtained from the Pentasol fractionation procedure given above for separation of amylose.

Light-Scattering and Viscosity Measurements

Intrinsic viscosities were measured in No. 50 Cannon-Fenske viscometers, which had flow times of approximately 300 sec. for water. Kinetic energy corrections were not applied, since they were negligible at these flow times. The temperature of the viscosity bath was maintained at $25.00^\circ \pm 0.02^\circ\text{C}$. Intrinsic viscosities were obtained from the relationship

$$\lim_{C \rightarrow 0} [\eta/\eta_0 - 1]/C.$$

The amylose, amylopectin, glycogen, and dextran were first dissolved in 1M KOH and then neutralized with the appropriate amount of HCl to obtain a pH of 12 or 7. The correct amount of KCl was added to give the desired amount of added salt. In salt-free solutions, the alkali-dispersed amylose was diluted from 0.5M KOH to pH 12 and 0.1M KOH. In other studies the amylose or amylopectin was dissolved in 4M GCl for neutral pH study. Theta solvent was obtained by dissolving the amylose or amylopectin in 0.5M NaOH and then adding methanol until the concentration was 32% (v/v.).

Solutions were clarified for light-scattering and viscosity studies by centrifugation of the polysaccharide samples for 1 hr. at 40,000 r.p.m. ($105 \times 10^3 g$) in a Spinco model L ultracentrifuge. Supernatant materials were carefully removed by vacuum pipetting at a constant rate. The solution was further clarified for light-scattering by filtration through a No. 015 Selas glass filter directly into the dissymmetry cells. Cylindrical cells were used for the larger-MW components, to obtain Zimm plots. The light-scattering photom-

eter and the methods employed were the same as those previously described by Senti *et al.* (17).

RESULTS

The intrinsic viscosities at pH 7 and 12 for amylose, amylopectin, sweet-corn glycogen, rabbit-liver glycogen, and dextran are given in Table I for

TABLE I
INTRINSIC VISCOSITIES OF POLYSACCHARIDES AT pH 7 AND 12

POLYMER	\bar{M}_w	SOLVENT	$[\eta]$ (pH 7)	$[\eta]$ (pH 12.0)	PERCENTAGE CHANGE ^a
Potato amylose	520,000	{no added salt ^b	0.88	1.60	-82
		{0.50M KCl (+KOH)	.88	.57	35
		{0.75M KCl (+KOH)	.88	.54	39
Corn amylose	316,000	1.0M NaCl	.72	.42	42
Corn amylopectin	100×10^6	1.0M KCl	.88	.50	43
Sweet-corn glycogen	18×10^6	1.0M KCl	.070	.042	40
Rabbit-liver glycogen	4×10^6	{1.0M KCl	.050	.030	40
		{2.0M KCl	.050	.027	46
Dextran	2.5×10^6	{1.0M KCl	.82	.82	0
		{2.0M KCl	0.56	0.56	0

^aPercentage change equals $100([\eta]_7 - [\eta]_{12})/[\eta]_7$, where the subscript on $[\eta]$ refers to the pH value.

^bThe amylose was dissolved 0.5M KOH and this solution was diluted with distilled water to obtain a 0.1M KOH solution (pH = 12.3).

various solvent systems. The intrinsic viscosities for the sweet-corn glycogen were obtained from the points shown in Fig. 1 by extrapolation of the points to zero concentration. As seen in this plot, the concentration dependence was the same at pH 7 and 12. The extrapolations of the viscosity studies for rabbit-liver glycogen are given in Fig. 2. In these studies two concentrations of salt were used: 1.0M and 2.0M KCl. In this plot the slopes of the lines are slightly different at pH 7 and 12. This may be due to protein impurities. Both the rabbit-liver glycogen and the sweet-corn glycogen (Table I) have decreases in intrinsic viscosity of approximately 40% at pH 12 in comparison to their values at pH 7. The same result is obtained for corn amylopectin. This drop in viscosity is the same as that obtained for both corn amylose and potato amylose (Table I). As mentioned in the introductory section, this drop in viscosity corresponds to a destruction of the helices of amylose. This result was quite surprising for the branched polysaccharides, because of their high degree of branching. For example, the sweet-corn glycogen has a degree of branching of approximately 7% as shown previously (14,18), and the rabbit-liver glycogen has a degree of branching of approximately 8%. The corn amylopectin has about 4.0% branching (14,18).

To test whether this change in viscosity was due to ionization of hydroxyl groups on the branched polysaccharides, the intrinsic viscosity for dextran at pH 7 and 12 in 1.0 and 2.0M KCl was obtained, and the extrapolation of the results is shown in Fig. 3. The concentration dependence of the dextran for the 1.0M KCl is not linear; nevertheless, it does curve over to meet the linear line for the pH 7. This nonlinearity must be due to charged groups on the dextran. Considering this concentration dependence of dextran at

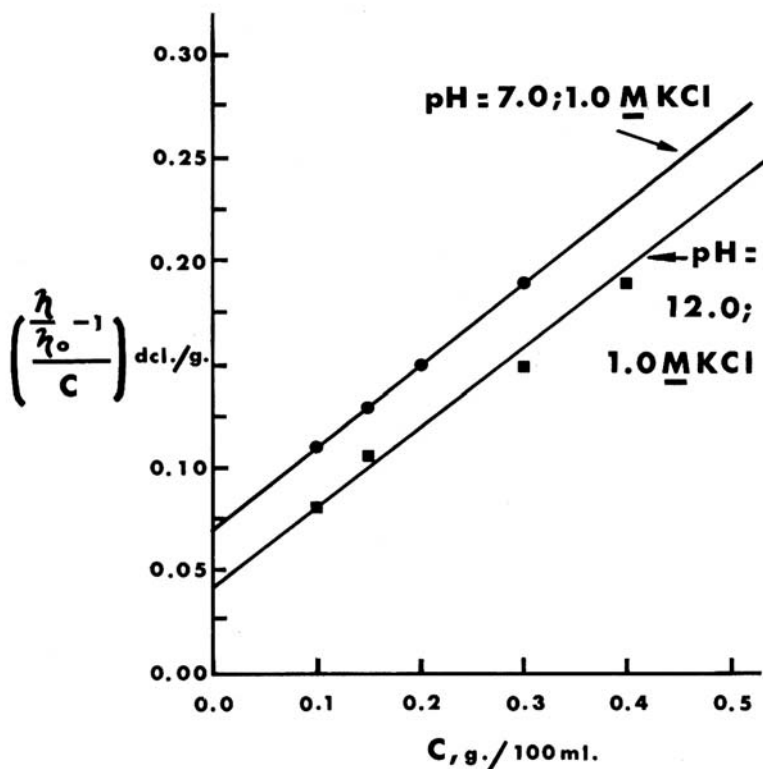


Fig. 1. Sweet-corn glycogen. Viscosities were obtained at pH 7.0 and pH 12.0 in 1.0M KCl.

pH 12, it is seen that $[\eta]$ is the same at pH 7 and 12 in 1.0M KCl. To eliminate these concentration effects, the specific viscosities of dextran were obtained in more concentrated salt solutions. As shown in Fig. 3, this concentration dependence is absent in 2.0M KCl. In both the 1.0M and 2.0M KCl solutions the intrinsic viscosities of the dextran are therefore the same at pH 7 and pH 12. Consequently, the drops in viscosity given above for the branched polyglucosides are not due to ionization of the hydroxyl groups.

Table I shows that at pH 12 for the potato amylose the intrinsic viscosity varies according to the amount of salt added. At pH 7 the intrinsic viscosity appears to be independent of salt concentration. In the absence of salt the intrinsic viscosity at pH 12 increases instead of decreases. This increase is almost twofold: 0.88 at pH 7 and 1.60 at pH 12. As the concentration of alkali is increased to 0.5M KOH, the value of $[\eta]$ increases to $[\eta] = 2.16$. At concentrations of KOH higher than 0.5M KOH in the absence of added salt, the intrinsic viscosity begins to decrease for a short span (19). Hence, in the absence of salt the intrinsic viscosity goes through a maximum at high concentrations of alkali, and the minimum at low concentrations of alkali (pH 12) is absent. This maximum for amylose was also observed by Maywald,

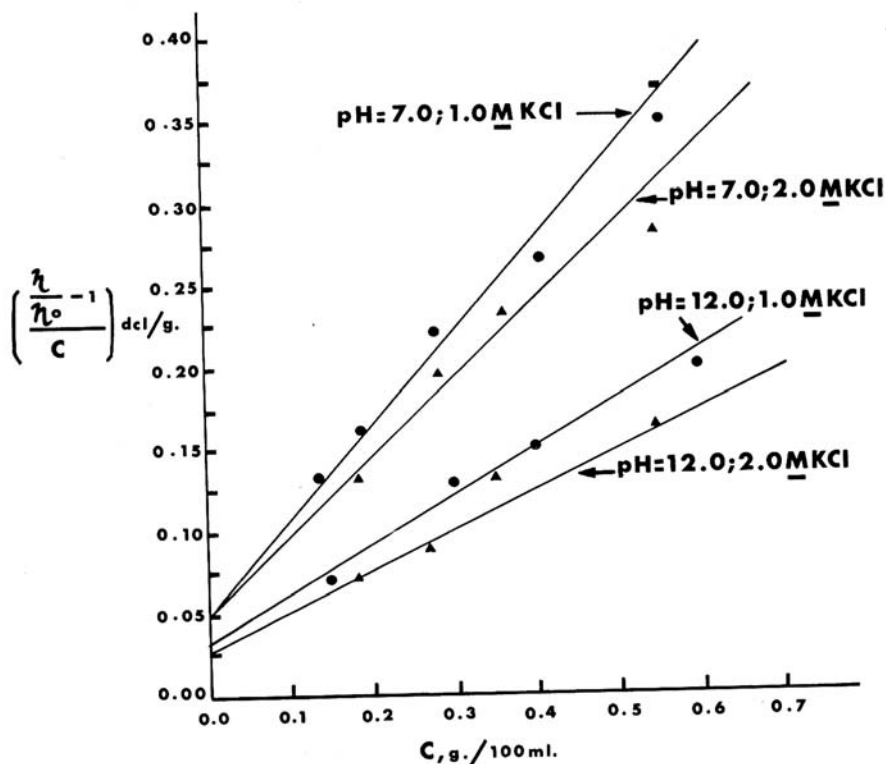


Fig. 2. Rabbit-liver glycogen. Extrapolation of reduced viscosities to zero concentration for rabbit-liver glycogen at pH 7.0 and pH 12.0. Two concentrations of salt were used: 1.0M and 2.0M KCl.

Schoch, and Leach (20). Addition of salt to the 0.5M KOH solution drops the value of $[\eta]$ to 2.06 for 0.5M KOH + 0.5M KCl. Again the same effect of salt is seen as above.

The effect of various solvents on the intrinsic viscosity of amylose is shown in Fig. 4 with a Kurata and Stockmayer (21) plot. It was shown previously (22) that the Flory-Fox-Schaeffgen (21,23) plot of $[\eta]^{2/3}/\bar{M}_w^{1/3}$ vs. $\bar{M}_w/[\eta]$ gives the same erroneous values of K_w for amylose in alkali solutions as in the case of atactic polypropylene. Hence, the Kurata-Stockmayer plot must be employed. With such a plot it was shown that extrapolated values of K_w (where $[\eta] = K_w \bar{M}_w^{a_w}$) are the same for amylose in the solvents 0.33M KCl, 4.2M GCl, and dimethyl sulfoxide (DMSO). Hence, in all of these solvents the same segment length and consequently the same conformation is obtained for amylose. Consequently, the points in Fig. 4 illustrate that the theta solvent 32% CH_3OH + 0.33M NaOH gives the same conformation of amylose as at pH 12 where the helix is destroyed. The points of Fig. 4 indicated by black dots also show how the amylose samples of Table I are changed from the helix (pH 7) to the more random conforma-

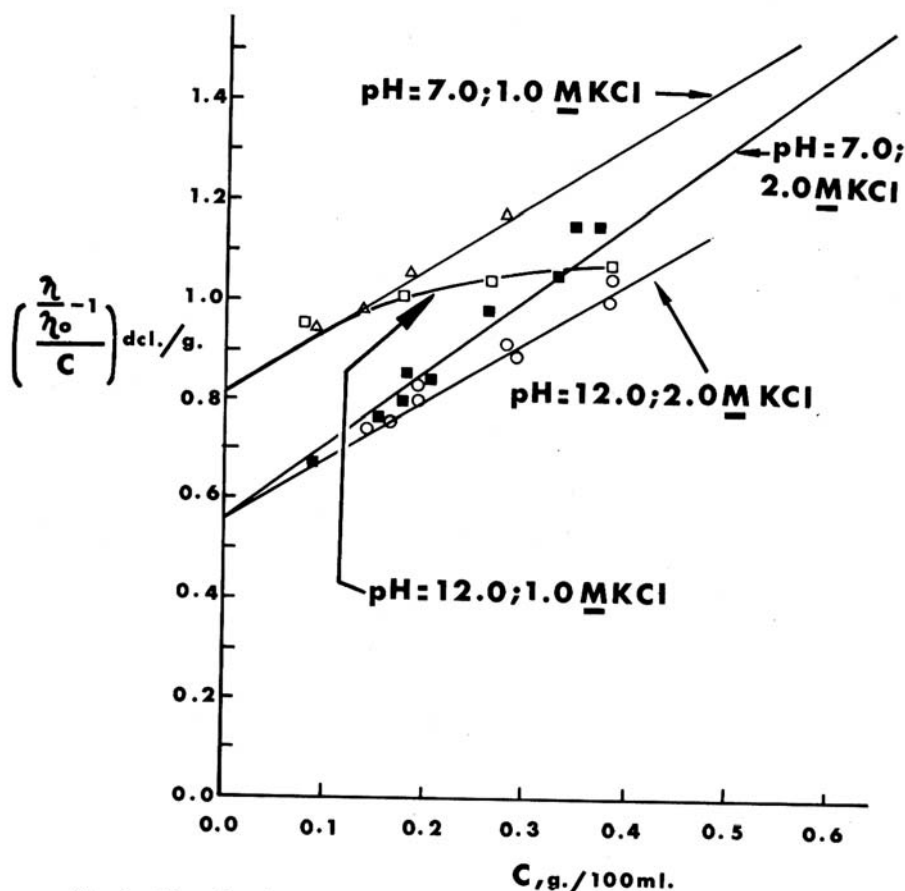


Fig. 3. Viscosity of dextran. The reduced viscosities were obtained at pH 7.0 and 12.0 for the dextran. Two concentrations of salt were employed: 1.0M and 2.0M KCl.

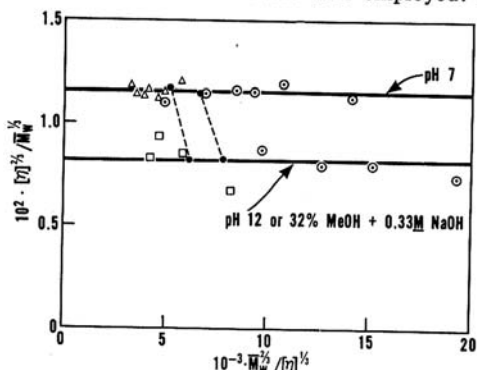


Fig. 4. Viscosity of amylose in various solvents as examined by a Kurata and Stockmayer (21) plot: Δ , amylose in 4.2M GHCl (pH 7); \bullet , points obtained in the solvent 0.75 and 0.50M KCl for pH 7 and 12; \circ , data obtained from Rao and Foster (3) in 0.33M KCl, pH 12; \square , points obtained in the theta solvent 32% (v/v.) $\text{CH}_3\text{OH} + 0.33\text{M NaOH}$.

tion (pH 12). The net result is that hydrogen-bond-breaking solvents (4.2M GCl and DMSO) retain the helix, whereas theta solvents in alkali solutions destroy the amylose helix. A similar drop in $[\eta]$ at pH 12 was observed by Doppert and Staverman (6), who in addition showed that this drop occurs also in 6M urea.

The intrinsic viscosity of amylopectin in the theta solvent 32% CH₃OH + 0.33M NaOH was also examined, and the results are compared to those of amylose in Table II. The drop in viscosity for amylose in going from the

TABLE II
INTRINSIC VISCOSITIES OF CORN AMYLOSE AND AMYLOPECTIN IN VARIOUS SOLVENTS

SAMPLE	\bar{M}_w	SOLVENT FOR $[\eta]$		PERCENTAGE CHANGE IN $[\eta]$
		4M GCl	32% CH ₃ OH + 0.33M NaOH	
Amylose	148,000	0.45	0.29	% 36
Amylose	287,000	0.66	0.42	37
Amylopectin	160 × 10 ⁶	0.97	0.54	45

neutral solvent 4M GCl to the above theta solvent in alkali solutions is essentially the same as that recorded in Table I for the pH 12 solvents when salt is present.

DISCUSSION

Similarity of Conformational Changes for Branched and Linear Alpha-1,4-Linked Polyglucosides

The results presented in Table I and Figs. 1 and 2 show that the same drop in intrinsic viscosity at pH 12 occurs for the branched polymers amylopectin and glycogen as for the linear polymer amylose. If the conclusions of Rao and Foster (5) are correct, then this drop in $[\eta]$ illustrates that these branched polymers are also in the helical conformation at neutral or acidic pH values and that this helix is destroyed at pH 12.

These possible helices could be stabilized by hydrogen bonds between the C-2 and C'-3 hydroxyl groups of adjacent glucose units in the α -1,4-linked chain. Such hydrogen-bonded helices have been shown to exist in amylose in DMSO solutions (9,24,25):

It is further known that the glucose units of both amylose and glycogen exist in the Cl chain conformation both in neutral and alkaline solutions (26,27)². Moreover, this Cl conformation gives a helical twist to the α -1,4-linked glucose chain. Consequently, the branched polymers as well as the linear polymers would have a natural helical twist in their chains which could readily promote the formation of a helix having hydrogen bonds between the C-2 and C'-3 hydroxyl groups.

The results given in Table II and Fig. 4 substantiate the above viscosity studies. Thus amylopectin as well as amylose undergoes a drop of approximately 40% in intrinsic viscosity in going from neutral pH to a 0.33M NaOH solution containing 32% methanol. Hence, the same type of conformational change is involved in this transition in alkali medium in the absence of added salt for both amylose and amylopectin.

²Glass, C. A., unpublished research.

The results on dextran, which is an α -1,6-linked polyglucoside, show that the above viscosity changes for amylose, amylopectin, and glycogen do not occur for dextran (Table I and Fig 3). Consequently, these changes cannot be due solely to ionization of the hydroxyl groups of the glucose units, or to the possible complexing of metal ions to these ionized groups, or to any other phenomena involved with the individual glucose units. Rather, the results on dextran illustrate that these changes in intrinsic viscosity must be due to conformational changes of the α -1,4-linked polyglucosidic chains. Furthermore, the same drop in $[\eta]$ for the 0.33M NaOH and 32% CH₃OH solution of amylose or amylopectin illustrates that ion-binding cannot be a factor. That is, in this case the drop cannot be attributed to the ion-binding of ions to individual glucose units because methanol—and not salt—was added. It can therefore be concluded that the conformational changes which occur for amylose in going from neutral to basic solutions involve the conformation of the glucosidic chain and that such changes also occur for amylopectin and glycogen. Furthermore, it is concluded that such changes involved a destruction of the helix because, as shown in the introductory section, the conformation of amylose remains the same after addition of I₃.

Analysis of the Conformational Change for Amylose in Alkali Solutions

The experiments of Doppert and Staverman (6,11) have shown that the hydroxyl groups of amylose become ionized somewhere near pH 11. And, as brought out in the introductory section, other experimental data given by them also show that amylose behaves as a polyelectrolyte at about pH 12 and above. The results (Table I) for amylose are therefore explainable if it is considered that two changes occur in the amylose molecules in going from neutral to alkali solutions: 1) the amylose is changed from a neutral polymer to a polyelectrolyte and 2) a helix-to-coil transition occurs at pH 12.

Because amylose behaves as a polyelectrolyte (6), the dramatic increase in $[\eta]$ at pH 12 in the absence of salt is understandable. That is, the destruction of the helix and the ionization of the hydroxyl groups occur simultaneously, and the resulting value of $[\eta]$ will depend on both factors. As shown elsewhere (28), the addition of salt reduces the electrostatic charge of the polymer by adding a counter-ion which can become associated with the ionic groups of the polyelectrolyte. In some cases where the polymer or colloid does not precipitate, a reversal of the polymer's electrostatic charge occurs; i.e., the charge changes from a negative to a positive charge (28). A reversal of charge does not occur at pH 12 because the amylose precipitates. Thus, because of the negative charge on amylose, the ionized groups of amylose will in the absence of salt repel one another and the amylose molecules will become more stiff. The reduction in $[\eta]$ will now depend upon how much salt has been added. The 0.75M KCl solution at pH 12 represents almost the maximum amount of added salt, since it was observed that further addition (1.0M KCl) salted-out the amylose. The 0.5M KCl solution gives a slightly higher value of $[\eta]$, as would be expected on the basis of the polyelectrolyte behavior of amylose. But it should be emphasized that the value of $[\eta]$ at pH 12 in the theta solvent (in the presence of sufficient salt or methanol) has dropped 40% from the value of $[\eta]$ in the theta solvent at pH 7. Conse-

quently, this drop shows that a change in conformation in the amylose (or in the branched polymers) has occurred; otherwise, the value of $[\eta]$ would be the same for all theta solvents.

Recently Maywald, Schoch, and Leach (20) examined the properties of amylose, amylopectin, and glycogen in alkaline solutions. They showed that $[\eta]$ increased for all of these polymers as alkali was added to a neutral solution. Our results (Table I) for amylose in the absence of *sufficient* salt are in agreement with theirs. It should be noted, however, that sufficient salt must be added to get the maximum drop in $[\eta]$. The results of Table I show that for the particular amylose studied, the change in $[\eta]$ for amylose with addition of various amounts of salt may range from maximum *increase* to $[\eta] = 1.60$ to a maximum *decrease* to $[\eta] = 0.54$ or to any point in between. The failure of Maywald *et al.* (20) to obtain a minimum was therefore caused by a lack of sufficient salt to reduce the electrostatic repulsive forces of the negatively ionized hydroxyls. Furthermore, the ability of a salt to reduce the charge on amylose should go according to the solubility sequence (28): $\text{Li}^+ < \text{Na}^+ < \text{K}^+ < \text{Cs}^+$. That is, the Na^+ ion forms a less soluble salt with the ionized hydroxyl group than the K^+ ion and hence is *less* capable of salting-out (28). Our studies show that a greater amount of Na^+ ion must be added to salt-out amylose at pH 12 than K^+ ion, and hence appear to confirm the above ionic sequence. That is, 1.0M KCl salts-out amylose, whereas 1.0M NaCl does not.

It is therefore concluded from the above results that the viscosity of amylose, amylopectin, or glycogen can either increase or *remain constant* in going from pH 7 to pH 12, because amylose behaves as a typical polyelectrolyte at pH 12. But again it is concluded that the *drop* in $[\eta]$ in the theta solvent cannot be due to this polyelectrolyte behavior of these polymers at pH 12 but must be due to the destruction of their helical conformation.

Further Verification of the Helix-to-Coil Transition

One of the main points brought out above is that the neutral molecule methanol when added to 0.5M NaOH solution of amylose produces a value of $[\eta]$ which is essentially the same as that for amylose in 0.75M KCl at pH 12. But the methanol itself does not destroy the helix, because it is well known that methanol can give a crystallized helical structure for amylose. Furthermore, it was observed by Tobin³ that addition of about 6% methanol or ethanol to an aqueous solution of amylose at pH 7 did not produce a change in $[\eta]$ as long as the correct η_0 was used. Yet it was observed that these alcohols helped stabilize the amylose from retrogradation. These results again illustrate that the alcohols stabilize the existing helical structure at neutral pH and that the hydrogen bonds involved in this helix are formed prior to addition of the alcohol, I_3^- , or any other complexing agent.

Additional verification that these hydrogen bonds exist before the helix is formed can be made by examining the ΔH and ΔS values involved in the interaction of amylose with I_3^- . By extending the observed ΔH values obtained for the interaction of I_3^- with oligosaccharides having six or less glucose resi-

³Tobin, R., private communication.

dues to large numbers of residues, Senti and Erlander (29) obtained theoretical values for ΔH and ΔS for large loops. These ΔS values for the loops were then compared to those values for the helix and are listed in Table III

TABLE III
COMPARISON OF ΔH AND ΔS VALUES FOR THE FORMATION OF EITHER A HELIX OR A LARGE THEORETICAL END-TO-END-CONNECTED LOOP FOR THE REACTION OF POLYGLUCOSIDES WITH I_3^- ^a

GLUCOSE UNITS	LOOP (L) / HELIX (H) - ΔH (L) / - ΔH (H)	- ΔS (L) / - ΔS (H)
7	7,100/ 7,000	13.1/13.7
8	8,400/ 8,300	16.6/17.4
9	9,600/ 9,500	19.9/21.8
10	10,800/10,800	23.2/24.7
11	12,000/12,200	26.6/28.7
∞	—/19,600	—/25.6

^aData obtained from calculations of Senti and Erlander (29) and results of Thoma and French (30).

(29,30). The surprising result is that the ΔH values for both the formation of the helix and the theoretical loop are the same. The difference in free energy for the formation of the helix and the loop is therefore not in different ΔH values but in different entropy (ΔS) values. That is, the seven-membered loop would have more flexibility and freedom of movement than the seven glucose units in a six-residue helix, because of the overlap of one glucose unit for the helix. Consequently, the decrease in entropy in going from the loop to the helix is accountable. However, the same value of ΔH for the helix and the theoretical loop shows that no more hydrogen bonds are being formed. In other words, the hydrogen bonds were already formed *prior* to the addition of the I_3^- complex. It should be noted that the theoretical loop is based on the results of oligosaccharides having four, five, and six glucose units (29). Therefore, these molecules must also have hydrogen bonds. And the only possibility for the existence of such bonds is when the C-2 and C-3 hydroxyl groups interact as in the case of *both* maltose and amylose in DMSO solutions (24). Consequently, these data on ΔH again support the above conclusions that α -1,4-linked polyglucosides have the helix structure in neutral aqueous solutions in the absence of complexing agents and that the helix is stabilized by hydrogen-bonding between adjacent C-2 and C-3 hydroxyl groups.

Further proof that amylopectin and glycogen have the same helical structure as amylose can be obtained by examining their dispersion curves and their partial specific volumes (\bar{V}). The dispersion curves of amylose and amylopectin as obtained from optical rotation studies by Neely (31) show that there are no detectable differences between these two components in DMSO as well as aqueous solution. Consequently, if hydrogen bonds exist in amylose, they must also exist in amylopectin. Also, the partial specific volume of amylopectin and amylose are approximately the same, which is in contrast with that for retrograded amylose (32). That is, $\bar{V} = 0.66$ for amylose and 0.65 for amylopectin, whereas $\bar{V} = 0.60$ for retrograded amylose (32). Hence, the optical rotation and \bar{V} studies again show that amylose and amy-

lopectin should have the same conformation. It should also be noted that ultracentrifugal studies (32) of both amylose and periodate-oxidized amylose show that the helix existed in the amylose molecules in aqueous solutions prior to oxidation.

Existence of the Helix in Hydrogen-Bond-Destroying Solvents

It was shown by Griffin *et al.* (22) that amylose has the same physical dimensions and properties in 4.2M GCl as in water or 0.33M KCl at pH 7. Moreover, the extrapolated value of (\bar{R}^2/\bar{M}) or the segment length in these two solvents is essentially the same as in DMSO, but is quite different from that obtained at pH 12 in the presence of salt. Hence, the helix appears to exist in hydrogen-bond-breaking solvents. This fact was further emphasized by the results of Doppert and Staverman (6), who observed that even in the presence of 6M urea the amylose still produced a drop in viscosity at pH 12. Their observed increase in viscosity at pH 7 must be due to the ability of urea to change water into a non-theta solvent. DMSO produces a similar increase in the viscosity of amylose, but as noted (22) the extrapolated value of (R^2/M) agrees with that in water. Hence, at pH 7 urea as well as DMSO, 4M GCl, and 0.33M KCl must maintain the amylose helix.

As noted (25), the bulky DMSO molecule stabilizes the hydrogen bond between the C-2 and C'-3 hydroxyl groups by becoming complexed with only one hydroxyl. Friedberg *et al.* (33) showed that hydroxyethyl cellulose aggregated most extensively in about 66% (v./v.) DMSO solutions (where the $\text{DMSO}\cdot 2\text{H}_2\text{O}$ complex exists). However, Cowie and Toporowski (34) have shown that amylose aggregates more in 25% (v./v.) DMSO. These and other results led Erlander and Tobin (25) to suggest that the $\text{DMSO}\cdot\text{H}_2\text{O}$ or $\text{DMSO}\cdot 2\text{H}_2\text{O}$ complex stabilizes the helix by forming a hydrogen bond between one of the starch hydroxyls (the C-2 or C'-3) and one of the complexed water molecules attached to DMSO. Thus one hydroxyl is left free to hydrogen-bond to the complexed hydroxyl.

The same principle can be applied to other hydrogen-bond-breaking solvents. That is, either the ion or its hydrated water can interact with one of the hydroxyl groups (the C-2 or C'-3 hydroxyl). The bulkiness of such agents and the close proximity of the C-2 and C'-3 hydroxyl groups would prohibit the destruction of the hydrogen bond between these groups by making it impossible for one or more molecules or ions to complex with both adjacent hydroxyls. Consequently, the urea, guanidinium ion, thiocyanate ion, DMSO molecule, or any other ion or polar molecule or its hydrate would stabilize the hydrogen bond by increasing the polarity of one of these hydroxyl groups. This increase in polarity would stabilize this hydrogen bond so that it is even stronger than it is in water. However, intermolecular hydrogen bonds could readily be destroyed, because both hydroxyls participating in such a bond would be susceptible to attack by the added ion or molecule. Consequently, the retrogradation of amylose is prevented in such solvents but the helix is stabilized.

CONCLUSIONS

The results presented here and elsewhere therefore show that amylose, amylopectin, and glycogen exist in the helical conformation in neutral and

acidic aqueous solutions, as well as in hydrogen-bond-breaking solvents. The helix-to-coil transition near pH 12 may be obscured if sufficient salt is not present. It should even be possible to maintain the intrinsic viscosity at pH 12 at the same value as obtained at pH 7 if the proper amount of salt were added. In other words, with the proper ionic strength the effects of the electrostatic repulsive forces and the destruction of the helical structure would cancel each other and $[\eta]$ would neither increase nor decrease.

In the absence of complexing agents, the helix in amylose as well as in amylopectin and glycogen most likely exists as seven or eight glucose residues per helical turn as suggested earlier (10), or possibly it may exist as a fractional number such as a 7.8-membered helix (8). Because the helix is stabilized by hydrogen bonds between adjacent glucose units (C-2 and C'-3 hydroxyls) rather than between individual helical loops (see "Discussion"), then it should be possible to have a fractional number for the number of glucose residues per helical turn. Indeed, the number may not be constant and may even vary slightly with different amylose molecules in the same medium. And most likely the seven- or eight-membered helix in the absence of complexing agents is readily converted to a six-membered helix in aqueous solutions when I_3^- is added. Support of such a variable helical structure is given by the data of Yamashita and Hirai (35); they observed that even in the *crystalline* state, conversion from a helix having seven or eight glucose units per residue to one having six glucose units occurred without alteration of the crystal structure.

Literature Cited

1. FOSTER, J. F., and ZUCKER, D. Length of amylose-iodine complex as determined by streaming dichroism. *J. Phys. Chem.* 56: 170-173 (1952).
2. HOLLO, J., and SZEJTLI, J. Structure of the amylose molecule in aqueous solution. *Stärke* 10: 49-52 (1958).
3. RUNDLE, R. E., FOSTER, J. F., and BALDWIN, R. R. The nature of the starch-iodine complex. *J. Am. Chem. Soc.* 66: 2116-2120 (1944).
4. KUGE, T. Molecular configuration of amylose in aqueous solutions. *Bull. Univ. Osaka Pref. (Ser. B)* 11: 121-125 (1961).
5. RAO, V. S. R., and FOSTER, J. F. Studies of the conformation of amylose in solution. *Biopolymers* 1: 527-544 (1963).
6. DOPPERT, H. L., and STAVERMAN, A. J. Polyelectrolyte character of amylose. I. *J. Polymer Sci. (Part A-1)* 4: 2367-2372 (1966).
7. RAO, V. S. R., and FOSTER, J. F. Studies of the conformation of carboxymethyl amylose in aqueous solutions. *Biopolymers* 3: 185-193 (1965).
8. SZEJTLI, J., RICHTER, M., and AUGUSTAT, S. Molecular configuration of amylose and its complexes in aqueous solutions. Part II. Relation between the DP of helical segments of the amylose-iodine complex and the equilibrium concentration of free iodine. *Biopolymers* 5: 5-16 (1967).
9. ERLANDER, S. R., and TOBIN, R. The stability of the helix of amylose and amylopectin in DMSO and water solutions. *Makromolec. Chem. (in press)*.
10. ERLANDER, S. R., GRIFFIN, H. L., and SENTI, F. R. The destruction of amylose helices by periodate oxidation. *Stärke* 17: 151-158 (1965).
11. DOPPERT, H. L., and STAVERMAN, A. J. Polyelectrolyte character of amylose. II. *J. Polymer Sci. (Part A-1)* 4: 2373-2380 (1966).
12. SCHOCH, T. J. The fractionation of starch. *Advan. Carbohydrate Chem.* 1: 247-277 (1945).
13. ERLANDER, S. R., and FRENCH, D. Dispersion of starch granules and the validity of light-scattering results on amylopectin. *J. Am. Chem. Soc.* 80: 4413-4420 (1958).
14. ERLANDER, S. R., and GRIFFIN, H. L. Production of glycogen in various genetic corn endosperms. *Stärke* 19: 34-41 (1967).

15. PUCKER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B. Determination of starch in plant tissue. *Anal. Chem.* 20: 850-853 (1948).
16. KUNETZ, J. E. A study of the diurnal variation of amylose and amylopectin in tobacco by a colorimetric method. M.S. Thesis, Pennsylvania State College, State College, Pa.
17. SENTI, F. R., HELLMAN, N. N., LUDWIG, N. H., BABCOCK, G. E., TOBIN, R., GLASS, C. A., and LAMBERTS, B. L. Viscosity, sedimentation and light-scattering properties of fractions of an acid-hydrolyzed dextran. *J. Polymer Sci.* 17: 527-546 (1955).
18. ERLANDER, S. R., and FRENCH, D. Acid hydrolysis and molecular weights of various corn amylopectins and glycogen. *J. Polymer Sci.* 32: 291-316 (1958).
19. ERLANDER, S. R., and PURVINAS, R. M. The polyelectrolyte behavior of amylose and its helix-to-coil transition in aqueous alkaline solutions. *Stärke* (in press).
20. MAYWALD, EILEEN C., SCHOCH, T. J., and LEACH, H. W. Expansion and contraction of starch molecules in solution. I. Effects of temperature, pH, and alkali. Presented at 52nd Annual Meeting, Los Angeles, Calif., April 1967.
21. KURATA, M., and STOCKMAYER, W. H. Intrinsic viscosities and unperturbed dimensions of long chain molecules. *Fortschr. Hochpolym. Forsch.* 3: 196-312 (1963).
22. GRIFFIN, H. L., ERLANDER, S. R., and SENTI, F. R. Structural relationships, kinetics, and molecular properties in the acid depolymerization of amylose. *Stärke* 19: 8-17 (1967).
23. WALES, M., MARSHALL, P. A., and WEISSBERG, S. G. Intrinsic viscosity-molecular weight relationships for dextran. *J. Polymer Sci.* 10: 229-240 (1953).
24. CASU, B., REGGIANI, M., GALLO, G. G., and VIGEVANI, A. Hydrogen bonding and conformation of glucose and polyglucoses in dimethyl sulfoxide solution. *Tetrahedron* 22: 3061-3083 (1966).
25. ERLANDER, S. R., and TOBIN, R. The effect of aqueous salt solutions on the conformation and thermal motion of molecules and polymers such as starch. *Makromolec. Chemie* (in press).
26. CASU, B., and REGGIANI, M. Infrared spectra of amylose and its oligomers. *J. Polymer Sci. (Part C)* 7: 171-185 (1964).
27. RAO, V. S. R., and FOSTER, J. F. The conformation of the pyranose rings in mono-, di-, and polysaccharides at high pH by proton magnetic resonance studies. *J. Phys. Chem.* 69: 636-640 (1965).
28. ERLANDER, S. R. Explanation of ionic sequences in various phenomena. II. Reversal of colloid charge. *J. Macromolec. Sci.* (in press).
29. SENTI, F. R., and ERLANDER, S. R. Carbohydrates. In *Non-stoichiometric compounds*, L. Mandelcorn, ed.; pp. 568-605. Academic Press: New York (1964).
30. THOMA, J. A., and FRENCH, D. The starch-iodine-iodide interaction. II. Potentiometric investigations. *J. Phys. Chem.* 65: 1825-1828 (1961).
31. NEELY, W. B. Optical rotatory dispersion studies on polysaccharides. III. Amylose, amylopectin, and methyl-cellulose. *J. Organic Chem.* 26: 3015-3016 (1961).
32. ERLANDER, S. R., and GRIFFIN, H. L. Physical properties of amylose, retrograded amylose, and periodate-oxidized amylose as studied by ultracentrifugal molecular weight determinations. *Stärke* 19: 139 (1967).
33. FRIEDBERG, F., BROWN, W., HENLEY, D., and OHMAN, J. A light-scattering study on hydroxyethyl cellulose in binary mixtures of water-dimethyl sulfoxide and water-formamide. *Makromolekul. Chem.* 66: 168-179 (1963).
34. COWIE, J. M. G., and TOPOROWSKI, P. M. Association in the binary liquid system dimethyl sulfoxide-water. *Can. J. Chem.* 39: 2240-2243 (1961).
35. YAMASHITA, Y., and HIRAI, N. Single crystals of amylose V-complexes. *J. Polymer Sci. (Part A-2)* 4: 161-171 (1966).

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