THE CONFORMATION OF AMYLOSE IN ALKALINE SALT SOLUTION*

W. BANKS AND C. T. GREENWOOD†

Department of Chemistry, The University of Edinburgh (Great Britain)

(Received February 8th, 1971; accepted for publication in revised form, July 12th, 1971)

ABSTRACT

The conformation of amylose in various solvents is discussed. It is shown that the changes in molecular volume of the polysaccharide (measured by viscosity) as potassium chloride is added to a solution of amylose at pH 12 are similar to those obtained on adding butan-1-ol to the solution. The viscosity number in both cases decreases to values less than that observed for amylose in water, in which Flory theta-conditions are approximated. The minimum value of the viscosity number, in fact, is identical to that observed on the addition of butan-1-ol and iodine to neutral aqueous solution of amylose — conditions known to result in a helical complex. It is concluded that amylose undergoes a coil-to-helix transition as potassium chloride is dadde to solutions of the polysaccharide at pH 12.

The conformation of amylose in solution is still a matter of dispute. Our hydrodynamic studies[1-5] suggest that amylose behaves as a random coil in neutral, aqueous salt solution, formamide, methyl sulphoxide, and 0.15M potassium hydroxide, and that in these solvents the extensive intramolecular hydrogen-bonding which is thought to be necessary for pronounced helical character is absent. However, it has been suggested that the molecule may have considerable helical character, and still behave as a random coil[6-10]. In such an "interrupted helix", the macromolecule is postulated to consist of helical segments, each containing 100–120 "anhydroglucose" units, with short regions of random coil between them, the latter conferring the flexibility necessary for the molecule as a whole to behave as a random coil.

This model of the interrupted helix was apparently confirmed by Rao and Foster[7] who reported that, when the pH of an aqueous solution of amylose was varied, the limiting viscosity number of the amylose decreased to a minimum at pH 12 and then subsequently increased. It was suggested that in neutral, aqueous solution, the model of the interrupted helix was applicable, and that on adding alkali the hydrogen bonding stabilizing the helix was progressively destroyed, and the amylose macromolecule adopted a more random, and compact, conformation. At pH 12, the helical conformation was supposed to be completely absent. As the concentration of

---

*This is Part 61 in the series "Physicochemical Studies on Starches"; Part 60: *Biopolymers*, in press.

†Present address of both authors: Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts. WD3 5SH, Great Britain.

alkali was further increased, the hydroxyl groups underwent ionisation, swelling the polysaccharide molecule, and thus increasing the limiting viscosity number. Later work by Erlander[9,10] purported to support this theory of a helix–coil transformation for amylose, and it was suggested that the phenomenon was analogous to the chemical melting of the α-helix in polypeptides.

However, the analogy is not quite so apt as might at first sight appear. In the case of polypeptides, the limiting viscosity number increases on going from the helix to the random coil, whereas in amylose it is supposed to decrease. Unpublished calculations of Dimpfl, quoted by Brant and Min[11], support the intuitive reasoning that the conversion of amylose helix into random coil, for helical contents of up to 90%, will result in an increase in unperturbed chain dimensions, and thus in an increase in limiting viscosity number. A more serious objection was raised by Procter[12,13], who reported that the minimum in the limiting viscosity number as a function of pH was due to the presence of supporting electrolyte rather than to pH *per se*; in the absence of potassium chloride, no minimum was observed. In view of these facts, it would appear most unlikely that a helix–coil transition occurs in amylose at pH 12.

Our own interpretation of the extensive hydrodynamic measurements that we have carried out on amylose in neutral, aqueous salt solution is that the macromolecule has negligible helical content[1-5]. We have recently shown[14] that a coil–helix transition occurs in aqueous solution on the addition of complexing agents such as iodine and butan-1-ol. This work has now been extended to alkaline solution, and

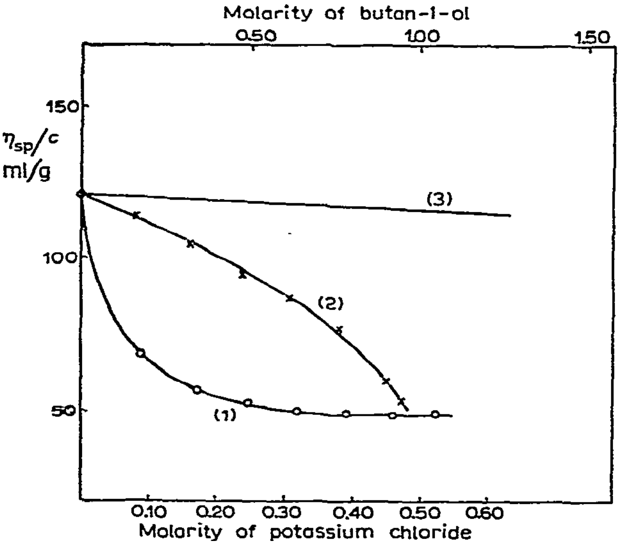


Fig. 1. The effect on the viscosity number (η_{sp}/c) of amylose in 0.01M potassium hydroxide of adding (*1*) 3M potassium chloride in 0.01M potassium hydroxide, (*2*) butan-1-ol, and (*3*) 0.01M potassium hydroxide (control).

the changes in reduced viscosity which occur on the addition to a solution of amylose in 0.01M KOH of (*1*) 0.01M KOH+3M KCl, (*2*) butan-1-ol, and (*3*) 0.01M KOH are shown in Fig. 1.

The addition of potassium chloride to the solution causes an immediate, rapid decrease in the value of η_{sp}/c, in agreement with the results of Procter[12-13]. On the other hand, the effect of butan-1-ol is somewhat slower, but the end result is a greatly decreased value of η_{sp}/c, and finally the precipitation of the amylose as the butan-1-ol complex. The line shown for 0.01M KOH is merely to demonstrate the slight decrease in η_{sp}/c which is a result of the dilution of the amylose solution.

The concept of amylose as a helical macromolecule was first advanced by Hanes[15] to explain the blue complex formed on the addition of iodine. Freudenberg *et al.*[16] supported this hypothesis, because it would explain the ease of formation of the Schardinger dextrins. Subsequent optical[17,18] and X-ray[19-21] work by Rundle and co-workers confirmed the correctness of the Hanes model in both the crystalline state and in solution. These studies refer only to the complexes of amylose, and not to the polysaccharide itself. However, knowing the conformation of the polysaccharide molecule in the presence of complexing agents, we may use this as a reference point for deducing the shape of the polymer in other solvents by measuring the changes in some hydrodynamic parameter, such as limiting viscosity number, which occur as the complex is formed.

Fig. 1 shows that the addition of either butan-1-ol or potassium chloride to amylose at pH 12 causes a decrease in the viscosity number, the value of which eventually falls to approximately 50 $ml.g^{-1}$. In caustic alkali at pH 12, amylose undergoes solvent expansion[2,4,5], presumably due to the ionization of the hydroxyl groups, and consequently the excluded volume effect is appreciable. One result, therefore, of adding either butan-1-ol or potassium chloride to amylose at pH 12 would be to effectively suppress this ionization, and so cause the macromolecular coil to collapse until it attained its unperturbed dimensions. Neutral, aqueous solvents at room temperature provide thermodynamically ideal conditions for amylose—the light-scattering, second virial coefficient is zero[1,22], the exponent in the Mark–Houwink equation has been shown[1-3] to be 0.50, the corresponding exponent in the equation relating the sedimentation coefficient to molecular weight has been shown[1,5] to be 0.50, and the mean-square radius of the polymer coil is proportional to the molecular weight—and therefore the unperturbed dimensions are observed in these solvents. For the amylose used in this work, the viscosity number in water and in neutral, 0.33M potassium chloride is 70 $ml.g^{-1}$. It would therefore be expected that the minimum value of the viscosity number in Fig. 1 would be 70 $ml.g^{-1}$; in fact, it is 50 $ml.g^{-1}$. We must attempt therefore to explain, in conformational terms, the changes that have occurred in the amylose molecule to cause such a profound alteration in hydrodynamic behaviour.

The decrease in the viscosity number observed on the addition of butan-1-ol is almost certainly due to the formation of a helical complex. Support for this contention is to be found in our recent work[14] with the identical sample of amylose, where we found a decrease in viscosity number from 70 to 50 ml.g^{-1} on the addition of either iodine or butan-1-ol to neutral solutions of the polysaccharide. In the case of iodine, the minimum viscosity number was observed at an iodine concentration of approximately 19 mg/100 mg of amylose, *i.e.* at the saturation uptake of iodine. Rundle's work[17–21] shows quite conclusively that the complexes of amylose with iodine and butan-1-ol are helical, and we conclude that the decrease of the viscosity number of the polysaccharide to values substantially lower than that observed for amylose in neutral solution is indicative of the assumption by the macromolecule of a helical conformation. On this basis, the addition of potassium chloride to an aqueous solution of amylose at pH 12 must cause the polysaccharide to become helical.

Whilst it is in no way surprising that the addition of butan-1-ol to amylose in alkaline solution should result in a helical complex being formed, the explanation advanced here for the phenomenon observed on the addition of potassium chloride to amylose solutions at pH 12 is novel and will therefore be dealt with in more detail.

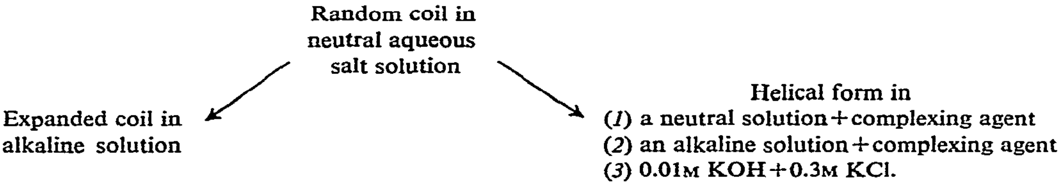
It has been suggested[23,24] that the conformation of the constituent D-glucose residues in amylose is a function of the degree to which they are ionized, and calculations have shown[25] that the unperturbed dimensions of amylose would be grossly dependent on the conformation of the monomer unit. However, more-recent n.m.r. studies[26,27] have shown that the D-glucose residues are invariably present in the *C1*(D) conformation. The observed solution behaviour of amylose must be explained, in fact, without recourse to a model in which the constituent monomer units may change their conformation.

Rao and Foster[7] were the first to report that, under specific conditions of pH, the viscosity number of amylose could fall to values much lower than observed in water. They explained the phenomenon by suggesting that, as the hydroxyl-ion concentration was increased, the forces stabilizing the helix collapsed, and at pH 12 the macromolecule assumed the form of a random coil, *i.e.* a postulate diametrically opposed to that presented here. Their argument depends on the two premises, (*1*) that amylose in neutral solution must be predominantly helical, because the addition of iodine does not cause a measurable change in its viscosity[6], and (*2*) that the change noted at pH 12 was mediated solely by alkali. Both these premises have been shown[12–14] to be incorrect; the first because Holló and Szejtli[6] graphed the relative viscosity, rather than the viscosity number, and the second because Rao and Foster[7] neglected the presence of substantial amounts of potassium chloride in their solutions. Conclusions drawn on the basis of such erroneous premises must be regarded therefore as of doubtful significance.

There are also two further, major discrepancies with the Rao and Foster model. The first is that, within the limitations of the present theoretical methods for treating the excluded-volume effect, the skeletal structure of amylose is the same in both neutral, and alkaline, aqueous solution[2,4,5], but completely different at pH 12 in the presence of potassium chloride (and in the presence of complexing agents). On the basis of the Rao and Foster model, one would expect the similarity to be between conditions at pH 12 plus potassium chloride, and higher pH values, with amylose in neutral solution presenting the unique case. The second discrepancy is that, accepting these authors' model, it has to be concluded that the hydrodynamic behaviour of the alleged coil is, by chance, identical to that of known-helical complexes.

The possibility has also to be considered that the phenomenon of spontaneous crystallization, or retrogradation, has affected our observations. Indeed, Schoch *et al.*[28] have suggested that the results at pH 12 in the presence of potassium chloride are due largely to retrogradation of the amylose. Retrogradation is a complex phenomenon, depending on many parameters such as pH, molecular size, concentration, and temperature, and by judicious choice of these variables, we have never found retrogradation to occur in our solution studies. In the present instance, we have found that the amylose may be precipitated at pH 12 by further increasing the concentration of potassium chloride, but this material is readily soluble in water. This behaviour is similar to that exhibited by the helical amylose–butan-1-ol complex, and it is well-known that retrograded material, on the other hand, is characterized by its insolubility in water.

We suggest that the results presented here are in agreement with the following conformational states of amylose in various aqueous solvents:



Random coil in
neutral aqueous
salt solution

Expanded coil in
alkaline solution

Helical form in
(*1*) a neutral solution + complexing agent
(*2*) an alkaline solution + complexing agent
(*3*) 0.01M KOH + 0.3M KCl.

In going from neutral solution to alkaline salt solution, amylose undergoes a coil to helix transformation, and not, as postulated by other investigators, a change in the opposite sense.

It should be noted that, recently, Brant and co-workers[29–31] have concluded, on the basis of both experimental evidence and from computer model-building, that amylose in aqueous solution behaves as a statistical coil with no helical character, unless complexing agents are present; this conclusion fully supports our own work[1–5,14].

REFERENCES

1 W. Banks and C. T. Greenwood, *Makromol. Chem.*, 67 (1963) 49.
2 W. Banks and C. T. Greenwood, in G. N. Ramachandran (Ed.), *Conformation of Biopolymers*, Academic Press, London, 1967, p. 739.
3 W. Banks and C. T. Greenwood, *Carbohyd. Res.*, 7 (1968) 349.
4 W. Banks and C. T. Greenwood, *Carbohyd. Res.*, 7 (1968) 414.
5 W. Banks and C. T. Greenwood, *Eur. Polymer J.*, 5 (1969) 649.
6 J. Holló and J. Szejtli, *Staerke*, 10 (1958) 49.
7 V. S. R. Rao and J. F. Foster, *Biopolymers*, 1 (1963) 527.
8 J. Szejtli and S. Augustat, *Staerke*, 18 (1966) 38.
9 S. R. Erlander and H. L. Griffen, *Staerke*, 19 (1967) 139.
10 S. R. Erlander and R. M. Purvinas, *Staerke*, 20 (1968) 37.

11 D. A. Brant and B. K. Min, *Macromolecules*, 2 (1969) 1.
12 R. Geddes, C. T. Greenwood, and A. R. Procter, *Abstr. Int. Symp. Carbohyd. Chem.*, Münster, July 1964, pp. 26–27.
13 A. R. Procter, Ph.D. Thesis, University of Edinburgh, 1965.
14 W. Banks and C. T. Greenwood, *Polymer*, 12 (1971) 141.
15 C. S. Hanes, *New Phytologist*, 36 (1937) 189.
16 K. Freudenberg, E. Schaaf, G. Dumpert, and T. Ploetz, *Naturwissenschaften*, 27 (1939) 850.
17 R. E. Rundle and R. R. Baldwin, *J. Amer. Chem. Soc.*, 65 (1943) 554.
18 R. E. Rundle and D. French, *J. Amer. Chem. Soc.*, 65 (1943) 558.
19 R. E. Rundle and D. French, *J. Amer. Chem. Soc.*, 65 (1943) 1707.
20 R. E. Rundle and F. C. Edwards, *J. Amer. Chem. Soc.*, 65 (1943) 2200.
21 R. E. Rundle, *J. Amer. Chem. Soc.*, 69 (1947) 1769.
22 W. Banks, C. T. Greenwood, and J. Sloss, *Carbohyd. Res.*, 11 (1969) 399.
23 R. E. Reeves, *J. Amer. Chem. Soc.*, 76 (1954) 4595.
24 J. Holló, J. Szejtli, and J. Toth, *Staerke*, 13 (1961) 222.
25 W. Burchard, *Makromol. Chem.*, 42 (1960) 151.
26 V. S. R. Rao and J. F. Foster, *J. Phys. Chem.*, 69 (1965) 63.
27 R. Geddes, Ph.D. Thesis, University of Edinburgh, 1965.
28 E. C. Maywald, H. W. Leach, and T. J. Schoch, *Staerke*, 20 (1968) 189.
29 K. D. Goebel and D. A. Brant, *Macromolecules*, 3 (1970) 634.
30 C. V. Goebel, W. L. Dimpfl, and D. A. Brant, *Macromolecules*, 3 (1970) 644.
31 D. A. Brant and W. L. Dimpfl, *Macromolecules*, 3 (1970) 655.

Carbohyd. Res., 21 (1972) 229–234.