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Investigations on the Molecular Weight of Aqueous Solutions of Potato Amylose by Osmotic Pressure

B. B. JØRGENSEN and O. B. JØRGENSEN

*Department of Technical Biochemistry, Royal Technical University, Copenhagen *, and the University Institute of Physical Chemistry, Copenhagen, Denmark*

The colloid osmotic pressure of aqueous amylose solutions is measured. In concentrations of 0.2–0.6 % amylose the reduced osmotic pressure, p/c, is constant within the experimental error. The influence of heating in alkaline solution on the osmotic pressure and viscosity of amylose solutions is examined. The osmotic pressure of the potato amylose examined corresponds to a molecular weight $\sim 2.02-2.10 \times 10^5$ and to a D.P. $\sim 1\,250-1\,300$.

On account of the difficulties in making stable aqueous solutions of amylose most of the physical determinations of molecular weight of amylose are performed on amylose derivatives in a suitable solvent. Reviews on this subject are given by Greenwood [1] and Whelan [2].

By means of the inverted microosmometer described by Christiansen and Jensen [3] it is possible to measure small osmotic pressures in rather short times. The purpose of this investigation is to examine the possibility of measuring the osmotic pressure of aqueous solutions of amylose by means of the inverted microosmometer before retrogradation of amylose takes place. As the amylose was brought in solution in alkali it was necessary to examine how alkali treatment influenced the osmotic pressure of the amylose solution.

EXPERIMENTAL

Amylose. The amylose examined was prepared from potato starch by precipitation with thymol after the precipitation of the amylopectin as described by Hobson *et al.*[4] The amylose showed a "blue value" = 1.38.

Amylose solution. The amylose solutions were prepared by dissolving a suitable amount of amylose in 0.4 N KOH by shaking for 1 ½ h and filtering through a G4 sintered-glass filter. The solutions were then heated the time wanted (0–16 min) in a boiling water bath. During the shaking and heating the amylose solutions were protected against oxygen by an atmosphere of nitrogen. After the heating the solutions were cooled in running tap water. Immediately before the osmotic measurements a suitable volume of the solutions was neutralized with 0.4 N HCl and eventually diluted with 0.2 M KCl.

* Address: Odensegade 14, Copenhagen Ø.

The exact concentrations of amylose in the solutions were determined by acid hydrolysis according to Pirt and Whelan [5] followed by determination of glucose by the method of Somogyi [6] and Nelson [7].

Osmotic pressure measurements. The colloid osmotic pressures were measured by means of the inverted microosmometer described by Christiansen and Jensen [3]. The semipermeable membranes were prepared as described by Jensen and Marcker [8]. On account of the instability of neutral amylose solutions the measurements were started immediately after the equilibrium was established. The measurements were performed at 21°C. The outer liquid in the osmometer was 0.2 M KCl.

Viscosity measurements. The specific viscosities of the amylose solutions were measured at 25°C in an Ostwald viscosimeter. The measurements of the viscosity were made on the same alkaline solutions as were used for the osmotic measurements (heated different times) after dilution 1:10 with water.

RESULTS

Molecular weight calculations from osmotic pressures were made by means of the equation: $M = k\frac{c}{p}$, where c = concentration of amylose in g/ml and p = osmotic pressure in cm water column. At 21°C the value of k is 247.6×10^5 (Christiansen and Jensen [3]). D.P. = $M/162$.

It was examined how increasing heating times of the alkaline amylose solution influenced the osmotic pressure and the specific viscosity of the amylose solution, see Fig. 1.

Fig. 1 shows that the apparent D.P. obtains a rather constant value after heating of the amylose solution for about 5 min and only decreases slightly for longer heating times.

It is further seen from Fig. 1 that the specific viscosity of the solution, which was used for osmotic measurements but not neutralized after dilution

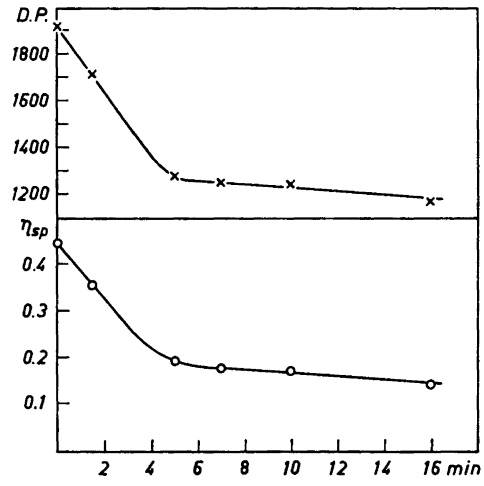

Fig. 1. The graph shows the relation between apparent D.P. and time of heating, t, of amylose in 0.4 N KOH, $c = 4.28 \times 10^{-3}$ g/ml after neutralisation, × — × — ×, and the relation between specific viscosity, η_{sp}, and time of heating of amylose in 0.4 N KOH, $c = 0.43 \times 10^{-3}$ g/ml 0.04 N KOH, ⊙—⊙—⊙.

Table 1. Experiments I—IV show the osmotic pressure of different dilutions of an amylose solution. Experiment V shows the osmotic pressure of the same amylose preparation but in another solution and with another membrane. Experiments VI and VII show the osmotic pressure of another preparation with a known D.P.
c is concentration of amylose in the neutral solution, p is osmotic pressure in cm water column, M is molecular weight and D.P. is degree of polymerisation.

Exp.	$c \times 10^3$ g/ml	p cm	p/c	$M \times 10^{-5}$	D.P.
I	2.15	0.30	140	1.77	1 090
II	2.86	0.41	143	1.73	1 070
III	4.29	0.60	140	1.77	1 090
IV	5.73	0.80	140	1.77	1 090
V	5.02	0.69	137	1.81	1 120
VI	5.14	0.62	121	2.05	1 270
VII	4.96	0.60	121	2.05	1 270

1:10 with water, shows a quite analogous variation. It was further found that η_{sp}/c was independent of c ($c \leq 0.43 \times 10^{-3}$ g/ml), the amylose concentration, by further dilution both in heated and in unheated solutions.

These results seem to show that it is necessary to heat the alkaline amylose solutions at least for 5 min before the osmotic pressure and the viscosity of the solutions are well defined.

It was examined if the proportion p/c, the so-called reduced osmotic pressure, was dependent on the concentration.

In Table 1 are shown the results of these measurements. The alkaline amylose solution was heated for 5 min (these experiments were performed without protection by N_2), and the concentration was varied from 0.215 % to 0.573 % by dilution (Expts. I—IV). It is seen from the table that p/c does not change with concentration in the range of concentration examined; accordingly the molecular weight can be calculated directly from p/c without extrapolating to $c = 0$. This means that the second virial coefficient is negligible at 21 °C in agreement with the light scattering measurements by Everett and Foster [11].

Experiment V, Table 1, shows an experiment, where the osmotic pressure of another solution of the same preparation is measured with another membrane than in experiments I—IV. It is seen that the measurements are reproducible.

The osmotic pressure was then determined of an amylose preparation, that kindly was placed at our disposal by dr. W. J. Whelan, and the D.P. of which was determined by dr. Whelan by periodate oxidation to 1 250. Our results of two independent measurements of the osmotic pressure (without use of N_2) are given as experiments VI and VII in Table 1.

DISCUSSION

It is seen from the reproducibility of the measurements that it is possible, with the technique used, to measure the osmotic pressure of neutral amylose solutions, before the amylose retrogrades.

Fig. 1 shows that the apparent D.P. falls very fast with the time of heating in alkali for the first short heating period, while further fall in apparent D.P.

for heating times longer than 5 min is very slight. As the amylose solutions were protected against oxygen by an atmosphere of nitrogen this effect is hardly due to oxidation. The slow fall for heating times longer than 5 min is possibly due to an alkaline degradation of amylose. The fast fall for shorter heating times may be due to another effect. Either the amylose contains a few very alkali-labile bonds — having eventually been introduced into the amylose during the preparation (Baum and Gilbert [9]) — or the heating in alkali causes a disaggregation of the associated amylose molecules, the amylose only being of molecular form after a heating time of about 5 min. Paschall and Foster [10] have shown that amylose disaggregates by standing in 1 N KOH at room temperature, but this effect is not pronounced for potato amylose. As the potato amylose in our examinations has been heated in alkali it is possible that the phenomenon may be explained as a disaggregation of the amylose.

The most well-defined molecular weight of amylose is found after a heating time of about 5—10 min in 0.4 N KOH under N_2. It is seen from Fig. 1 that D.P. of the amylose preparation examined under these conditions is about 1 250. Extrapolating to a heating time of 0 min a D.P. of about 1 300 is found.

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