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Comparison of molecular association of dextran and periodate-oxidized dextran in aqueous solutions

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A viscosimetric method was used to investigate the molecular association of dextran, 20% and 40% periodate-oxidized dextrans in aqueous solutions. Intrinsic viscosity numbers, $[\eta]$, found by Huggins and Kraemer equations, decreased in the presence of denaturing agents like urea, thiourea and guanidinium sulfate. Intrinsic viscosity values also decreased with increasing temperature. This decrease has been even less in the presence of denaturing agents. © 1997 Elsevier Science Ltd

INTRODUCTION

Water-soluble polymers have received great attention in recent years, due to their use in mineral processing, pharmaceuticals, detergents, cosmetics and textiles production (Molyneux, 1984). The solubility of these polymers in aqueous medium can be explained by the strong hydrogen bonding between these compounds and water molecules.

Hydrogen bonding, existing between the polymer and water molecules, can be changed in the presence of different cosolutes: (i) organic; and (ii) inorganic additives. Among the organic, additive denaturing agents (e.g. urea, thiourea, guanidinium salts) are of special importance. Denaturing agents have, in their structure, hydrogen-donor and hydrogen-acceptor groups, which are capable of hydrogen bonding with the suitable functional groups in the polymer.

In previous works it has been observed that these denaturing agents have been effective compounds on breaking the inter- and/or intra-molecular association in the aqueous solutions of some water-soluble polymers like poly(ethylene oxide) (Güner & Güven, 1978) and poly(*N*-vinyl-2-pyrrolidone) (Güven & Eltan, 1981). Dextran is a polysaccharide and a water-soluble polymer. Dextrans are of great interest from a scientific, practical and technological point of view and they are mainly used for medical applications (Tomiko *et al.*, 1994; Mocanu *et al.*, 1993; Haldane & Logan, 1994). Dextran has, in its structure, a ring-oxygen atom and a bridge-oxygen atom with hydrogen-acceptor property and a hydroxyl group with hydrogen-donor and acceptor properties (Vinogradov & Linnell, 1971). It is known that dextran, in aqueous solution, makes hydrogen bonding with water molecules (Koulkes-Pujo & Tran Thi, 1985) and also shows molecular association (Murphy & Whistler, 1973). The effects of denaturing agents and temperature on the rupture of molecular association in aqueous solutions of dextran have already been investigated by viscosimetric studies (Güner, 1995).

Dextran dialdehyde can be obtained by the oxidation of dextran and has been used in various investigations (Schactit *et al.*, 1993; Badtoshevich *et al.*, 1989; Mikihiko & Kazumi, 1993).

In this work, the molecular association and the effects of denaturing agents and temperature on this molecular association, in aqueous solutions of dextran dialdehyde (20% oxidized and 40% oxidized) have been investigated.

EXPERIMENTAL

The polymer sample used in this study was commercial dextran with a molecular weight of 70 000 (T 70), obtained from Pharmacia Fine Chemicals AB (Uppsala, Sweden). Denaturing agents used: urea,

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thiourea and guanidinium sulfate were obtained from Fisher Scientific Company. Ethylene glycol (AnalaR) and sodium metaperiodate (AnalaR) were from BDH, respectively. All these commercial compounds were used without further purification. Polymer sample was dried and stored in a desiccator over $CaCl_2$. Polymer solutions were used immediately after preparation.

Dextran dialdehyde was synthesized by treating dextran (1.2 g, 1.71×10^{-5} mol) in water (25 ml) with 0.2 M sodium metaperiodate (i) 50 ml for 40% oxidation (2.14 g, 0.01 mol); (ii) 25 ml for 20% oxidation for 24 h at room temperature at pH 4. These reactions were carried out in glass-stoppered flasks in the dark. Excess periodate was destroyed with ethylene glycol (0.55 ml, 0.01 mol). The solution was dialyzed first against running water for 24 h and then against acetate buffer at pH 3 for 3 h in order to remove products with molecular weights < 13 000. Acetate ions were removed by dialyzing the solution against water for 4 h. The resulting solution was then lyophilized.

20% and 40% periodate-oxidized dextrans were characterized by UV and IR spectra. The spectrophotometric measurements were obtained on a Hitachi 100-60 Model UV-Visible double-beam spectrophotometer at room temperature. IR spectra were recorded on a Nicolet 520 FTIR spectrophotometer at room temperature.

For 20% and 40% oxidized dextran solutions, the UV absorption bands were observed at 264 and 227 nm, respectively, at pH 3. The IR spectra of periodate-oxidized dextran show a typical aldehyde peak at 1740 cm^{-1} at pH < 4 and > 5.2 (Drobchenko *et al.*, 1993). In this study, the IR spectra were obtained in KBr discs at room temperature. Carbonyl absorption for 20% and 40% oxidized dextrans was observed at 1724.5 cm^{-1} and 1711.3 cm^{-1}, respectively.

An Ubbelohde viscometer was used for viscosimetric measurements. The temperature of the thermostat was controlled to within ±0.1°C, and the flow-times were measured with an accuracy of ±0.1 s. Kinetic energy correction was not necessary for the viscosity since the molecular weight of the polymer was not too high.

The concentration dependence of the viscosity of dilute polymer solutions is described by the well-known Huggins equation:

$$\eta_{sp/c} = [\eta] + k_H[\eta]^2c$$

and Kraemer equation:

$$\ln\eta_{r/c} = [\eta] - k_K[\eta]^2c.$$

Here, $[\eta]$ is the intrinsic viscosity and, k_H and k_K are the Huggins and Kraemer constants. The intrinsic viscosity is a measure of the effective hydrodynamic volume of the polymer in solution and both Huggins and Kraemer constants are measures of polymer–solvent interaction.

RESULTS AND DISCUSSION

The behaviour of the polymer coil in solution is usually explained by hydrodynamic properties. Hydrodynamic properties can be obtained from different experimental techniques (gel permeation chromatography, and viscosimetry). Between these, viscosimetric technique is frequently used owing to its simplicity as well as to its high sensitivity to aggregation (Pavlov *et al.*, 1990; Priel & Silberberg, 1970; Tam *et al.*, 1992).

It has already been mentioned that the polymer–solvent interactions in the aqueous solutions of dextran is via hydrogen bonding. Change in the polymer hydrodynamic volume (dependent on the intensity of the association) can be followed by the intrinsic viscosity change. In the presence of additives, depending on its type and concentration, the disruption of association between the polymer and solvent molecules can be followed by a decrease in intrinsic viscosity.

The intrinsic viscosity values of dextran in its aqueous solution at 25°C have been investigated. It has been observed that, in the presence of denaturing agent, intrinsic viscosity values from Huggins equation decreased (Fig. 1). The intrinsic viscosity values calculated from the Kraemer equation were very close to those calculated by the Huggins equation. It has been observed that the dialdehyde structure obtained from the periodate oxidation of glucans displayed strong interactions with the amino groups of various compounds (Kobayashi & Ichishima, 1991). Considering the structure of the denaturing agents, urea contains two amino groups with hydrogen-donor and acceptor properties and a hydrogen-acceptor carbonyl group. It is known that the amino groups form hydrogen bonds with water, thereby destroying the ice-like structure of water (Wen & Saito, 1965;

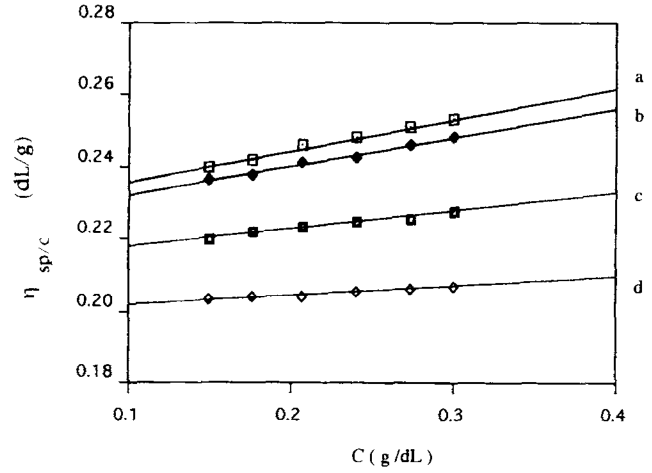

Fig. 1. Change of reduced viscosity of dextran in (a) water; (b) urea; (c) thiourea; and (d) guanidinium sulfate at 25°C (from Huggins equation).

Wolrafen, 1966). In addition, both the amino groups and the carbonyl group can associate with the suitable functional groups of the polymer. Urea–polymer association can in fact prevent the polymer associating with the water molecules. In thiourea, besides the two amino groups there is a stronger hydrogen-acceptor thio carbonyl group. In the two previous studies, in poly(*N*-vinyl-2-pyrrolidone)/water (Güven & Eltan, 1981) and dextran/water systems (Güner, 1995), it has been observed that thiourea has been more effective than urea in decreasing the intrinsic viscosity. Guanidinium sulfate contains six amino groups per molecule. Sulfate and bisulfate ions form when guanidinium sulfate dissolves in water. It is known that the presence of ionic species in aqueous medium destroys the hydrogen-bonded structure of water. Here, of course, the ion's charge and size are important. It is already known that the divalent sulfate ion is more effective in destroying the hydrogen bonding of water than the monovalent bisulfate ion (Molyneux, 1975). The association of the amino groups of guanidinium with the polymer might become more important when anionic species disrupt the hydrogen-bonded structure of water. This is seen from the decrease of the hydrodynamic volume and contraction of the polymer coil.

Intrinsic viscosity values for the aqueous solutions of 20% and 40% periodate-oxidized dextrans at 25°C are given in Table 1. The decreasing order of effectiveness of the denaturing agents in reducing the intrinsic viscosity was the same as observed in the solutions of dextran:guanidinium sulfate > thiourea > urea (Fig. 2).

The Huggins constant is a measure of the polymer–solvent interaction and it is approx. 0.35 in good solvents. However, in some other polymer–solvent systems, higher values have been observed and this was interpreted to be due to molecular association (Güner, 1995; Sütterlin, 1975; Bohdanecky & Kovar, 1982). Huggins constants can be obtained from the slopes of the straight lines of $\eta_{sp/c}$ vs c and similarly, Kraemer constants can be calculated from the slopes of the straight lines of ln $\eta_{r/c}$ vs c. Values obtained from the linear regression analysis are shown in Table 1. Decreases in the intrinsic viscosities as well as in the Huggins and Kraemer constants are indications of the diminishing molecular association of the polymer in solution.

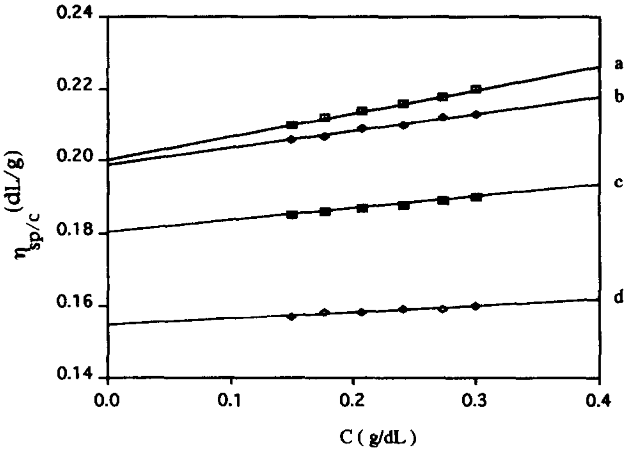

Fig. 2. Change of reduced viscosity of 20% periodate oxidized dextran in (a) water; (b) urea; (c) thiourea and (d) guanidinium sulfate at 25°C (from Huggins equation).

Temperature is also sometimes effective in breaking the molecular association. The intrinsic viscosity values measured at 40°C for the original and for the 20% and 40% oxidized dextran in the presence of 1 M denaturing agent are given in Table 2. A temperature increase of 15 deg brings about an intrinsic viscosity change ($\Delta[\eta] = [\eta]_{25°C} - [\eta]_{40°C}$) of 0.019 dl g^{-1} for dextran. For 20% and 40% oxidized dextran samples this change is 0.013 and 0.011 dl g^{-1}, respectively. The intrinsic viscosity change is less in solutions containing the denaturing agents. The intrinsic viscosity changes in 1 M urea solutions of the original dextran and of the

Table 1. Viscosity characteristics of dextran, 20% and 40% periodate-oxidized dextran in 1 M denaturing agent at 25°C

Dextran	$[\eta]$* (dl g^{-1})	k_H	k_K
Water	0.23	1.70	1.05
Urea	0.22	1.60	0.99
Thiourea	0.21	1.08	0.51
Guanidinium sulfate	0.20	0.64	0.29
20% oxidized dextran			
Water	0.20	1.68	1.05
Urea	0.20	1.21	0.63
Thiourea	0.18	1.01	0.52
Guanidinium sulfate	0.16	0.62	0.13
40% oxidized dextran			
Water	0.17	1.45	0.84
Urea	0.17	0.99	0.44
Guanidinium sulfate	0.12	0.60	0.11

*From Huggins equation.
Correlation coefficients are 0.997±0.001.

Table 2. Viscosity characteristics of dextran "1", 20% oxidised dextran "2" and 40% oxidised dextran "3" solutions

	$[\eta]^*_{40°C}$, "1"	$[\eta]^*_{40°C}$, "2"	$[\eta]^*_{40°C}$, "3"
Water	0.21	0.19	0.16
Urea	0.22	0.19	0.16
Guanidiniumsulfate	0.20	0.15	0.12

Correlation coefficients are 0.997±0.001, except in $[\eta]^*_{40°C}$ urea solution.

20% and 40% oxidized dextran were 0.008, 0.006 and 0.005 dl g^{-1}, respectively. However, in the presence of guanidinium sulfate (the most effective denaturing agent) temperature increase only changes the intrinsic viscosity of these three mentioned samples by 0.002 dl g^{-1}. Polymer samples not showing a large decrement in their intrinsic viscosity values in 1 M guanidinium sulfate could be taken as an indication of the effectiveness of the denaturing agent in breaking the hydrogen bonds.

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