Effects of Denaturing Agents on the Molecular Association of Dextran. I. Aqueous Solutions

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SYNOPSIS

Molecular association of aqueous dextran solutions have been investigated by viscosimetric measurements. Intrinsic viscosities of different concentrations of aqueous solutions of dextran with a variety of denaturing agents have been determined. Intrinsic viscosity numbers and Huggins constants of dextran solutions decreased with the addition of denaturig agents. The reducing effect of denaturing agents on the intrinsic viscosities and Huggins constants decreased in the order guanidinium sulfate > guanidinium carbonate > guanidinium chloride > thiourea > urea. The effect of temperature on the intrinsic viscosities of dextran and dextran denaturing agent solutions have also been investigated. A small change in the intrinsic viscosity values was observed for dextran in $1M$ of guanidinium carbonate and sulfate solutions with the increase of the temperature from 25 to 40°C. Aqueous dextran solutions, however, showed a significant decrease in the intrinsic viscosities in the same temperature range. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Inter- or intramolecular association of polymers with aqueous or nonaqueous solvents have been the object of many investigations. Hydrogen bonding, dipol-dipol, ion–dipol, and hydrophobic interactions are the main sources of associations of polymers in different media.

Molecular association of some water-soluble polymers, poly(ethylene oxide)[1] and poly(vinyl pyrrolidone),[2] aqueous solutions have been previously studied by viscosimetric methods. Of these studies, hydrogen bonding being responsible for these inter- or intramolecular association had been reported.

Dextran is the collective name of a large class of polysaccharides composed exclusively of D-glucose units. Dextran, a water-soluble polymer with a repeating unit, the structure of which is shown below, has a number of applications in biochemistry, food, and pharmaceutical industries. It is also known that dextran solutions may be used as a blood plasma substitute.
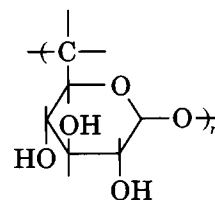
In all known solvents for dextran, hydrogen bonds are formed between the polymer and solvent molecules.[3,4] Murphy and Whistler[5] argued that many properties of dexran solutions might be explained by the presence of molecular association. Most of the water molecules are presumably associated with the dextran molecules by hydrogen bonding.[6]

In the present study, the viscosimetric behavior of aqueous dextran solutions and the addition of some denaturing agents on the molecular association of dextran solutions have been investigated.

EXPERIMENTAL

Dextran, with an average molecular weight of 496,000, was obtained from Pharmacia Fine Chemicals AB Uppsala, Sweden. Molecular weight of dextran has been determined by light-scattering mea-

surements. Molecular weight disribution is given to be $M_w/M_n = 2.59$. Denaturing agents used, thiourea and guanidinium carbonate, were obtained from BDH; the others, urea, guanidinium chloride, and guanidinium sulfate, were obtained from Fisher Scientific Company, Riedel-de Haen AG, and Merck, respectively. All the chemicals and the polymer sample were used without further purification. They were dried and stored in a dessicator over $CaCl_2$. An Ubbelohde type of viscosimeter was used. The temperature of the thermostat was controlled within a range of $\pm 0.1°C$, and the flow times were measured with an accuracy of ± 0.1 s.

RESULTS AND DISCUSSION

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

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

Here, $\eta_{sp/c}$ is the reduced specific viscosity, $[\eta]$ is the intrinsic viscosity, and k_H is the Huggins constant or Huggins slope coefficient. The intrinsic viscosity is a characteristic function for the single molecule in solution. It depends on molar mass, structure, and conformation of the polymer molecules, on the solvent power, and temperature. The dimension of $[\eta]$ is a measure of the effective hydrodynamic volume of the polymer in solution, while Huggins constant is a measure of polymer–solvent interaction.

Some of the available crystallographic studies on the structure of polysaccharides are as follows: (1) the ring-oxygen atom and bridge-oxygen atom are invariably hydrogen-bond acceptors; (2) here, each hydroxyl group is associated with two hydrogen bonds, one a donor and one an acceptor bond.[7] Dextran attracts the hydrogen and oxygen atoms of water molecules, and hydrogen bonding is one of the major type of forces causing association in aqueous solutions.

The association of polymer–solvent molecules by hydrogen bonding is possibly disrupted by using some denaturants such as urea,[1,8] thiourea,[2] and guanidinium compounds.[2,9] The requirement of high concentrations of these denaturants is generally interpreted to be due either to weak binding of the denaturant molecule to the polymer or to changes in the local water structure surrounding the polymer.

In this study, urea (U), thiourea (TU), guanidinium chloride (GCl), guanidinium carbonate (GCO_3), and guanidinium sulfate (GSO_4) are used as denaturing agents. Addition of these denaturants has lead to a decrease in the viscosities of aqueous dextran solutions at 25°C. The most important effects of these denaturants on the decrease of the intrinsic viscosity of dextran solutions are:

1. Urea has the smallest effect on the decrease of the intrinsic viscosity of dextran solutions (Figs. 1 and 3). This value is dropped to 0.518 in $1M$ of U and 0.492 in $1.5M$ of U solutions, while for the aqueous dextran solutions $[\eta]$ = 0.530 dL/g.

2. Guanidinium sulfate has the greatest effect on the decrease of the intrinsic viscosity of polymer solution (Figs. 2 and 3). The value of $[\eta]$ = 0.530 dL/g in aqueous solution is dropped to 0.419 in $1M$ of GSO_4 and 0.355 in $1.5M$ of GSO_4 solutions.

3. The decrease in the intrinsic viscosities of polymer solutions depends on the molar concentrations of denaturants. Intrinsic viscosity values decrease by increasing the denaturant concentrations (Fig. 3).

4. The decreasing order of effectiveness of the denaturing agents in reducing the intrinsic viscosity is $GSO_4 > GCO_3 > GCl > TU > U$ (Fig. 3). Intrinsic viscosities for $1M$ of solution of denaturing agents are calculated as: 0.419, 0.420, 0.473, 0.482, and 0.518 dL/g for GSO_4, GCO_3, GCl, TU, and U, respectively.

The observed decreases in the intrinsic viscosities can be considered as the distruption of the hydrogen bonding of dextran in aqueous solutions and the association among polymer chains.

The denaturing agents urea and thiourea have hydrogen-acceptor carbonyl and sulfonyl groups, and also hydrogen-donor and acceptor amino groups. Amino groups interact with adjoining water molecules through hydrogen bonding in that they break the ice-like structure of water.[10,11] The interaction of urea and thiourea molecules with polymer molecules may also result from the blocking of hydrogen bonding of dextran chains with water molecules.

The denaturing effect of guanidinium compounds is larger than urea and thiourea. This is due to the hydrogen bond disrupting effect of these compounds (GCl, GCO_3, and GSO_4 have three and six amino groups per molecule of compound). These denaturants provide chloride, carbonate, sulfate, bicarbonate, and bisulfate ions for the aqueous media. The last two ions are hydrogen-bond acceptor and donor in character. When the first three ions—chloride,
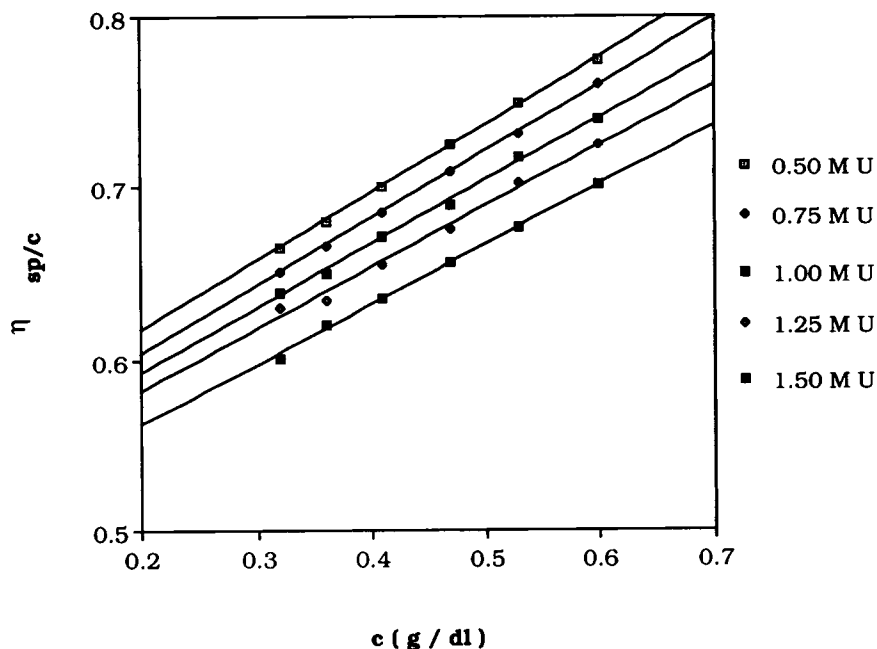
Figure 1 Change of reduced specific viscosity of dextran solutions for different urea concentrations at 25°C (correlation coefficients are 0.998 ± 0.002, except in 1.25M of U).

carbonate, and sulfate—are compared, carbonate and sulfate ions are doubly charged and they interact strongly with hydrogen-bond donors.

It is known that ions change the hydrogen-bonded structure of water. The ability to destroy the hydrogen-bonded structure of water depends mainly on the charge and size of the ion. Divalent ions (in this study, carbonate and sulfate) can destroy the hydrogen-bonded structure of water more than the univalent ions (in this study, chloride).[12-14] We assume that ions disrupting the hydrogen-bonded structure of water may also affect the dextran–water interaction.
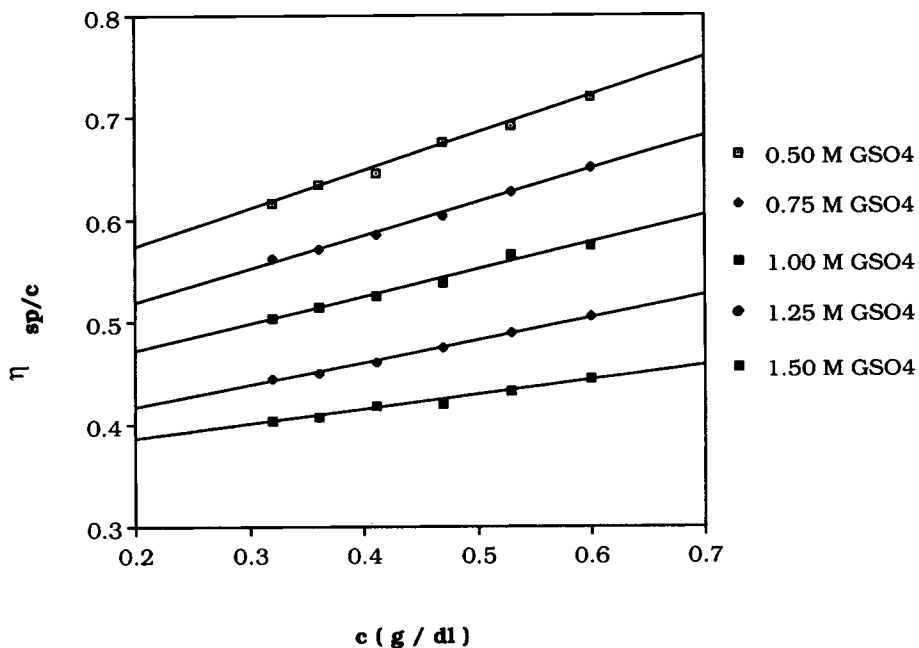

Figure 2 Change of reduced specific viscosity of dextran solutions for different guanidinium sulfate concentrations at 25°C (correlation coefficients are 0.996 ± 0.002).
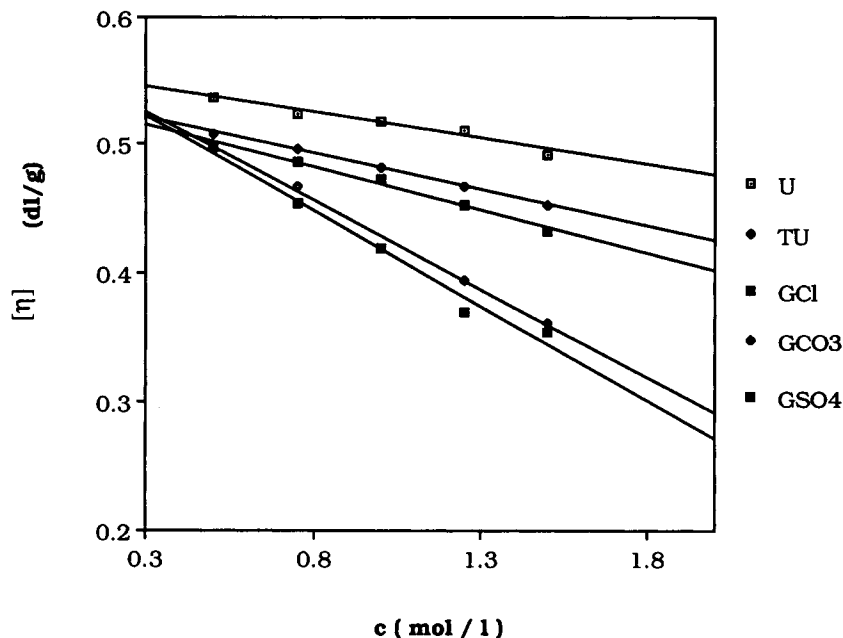
Figure 3 Change of intrinsic viscosity of dextran with denaturing agent concentration at 25°C.

The guanidinium carbonate and sulfate provide two guanidinium cations per mol of compound as compared to guanidinium chloride. A kind of interaction may arise between hydrogen-bond acceptors and the cations,[15-17] and it decreases the intrinsic viscosity of dextran in aqueous solutions.

The accepted value of Huggins constant for polymer molecules in good solvents is about 0.35. In some polymer–solvent systems, Huggins constant assumes high values,[18,19] which are usually due to the doubling of molecules in these solvents. There is one condition that must be fulfilled if double molecule formation is to become manifest in the Huggins constant. The process must be concentration dependent, i.e., association must be destroyed by diluting the system and reformed by concentrating it.

Huggins constant can be calculated from the slope of $\eta_{sp/c}$ vs. c line easily. For the aqueous dextran solutions, the k_H value was 1.526, while this value dropped to 1.442 in $1.5M$ of U solution.

On the other hand, the guanidinium sulfate has the greatest effect on the intrinsic viscosity decrement. The slopes of the straight lines show a continuous decrease with the increase of denaturant concentrations. The Huggins constant of dextran in $0.5M$ of GSO_4 is 1.496 (Fig. 2). It drops to 1.159 in $1.5M$ of GSO_4.

Changing the urea concentration $(0.5–1.5M)$ brings about a change of 0.05 to the k_H value. The decreasing order of effectiveness of the other denaturing agents in reducing of Huggins constants (with the values) is guanidinium sulfate (0.367) > guanidinium carbonate (0.358) > guanidinium chloride (0.218) > thiourea (0.117) for the same concentration range.

The observed decrease in both the intrinsic viscosity and the Huggins constant values can be taken as an indication of the decrease of association among polymer chains.

The effect of the temperature on the intrinsic viscosity of dextran solutions is also investigated. It is well known that there is an equilibrium between association and dissociation through hydrogen bonding, and that this equilibrium is affected by the change of temperature. A rise in temperature is a thermal stress and the equilibrium will, therefore, shift to relieve the stress by absorbing heat. Heat is absorbed by breaking hydrogen bonds. The temperature increase decreases the number of hydrogen bonds and, consequently, decreases the association, i.e., the equilibrium shifts towards dissociation.

The results of the intrinsic viscosity values of dextran solutions in water and in $1M$ of denaturants heated at 25, 30, 35, and 40°C are shown in Table I. The intrinsic viscosity of aqueous dextran solutions decreases on heating from 25 to 40°C. The intrinsic viscosity decrement was found to be 0.074 $(\Delta[\eta] = [\eta]\ 40°C - [\eta]\ 25°C)$. The same temperature increment was applied to polymer-denaturant solutions; the intrinsic viscosity decrements were

Table I Effect of Temperature on the Intrinsic Viscosity of Dextran Solutions

	$[\eta]$ 25°C	$[\eta]$ 30°C	$[\eta]$ 35°C	$[\eta]$ 40°C
Water	0.530	0.511	0.490	0.456
Urea[a]	0.518	0.515	0.510	0.504
Thiourea[a]	0.482	0.478	0.473	0.470
Guanidinium chloride[a]	0.473	0.471	0.469	0.468
Guanidinium carbonate[a]	0.420	0.419	0.419	0.419
Guanidinium sulfate[a]	0.419	0.419	0.418	0.418

[a] $1.0 M$ of solutions.

calculated to be: 0.014, 0.012, 0.005, 0.001, and 0.001 for $1M$ of U, TU, GCl, GCO_3, and GSO_4, respectively. The intensitivity of guanidinium carbonate and sulfate solutions to temperature increment in the range studied is the result of the already disrupted hydrogen bonds due to the action of the denaturing agent.

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