# Intrinsic Viscosity of Bovine Serum Albumin in Water–Dioxan

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*Summary* The intrinsic viscosity of bovine serum albumin in dioxan–water (1 : 3 v/v) was found to be 0·019 dl g$^{-1}$, far below the range 0·033—0·040 dl g$^{-1}$ expected for most globular proteins; the result indicates that serum albumin approximates closely to an ideal Stokes–Einstein sphere in this solvent system.

THE intrinsic viscosity of most globular proteins in aqueous solutions at the isoionic point and 25° is in the range 0·033—0·040 dl g$^{-1}$. Herein, we report on a protein whose intrinsic viscosity is far below this range. For bovine serum albumin (BSA) in dioxan–water (1 : 3 v/v) at the isoionic point (pH 5·63), ionic strength *I* 0·03, and 25°, the intrinsic viscosity was 0·019 dl g$^{-1}$ (see in Figure). For comparison, viscosity experiments were also carried out for BSA in ethanol–water (1 : 3 v/v) and in water alone under the same conditions (see Table).

*Effects of organic solvents on the intrinsic viscosity of* BSA *at the isoionic point, ionic strength*[a] 0·03, *and* 25°

| Solvent | pH[b] | [η] in dl g$^{-1}$ |
|---|---|---|
| Water .. .. .. .. | 5·30 | 0·036 ± 0·001 |
| Ethanol–water (1 : 3) .. .. | 5·74 | 0·035 ± 0·002 |
| Dioxan–water (1 : 3) .. .. | 5·63 | 0·019 ± 0·002 |

[a] Adjusted with KCl.
[b] The pH values as read from the glass electrode pH meter were not corrected for the presence of organic solvent.[8] The intrinsic viscosity of BSA does not change over the pH range 4·3—10·5.[9,10]

The intrinsic viscosity of BSA in water is in good agreement with the literature,[1,2] and the value in ethanol–water (1 : 3) is virtually the same as in water alone. The low value for BSA in water–dioxan is surprising, however, and, if correct, would mean that serum albumin approximates closely to an ideal Stokes–Einstein sphere in this solvent system.

Viscosity measurements were carried out with a Cannon–Ubbelohde No. 50 viscometer. The experimental procedure, kinetic energy correction, and calculation of solution densities have been described previously.[3]

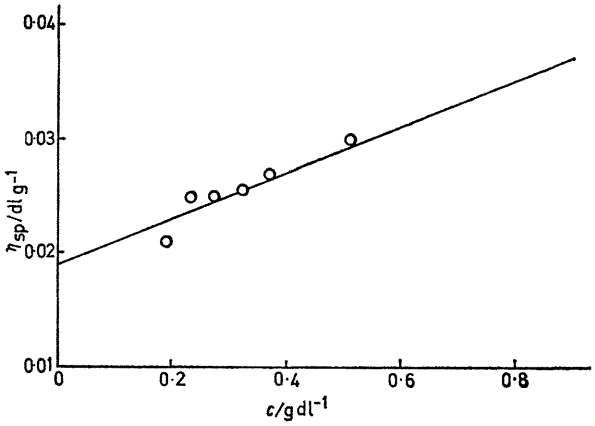
The low value could not be attributed to artefacts. In extremely dilute solutions (< 1 mg/ml) the protein may be adsorbed on the walls of the capillary, thus narrowing its radius, *r*, and thereby reducing the intrinsic velocity since $\eta \propto r^4$ (ref. 4). However, this could not be the case in our measurements, since we used a concentration range of 0·19—1·00 g dl$^{-1}$, and obtained similar results over the whole range. Further, solutions of this concentration are hardly extremely dilute. Adsorption most probably did not occur, but, even if it did, the value of 0·019 is far below the value of 0·032 obtained by correcting the value of 0·036 by 10%, the correction itself being an overestimation.

This shows that the low value is not due to experimental error.

The problem lies in the interpretation of the low value. The intrinsic viscosity of a protein is believed to be a measure of the asymmetry of the protein molecule as well as its effective volume,[5] and a change in the asymmetry, or the effective volume, or both, could explain the low value.

To determine which was responsible we measured o.r.d. spectra and the sedimentation velocity.

O.r.d. measurements (on a Cary 60 spectropolarimeter) showed that there was no change of helical content of BSA in water–dioxan compared with BSA in water alone. In both solvent systems the characteristic Cotton effect was observed [195 (peak), 233 (trough), and *ca.* 215 (shoulder) nm].




*Determination of the intrinsic viscosity of BSA in dioxan–water* (1 : 3) *at the isoionic point, ionic strength* 0·03, *and* 25°.

The sedimentation velocity coefficients, corrected to the reference state (water at 25°) were similar: for BSA in water alone, $s_{25,w}$ was 4·74 × 10$^{-13}$ s and for BSA in water–dioxan it was 4·78 × 10$^{-13}$ s. The difference could be due to experimental error, and both values were slightly lower than those reported.[3] Since the sedimentation coefficient is inversely proportional to the effective hydrodynamic radius of the molecule, our results suggest that the hydrodynamic radius of BSA in water is similar to that of BSA in water–dioxan. It is, therefore, reasonable to assume that there is no change in the effective volume of the BSA molecule as the solvent is changed from water to water–dioxan.

Therefore, we conclude that the low value of intrinsic viscosity may be attributed to the change of the asymmetry of the molecule. In 25% dioxan, the minor axis, *b*, of the BSA molecule increases while the major axis, *a*, decreases, thus causing the molecule to become more spherical. The change of axial ratio was also observed for BSA in dioxan–water (1 : 3) at pH 2·2, *I* 0·03, and 25°, but the change was in the opposite direction.[6] In the acidic solution, the major axis lengthens while the minor axis shortens; the molecule is elongated, becoming less spherical. In addition, while the effective volume is virtually constant for BSA in the isoionic water–dioxan mixture, it changes for BSA in acidic water–dioxan.

On the basis of the above observations, we deduce that BSA is basically a compact, but not quite rigid, molecule in aqueous solution. As dioxan is added the forces that hold

the molecule to a certain form are disturbed, possibly owing to the competition of dioxan with water in hydrogen bonding, to electrostatic interaction, or to proton transfer.[7] At the isoionic point where the protein molecule is not unfolded, dioxan causes the BSA molecule to exist in a more compact spherical form. In acidic solution where at least 35% of the polypeptide of BSA molecule unravels and the internal structure of the molecule becomes less rigid, dioxan causes the increase of the end-to-end root mean square distance of the molecule;[6] the molecule is elongated.

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