Light Scattering Measurements on Proteins in Water-Organic Solvent Mixtures

FELIX FRIEDBERG* and JÖRGEN ÖHMAN

Institute of Physical Chemistry, University of Uppsala, Uppsala, Sweden

Molecular weights of macromolecules determined by light scattering are affected by mixed solvents if these solvents have different refractive indices. The apparent variations in molecular weight are attributed to the preferential adsorption of molecules of one of the solvent components by the solute. Recently, this concept has been employed for measurements of the number of molecules selectively adsorbed, *i.e.* for gaining information on the degree of solvation [1-3]. The study described here was undertaken to learn whether values for the hydration of proteins might be obtained in a similar manner. The mixed solvent systems chosen were water-chloroethanol, water-glycerol and also water-ethylene glycol. Creatine-phosphotransferase of rabbit muscle and bovine serum albumin were the proteins evaluated.

For both proteins, the slope of the line in the plot Kc/R_{90} *versus* concentration increases to a maximum (at approximately 55 % by volume of chloroethanol) and then decreases again as the volume percent of the chloroethanol in the solvent mixture increases (Figs. 1 and 2). At about 50 volume percent concentration, chloro-

* Permanent address: Howard University, Washington D.C., U.S.A.

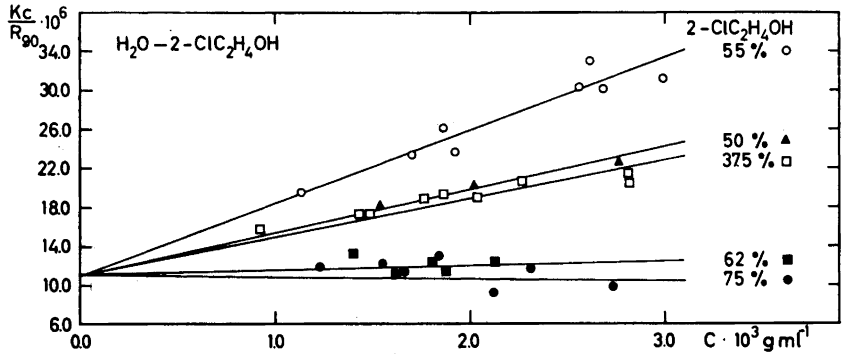

Fig. 1. Kc/R_{90} *versus* concentration for creatine-phosphotransferase in water (actually 0.1 M acetate buffer, pH 4.4)-2-chloroethanol mixtures of various volume percent composition. Solutions were cleaned by centrifugation for 2 h at 35 000 r.p.m. and for further 2 h at 20 000 r.p.m. in Dandliker cells using the Spinco preparative ultracentrifuge with rotors 40 and SW−25, respectively. Measurements were made at 436 mμ with benzene as the standard (R_{90} = 46.5 × 10^{-6} cm^{-1}). Extrapolation to zero angle is omitted because identical values were obtained at all angles examined (45°−135° at 10° intervals).

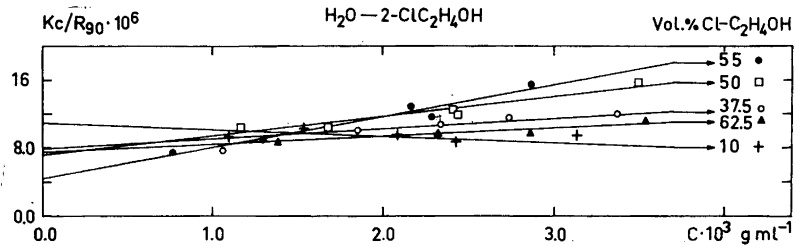

Fig. 2. Same as Fig. 1 but for bovine serum albumin.

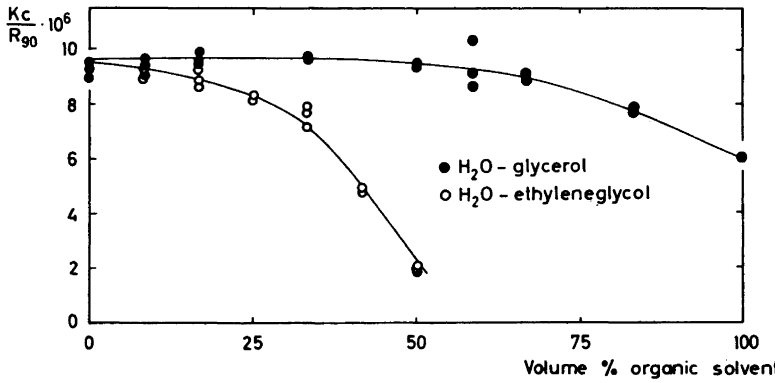

Fig. 3. Kc/R_{90} *versus* volume percent glycerol or ethylene glycol in the water (actually 0.2 M phosphate buffer, pH 6.0)-glycerol or ethylene glycol mixtures. Experimental conditions are the same as described for Fig. 1. Extrapolation to zero concentration of creatine-phosphotransferase is also omitted here, because at the conditions of the experiment the slope of the line Kc/R_{90} *versus* concentration is almost zero. The concentration range of protein used is identical to that given in Figs. 1 and 2. For each volume percent of organic solvent examined, values obtained with three different protein concentrations have been plotted.

ethanol induces major conformational changes, *i.e.* helix coil transitions in globular proteins [4]. According to Tanford *et al.* [5,6] the effect of chloroethanol on ribonuclease and β-lactoglobulin results not in a single alteration but rather in two successive processes of different character: First, disruption of the native structure of the molecule and then, refolding into a right handed helix. Solute-solvent interactions might be most pronounced when the molecules reach the stage of maximum unfolding. (It should be mentioned, however, that attempts made by us to show a similar phenomenon in the case of ribonuclease exposed to water-chloroethanol mixtures in various proportions have remained unsuccessful.) The scatter-

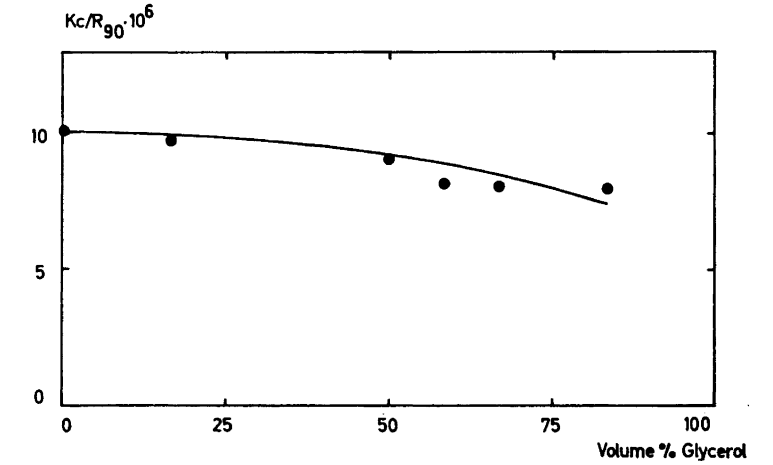

Fig. 4. Same as Fig. 3 but for bovine serum albumin in water (actually 0.1 M acetate buffer, pH 4.4)-glycerol mixtures.

ing of the experimental points, particularly when the slope increases greatly would make it hazardous to utilize an apparent decrease in molecular weight for calculation of a solvation layer.

Flow birefringence studies of Edsall and Foster [7] indicate that γ-globulin and serum albumin, too, are little affected by high glycerol concentrations. It has also been shown by optical rotation and sedimentation studies that γ-globulin retains its native conformation in ethylene glycol-water mixtures up to a glycol concentration of at least 80 % by volume except that some aggregation occurs. Ethylene glycol is similarly inert toward β-lactoglobulin [8]. From the data presented as Fig. 3, however, it appears that there is an increase in molecular weight because aggregation occurs upon addition of ethylene glycol (even in small amounts) to aqueous solutions of creatine-phosphotransferase. The tolerance exhibited by this enzyme towards glycerol is higher: Changes begin only after approximately 50 volume percent organic solvent have been attained. In the case of bovine serum albumin, dissolved in glycerol – water mixtures, the results are similar (Fig. 4). Again, these solvents cannot be employed to gain information on the degree of hydration of the protein molecules.

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Studies on Glucomannans from Norwegian Spruce *

4. Enzymic Hydrolysis

HANS O. BOUVENG, TOICHI IWASAKI, BENGT LINDBERG and HANS MEIER **

Träkemiska avdelningen, Svenska Träforskningsinstitutet, Stockholm, Sweden

The glucomannans from coniferous woods have been extensively studied during the last decade. The results, reviewed by Aspinall [1] show an essentially linear structure with (1→4)-linked β-D-glucose and β-D-mannose residues, usually in the proportion 1:3-4. The native polysaccharides also contain *O*-acetyl groups [2-4]. In some preparations glucose and mannose residues are to some extent substituted in the sixth position by α-D-galactopyranose residues [1].

There are indications that besides the galactosidic side-chains additional branching occurs in some coniferous wood glucomannans but conclusive evidence for this is lacking. It is further not known whether or not the glucose and mannose residues are arranged according to a regular pattern. The isolation and characterisation of the oligosaccharides formed upon enzymic hydrolysis of a glucomannan using a specific enzyme would give information of this. A crude hemicellulase has previously been used by Perila and Bishop [5] for enzymic hydrolysis of Jack pine (*Pinus banksiana* Lamb.) glucomannan.

A glucomannan from Norwegian spruce (*Picea abies* Karst.), containing 27 % glucose, 72 % mannose, and 1 % galactose, was accordingly hydrolysed with a commercial cellulase preparation from *Penicillium chrysogenum notatum* Astra 200 and the products were fractionated by paper and carbon column chromatography. The results are summarised in Table 1. Inspection of the table shows that the enzymic preparation was not specific for (1→4)-β-D-glucosidic linkages and the purpose of the investigation was therefore not attained. The glucosidic linkages were, however, more readily hydrolysed than the mannosidic, as indicated by the relatively high

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** Present address: Botanisches Institut, Universität Fribourg, Schweiz.