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Note

Hydrophobic interaction in sugar solutions

Results from gel interaction study

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Sugars can prevent the thermal denaturation of proteins in solution[1–4] as well as reduce the extent of their denaturation by urea[5]. The possible mechanism by which sugars induce stability to denaturation in protein molecules is not known.

We have recently observed[6] that the solubility of N-acetyl ethyl esters of aromatic amino acids, which have been considered as models[7] for the relevant portion present in a protein molecule, is lower in glucose and sucrose solutions than in water. This decreased solubility has been termed the "sugaring out" effect by analogy with the "salting out" phenomenon which describes the decrease in solubility of non-electrolytes in salt solutions. The increased activity coefficients of the esters in sugar solutions and the positive free energy of transfer of the molecules from water to these solutions have been interpreted as arising from the increased hydrophobic interaction which is probably responsible for the increased stability of the proteins in sugar solutions.

In a recent communication[8], the elution of the N-acetyl amino acid ethyl esters from aqueous Sephadex LH-20 (hydroxypropyl derivative of Sephadex G-25) was found to depend on the hydrophobic interaction between the gel and the ester molecules. The conclusion was obtained from an observed increased retention of the esters in the gel at higher temperatures. The increased retention of the esters in the presence of electrolytes indicated an increased hydrophobic interaction between the esters and the gel; this supported Kauzmann's suggestion that the hydrophobic interaction increases in salt solution[9]. The present communication reports a study of the interaction between the ester molecules and the Sephadex LH-20 gel in sucrose, glucose and fructose solutions.

Sucrose and fructose, like glucose[10], are not retained in the gel. The void volume, V_0, of the gel is not effected in sugar solutions. Swelling of the gel is reduced in sugar solutions compared to its swelling in water (Fig. 1). Of the three sugars, sucrose reduces the swelling most, the effects of glucose and fructose being about equal.

The retention of the esters in sugar solutions increases, as can be seen from the upper part of Fig. 2, where the elution volume of N-acetyl-L-tyrosine ethyl ester (ATYE) in 1.5 *M* sucrose is 50 ml greater than its elution volume in water. To compare the retention at different sugar concentrations, an affinity number[11] $A = (V_e - V_t)/g$ is used where V_e is the elution volume of the solute, V_t is the bed volume of the

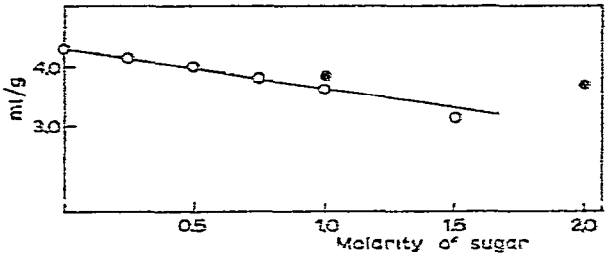

Fig. 1. The swelling of Sephadex LH-20 *versus* the concentration of sugar in the swelling solvents; ○, sucrose; ●, glucose and fructose pH 6.6 (distilled water); temperature, 25°.

gel, and g is the dry weight of the gel in grams (Fig. 2). The affinity of the esters for the gel (i) increases with sugar concentration, the increase being more rapid at higher concentrations of sugar, and (ii) increases in sucrose solution as compared with those containing glucose and fructose, the affinity in these latter two solutions being nearly equal. The retention pattern is similar to observations made in salt solutions[8]. The retention of the esters in the gel takes place by hydrophobic interaction. The increased retention in sugar solutions probably indicates stronger hydrophobic interaction

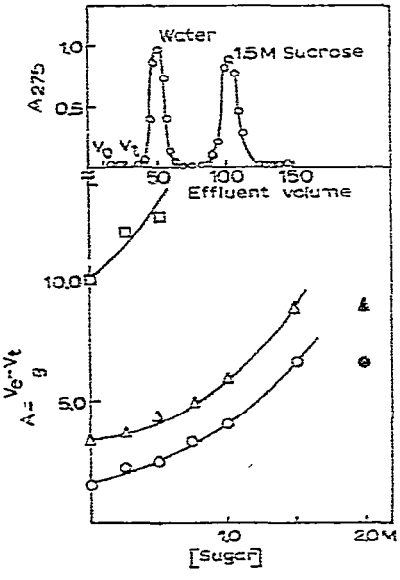

Fig. 2. Top: the elution of N-acetyl-L-tyrosine ethyl ester (ATYE) in water (50 ml) and 1.5 M sucrose solution (102 ml). V_0 = 11 ml; V_t = 28 ml. 3-ml fractions were collected except near the peak, where the fractions were 2.5 ml. Column dimensions 1.8 × 11 cm. V_0 was determined by Blue Dextran 2000, V_t by weighing the amount of water equal to the volume of the gel level in the column. Flow-rate, 25 ml/h; pH 6.6 (distilled water); temperature, 25°. Bottom: the affinity number, A, for N-acetyl-L-phenylalanine ethyl ester (APE), ATYE and N-acetyl-L-tryptophan ethyl ester (ATRE) chromatographed in Sephadex LH-20 *versus* the concentration of sugar in the elution; g = weight of dry gel. Open symbols, sucrose; closed symbols, glucose and fructose. Concentrations of the esters in the fractions were determined by measuring absorbance at 257, 275, and 278 nm for APE, ATYE and ATRE respectively. pH 6.6 (distilled water); temperature, 25°. □, ATRE; △, ▲, ATYE; ○, ●, APE.

between ester molecules and the gel. This indicates that hydrophobicity increases in sugar solutions, with the hydroxypropyl groups of the gel probably being the sites where the molecules interact. However, the possibility also exists that the solubilities of the esters in the mobile and stationary phases are different in the absence and presence of sugars, and this may explain the present results*.

The reduction in the swelling of the gel in sugar solution probably arises as follows. The ether oxygen in the Sephadex LH-20 is capable of hydrogen bonding with water molecules to form an oxonium structure which results in the swelling of the gel[12]. Sugar molecules, which are capable of forming hydrogen bonds even more strongly than water[13], compete favourably for water molecules with the ether oxygens to result in gel shrinkage. Alternatively, by analogy with the shrinkage of the gel at high temperatures due to increased hydrophobic interaction[12], the hydrophobic interaction of hydroxypropyl side chains in sugar solution may increase resulting in the observed gel shrinkage.

The present result indicates that addition of sugars to water increases hydrophobic forces between additional groups and molecules present in the system.

NOTE BY THE EDITOR

We doubt whether a general "sugaring out" effect exists. Experiments in quite another field, namely the paper chromatography of $HAuCl_4$ in aqueous 0.1 *N* HCl–glucose solutions, were entirely negative.

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