

A Near-Infrared Spectroscopic Investigation of Water in Solutions with Proline, Glycinebetaine, Glycerol and Albumin

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The ($\nu_2 + \nu_3$) combination band of water has been investigated in aqueous solutions of proline, glycinebetaine and glycerol and in a three component solution with each of these substances and albumin. It is shown that the hydrogen bonding strength between the water protons and proline or betaine is higher than between water and glycerol. Betaine exhibits a higher affinity versus the oxygen of water than does proline.

The water binding capacity of pure proline solutions is unchanged in a three-component solution with albumin. Proline neither enhances nor reduces the solubility of this highly soluble protein. In contrast, in a three-component solution with betaine, the solubility of both betaine and albumin is reduced. It is assumed that these solute particles compete for the same binding position on the water molecule. Concentrated glycerol solutions with very low water concentrations dissolve a considerable amount of albumin, which points to the fact that the protein must be partly dissolved in glycerol itself.

Introduction

In the meantime it is a well known phenomenon that several plant cells accumulate organic substances under water stress conditions: various polyols or the amino acid proline (reviewed by Hellebust [1] and Kauss [2]). Glycinebetaine is found in a large number of plants (Cromwell and Rennie [3], Storey *et al.* [4]) and a relationship between the glycinebetaine content and salt sensitivity has been described (Storey and Wyn Jones [5]).

In a former paper (Schobert [6]) the function of these substances has been interpreted to exert a regulatory effect on the cellular water structure, being altered during the state of a reduced water activity in the cell. The solution properties of proline and its influence on the solubility of several proteins has been described earlier (Schobert and Tschesche [7]), but additional investigations on the solution properties of the other compounds are lacking.

In the near infrared region spectroscopic investigations of the respective pure solutions and of their mixture with protein have been carried out, to obtain further information on the interrelationships of these substances with proteins and with water. Although these examinations of both the two- and three-component systems represent only very simple model systems, they can provide an insight into pro-

perties, being basic and important for further investigations of more complex cellular systems.

Materials and Methods

Glycinebetaine was a product of Fluka, Buchs, Switzerland, and proline was obtained from Bachem, Liestal, Switzerland in the purest grade available. Glycerol, p. a. grade, was procured from Merck, Darmstadt, GFR. Bovine albumin powder was a product of Sigma Chemical Company, St. Louis, USA. All the solutions were prepared with double-distilled water in calibrated tubes. Air bubbles in the viscous solutions were removed carefully by sonication in a cooled water bath. The density of the solutions were recorded by weighing a 100 μ l-aliquot in constriction pipettes. The water concentration and the molar extinction coefficient (ϵ) of water were calculated from the density. Each value is a mean of five parallel samples.

Infrared spectra were recorded, using the Cary Model 17 spectrophotometer (Varian); $t = 20^\circ\text{C}$. For each measurement the zero point was adjusted at 1050 nm. The results were established with three parallel samples.

Results and Discussion

General principles

The $\nu_2 + \nu_3$ combination band of water in the 1900 nm region proved to be convenient for the fol-

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lowing investigations, because there are few other interfering solute bands at this wavelength. In pure water this band includes a mixture of different water species. The higher the solute concentration, the lower is the water concentration and the more water of a definite species, interacting with the solute particles, is present in a solution. Therefore, only highly soluble solute substances are proper to this spectroscopic method, to obtain an effect of adequate quantity. Glycerol is miscible with water in all proportions and the solubility of proline, betaine and albumin proved to be of a sufficient magnitude to carry out the experiments. The shift of the combination band towards longer wave lengths (lower frequencies) indicates the existence of hydrogen bonds between water (as a proton donor) and the solute molecule (as a proton acceptor). The stronger and more numerous these hydrogen bonds are, the higher is $\Delta(\nu_2 + \nu_3)$ (Geiseler and Seidel [8], Bonner and Choi [9]).

The magnitude of the molar extinction coefficient of water (ϵ_{water}) is increased by virtue of hydrogen bonding between the lone pair electrons of the water oxygen (as a proton acceptor) and the solute molecule (as a proton donor) (Geiseler and Seidel [8]). Furthermore, any positively charged solute particle is able to polarize the lone pair electrons of the water oxygen and thus enhances ϵ_{water} . This has been confirmed with 4 M solutions of LiCl, NaCl and KCl (not shown). ϵ_{water} rises with the size of the cation, due to the increasing ionic interaction between these cations and the water oxygen.

Proline

The influence of proline on water is demonstrated in Fig. 1. The shift of the combination band towards longer wave lengths is more pronounced with increasing concentrations of proline and indicates that the hydrogen bond between the carboxylic anion and water is much stronger than between water-water. The enhanced ϵ_{water} is due to the hydrogen bonding between the $-\text{NH}_2^+$ group of proline and the water oxygen. Thus, the ability to act as a proton-donor and acceptor and to form hydrogen bonds with water is closely related to the respective acidity and basicity of the solute particle. Therefore, water binds very strongly and preferentially to the polar groups of proline, acting as a water-structure-former.

The biological function of proline has been described as a "mini-detergent", converting hydrophobic

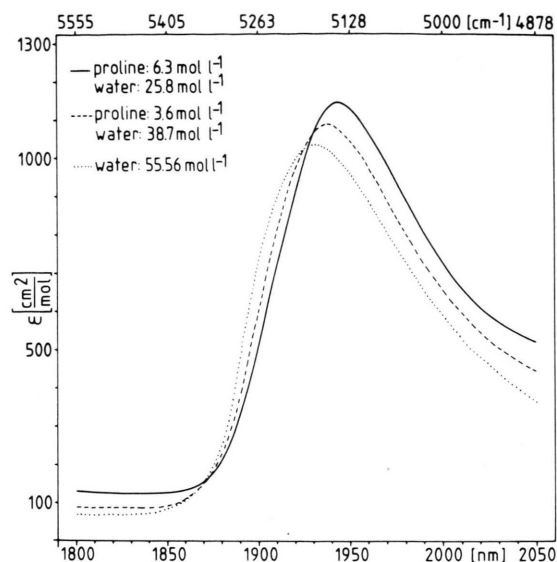


Fig. 1. Combination bands of water in solutions with proline at various concentrations.

areas of biopolymers into hydrophilic residues by a hydrophobic association of proteins with the amino acid and thus increasing the solubility of proteins (Schobert [6]; Schobert and Tschesche [7]). However, detergents and chaotropic solutes, increasing the solubility of many proteins, weaken the hydrogen bonds between water molecules, acting as water-structure-breakers. This in turn causes denaturation of the proteins. The water structure forming properties distinguish proline from detergents and they seem to be a prerequisite to preserve the integrity of proteins during the hydrophobic association with proline.

Glycinebetaine

As can be seen from Fig. 2, $(\nu_2 + \nu_3)$ is very similar in betaine and proline solutions, due to the basicity of the carboxylic anion. However, the value of ϵ_{water} is higher in betaine solutions compared to proline solutions of equal molar ratios with water or to solutions of equal water concentrations. This result shows that the interaction of the quaternary ammonium ion with the water oxygen is stronger than that of the $-\text{NH}_2^+$ -group in proline.

Glycerol

The first overtone of the OH-stretching mode of glycerol was found to interfere partly with the area

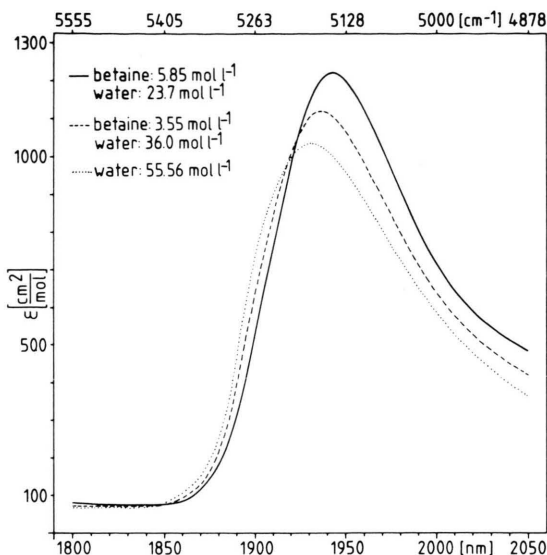


Fig. 2. Combination bands of water in solutions with glycinebetaine at various concentrations.

of the water band. Therefore, it is not possible to discuss ϵ_{water} in glycerol solutions, but only the shift of the combination band, which is represented in Fig. 3. It is indicated that the hydrogen bonding strength between glycerol and the water protons is stronger than between the water molecules in pure water, but less strong than the interaction between water and proline (in solutions of equal water concentrations). This result is in good agreement with assumptions, made formerly (Schobert [6]), that the OH-groups of glycerol behave “water-like” and therefore are able to replace water molecules.

Bovine albumin

Bovine albumin has also been investigated in pure water, to avoid any additional influence of buffer

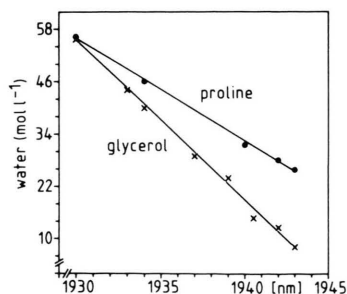


Fig. 3. Position of $(\nu_2 + \nu_3)_{\text{max}}$ in relation to the water concentration in solutions with glycerol and proline, respectively.

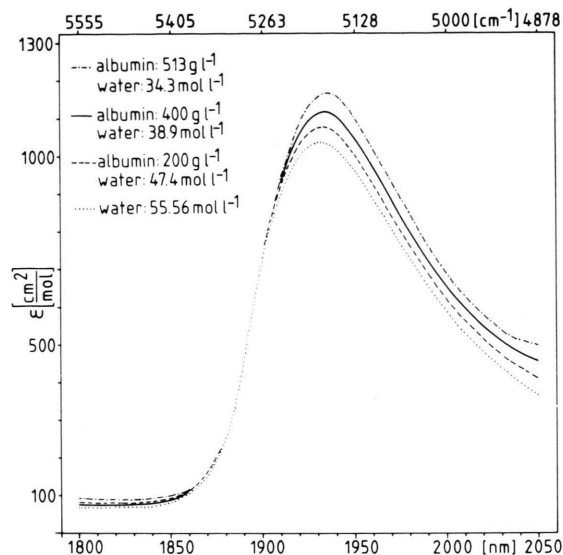


Fig. 4. Combination bands of water in solutions with bovine albumin of increasing concentrations.

salts. It is exhibited from Fig. 4 that increasing concentrations of albumin cause only a very little shift of $(\nu_2 + \nu_3)_{\text{max}}$, whereas ϵ_{water} is considerably enhanced. This indicates that albumin possesses a high H-donor potential and the interaction between the protein residues and water occurs preferentially via the water oxygen. This finding is further supported by the unchanged position on the left flank of the combination band. It points out that the number of the free water species, being included on this side of the combination band (towards higher frequencies), is not reduced by the presence of the dissolved albumin.

Proline and albumin

The postulated hydrophobic interaction between proline and unpolar residues of proteins includes an equilibrium state with bound and free proline molecules. Therefore, also the hydrophilic interrelationships between proline, water and the hydrophilic groups of proteins has to be considered. A three-component solution with albumin and proline was investigated (Fig. 5). In this solution, the concentration of both solute particles reaches the limit of their solubility and the definite mixture ratio results in the lowest water concentration, which can be obtained with this three-component system. This water concentration is lower than in the highest concentrated solutions of the respective pure substances (*cf.*

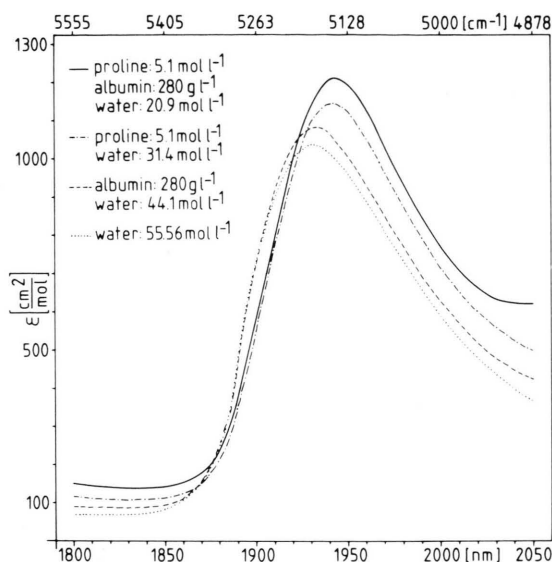


Fig. 5. Combination bands of water in a three-component solution with proline and albumin and in solutions of the respective pure substances.

Figs 1 and 4). However, the ratios of proline and albumin with water are approximately equal in both the three-component system (proline: 28.0 g/mol H₂O; albumin 13.4 g/mol H₂O) and in the highest concentrated solution with the pure substances (proline: 28.11 g/mol H₂O; albumin: 14.96 g/mol H₂O). This in turn means that the presence of proline neither enhances nor reduces the solubility of albumin. Furthermore, it is apparent that both solute species do not compete for water, but are able to “share” the water molecules present. Fig. 5 shows that the combination band is shifted towards longer wave lengths in the three-component solution. As can be seen from the pure proline solution (of equal solute concentration), this shift is due to the proline molecules present. In contrast, albumin contributes predominantly to ϵ_{water} . Therefore, it is highly probable that the extensive miscibility of both solute species is due to different binding sites with water. Proline, which has both H-donor and H-acceptor potential, obviously does not compete with the H-donor potential of albumin. The H-bonding strength between both solute particles and water seems to be of equal magnitude. If water is trapped between the polar groups of protein and proline, the hydration sphere around the protein is more tightly bound. This is probably an additional important function of proline in plant cells during water stress conditions.

Glycinebetaine and albumin

Betaine is less miscible with albumin than proline. The lowest possible water concentration in a three-component system with betaine and albumin of a definite mixture ratio is 26.1 mol/l. The concentrations, indicated for both solute components, are the limit of their solubility in that solution (Fig. 6).

The ratios of betaine and water (16.6 g/mol H₂O) and albumin and water (7.1 g/mol H₂O) are considerably lower in the three-component solution compared to the highest concentrated solution of the pure substances (betaine: 28.9 g/mol H₂O; albumin: 14.96 g/mol H₂O). Since betaine has been found to possess a strong affinity versus the oxygen of water and albumin exhibits a high H-donor potential, it is highly probable that betaine and albumin compete for the same position on the water molecules. Furthermore, betaine enhances the polarization of the water oxygen much more than does albumin. Therefore, water binds more preferentially to glycinebetaine, thus reducing the availability of water for albumin. This effect is essential in solutions of low water concentrations. Consequently, both solute components are dissolved only at higher water- and lower solute concentrations, respectively (compared to the three-component system with proline). Fig. 6 shows that the shift of $(\nu_2 + \nu_3)_{\text{max}}$ in the three-component solution corresponds to the concentration of betaine,

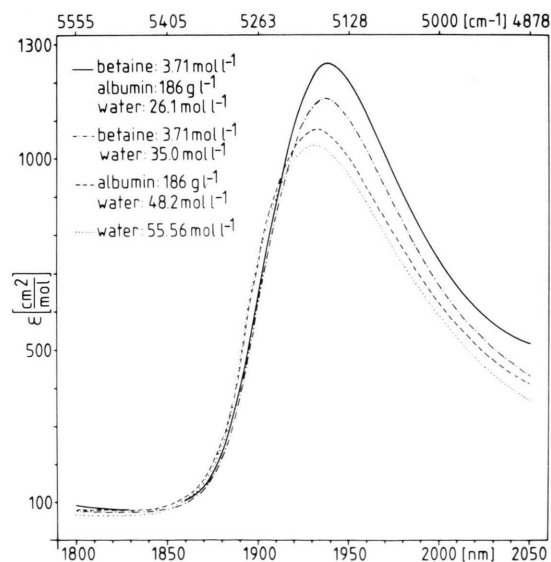


Fig. 6. Combination bands of water in a three-component solution with glycinebetaine and albumin and in solutions of the respective pure substances.

whereas $\varepsilon_{\text{water}}$ is higher than in the pure betaine solution of a comparable water concentration.

It has been suggested earlier (Schobert [6]) that the function of betaine in plant cells equals that of proline. However, since the hydrophilic effect of betaine turned out to be different and a hydrophobic interaction with proteins has not been established (not shown), this proposal does not seem to be correct.

Glycerol and albumin

Since glycerol is miscible with water in all proportions, very low water concentrations can be obtained even in three-component systems. The concentrations of albumin, indicated in Table I, represent the limit of its solubility in the respective solution.

As has been expected, the shift of $(\nu_2 + \nu_3)_{\text{max}}$ is lower in the three-component system with glycerol, compared to the mixed solutions with proline or betaine of the corresponding water concentration (Table I). This again indicates a relatively low hydrogen bonding strength between water and glycerol. It is further evident from Table I that the ratio of albumin and water, being 14.96 g/mol H_2O in the most concentrated albumin solution, is more than doubled

Table I. Position of $(\nu_2 + \nu_3)_{\text{max}}$ in three-component solutions with glycerol and albumin.

Glycerol [mol/l]	Albumin [g/l]	H_2O [mol/l]	$(\nu_2 + \nu_3)_{\text{max}}$ [nm]	Albumin: H_2O [g/mol]
2.8	467	25.9	1936	18.03 : 1
5.1	384	19.6	1939	19.59 : 1
6.3	332	14.8	1941	22.43 : 1
9.7	262	8.1	1944	32.34 : 1

in three-component solutions with a very low water concentration. Since a real enhancement of the albumin: water ratio seems impossible, this result leads to the conclusion that albumin is only partly dissolved in water and partly also in glycerol. This finding supports the assumption, made formerly (Schobert [6]), that glycerol is to some extent able to replace water molecules in their position and function.

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