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# SHORT COMMUNICATION

# CALORIMETRIC STUDY OF HUMAN SERUM ALBUMIN IN WATER–DIOXANE MIXTURES

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**The calorimetric isotherm of water sorption by solid human serum albumin (HSA) was measured in water–dioxane mixtures. This isotherm has been successfully described at low water contents in the solvent within the framework of the presented model. The adsorption equilibrium constant and the monolayer formation energy were estimated from this model. The interaction enthalpies of solid HSA with the solvent in water-rich mixtures do not differ essentially from the solution enthalpy of HSA in water. This means that the solvent water content may be sufficient for the intermolecular interactions of HSA suspended in a water–organic mixture to be similar to those in aqueous protein solution.**

## INTRODUCTION

The study of proteins suspended in organic solvents is of prime importance especially in order to understand the factors governing the state and stability of proteins in various unusual conditions. In addition, there is growing interest in enzymatic catalysis in organic solvents.[1–3] Hence the analysis of the thermodynamic aspects of the intermolecular interactions that occur when proteins are placed in organic liquids appears necessary. Evaluation of the interaction energies in such systems might prove of great interest.

An attempt at such an evaluation has been proposed earlier.[4] It involved the measurement of enthalpies corresponding to placing human serum albumin (HSA) in organic solvents and to the isothermal formation of heterogeneous systems. Intermolecular (or intramolecular) interactions that occur on introducing HSA into organic liquids lead to considerable heat effects, essentially depending on the nature of the solvent.

In this work, based on thermochemistry, we attempted to examine the nature of the interactions that occur on placing HSA in organic liquids. It has been shown[5–7] that in similar suspensions water adsorption can take place. Therefore, in this work the formation enthalpies of such suspensions were studied for HSA in dioxane and water–dioxane mixtures.

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## EXPERIMENTAL

Human serum albumin was obtained from Sigma (product No. A1887). A sample of protein was found to contain 8·5% of water. This value was determined from the weight loss of the protein sample in the presence of phosphorus pentoxide. Dioxane as the solvent was purified and dried by refluxing over sodium according to the recommended method.[8]

Measurement of the heat effects was performed at 298 K on a SETARAM BT-215 calorimeter. The calorimeter was calibrated using the Joule effect. In addition, the solution enthalpy of potassium chloride in water was determined to check the accuracy of the calorimeter. The solution enthalpy measured at a salt concentration of 0·0347 mol $l^{-1}$ corresponded to the recommended value.[9]

To measure the heat effects of suspension formation, a sample of HSA (2–8 mg) was placed in a metallic container with two PTFE washers which served as bottoms. A hermetically closed container was placed inside the calorimetric cell filled with the solvent (4·0 ml). After the cells had been thermostated, the PTFE washers were pricked with a needle 2 mm in diameter. As a result, the solvent came into contact with the sample of HSA.

For the general measured heat effect, a correction taking into account the heat effect of a blank test (pricking of the washers in an empty container) was

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introduced. The interaction enthalpy of HSA with a solvent was calculated as the ratio between the 'pure' heat effect of heterogeneous system formation and the weight of the dry protein.

The equilibrium concentration of water in the liquid phase containing no more than 1 mol $l^{-1}$ of water was determined after carrying out a calorimetric experiment by the volumetric method.[10] A known volume of the liquid phase was treated with calcium hydride and the volume of the hydrogen released was measured. No noticeable amounts of gas-generating impurities were observed in the calcium hydride, which was tested by measuring the water concentration in solutions with precisely added amounts of water. The equilibrium water content of the solutions containing water exceeding 1 mol $l^{-1}$ was considered to be the sum of the water concentration in the initial dioxane and of the added amount of water.

The lack of solubility of HSA in water–dioxane mixtures was confirmed by measurement on a Specord M-40 spectrophotometer at 280 nm. No significant variation in the absorbance of the liquid phase was observed after keeping the protein in water–dioxane mixtures.

## RESULTS AND DISCUSSION

Measured interaction enthalpies, $\Delta H_{int}^{HSA}$, corresponding to the formation of a protein–solvent heterogeneous system are plotted vs water concentration in the solvent in Figure 1. It can be seen that the obtained dependence consists of two parts. The beginning of the dependence corresponds to a rapid decrease in enthalpy at water concentrations in the range 0·03–2 mol $l^{-1}$. A second decrease in the interaction enthalpy is observed at water concentrations above 2 mol $l^{-1}$. Such changes in $\Delta H_{int}^{HSA}$ values indicate that the processes of water desorption and adsorption can contribute significantly to the interaction enthalpy.

The dependence in Figure 1 may be considered as a calorimetric isotherm of water sorption by the protein in an organic medium. We attempted a quantitative analysis of the data presented in Figure 1.

The adsorption of water by the protein can be assumed to occur at the initial stage according a simple equilibrium similar to the Langmuir isotherm:

$$\text{sorption site} + \text{water} \rightleftharpoons \text{sorption complex} \qquad (1)$$

The interaction enthalpy of the protein obeys the following expression:

$$\Delta H_{int}^{HSA} = Ah\left[\frac{K_c C_w}{1 + K_c C_w} - \theta_0\right] \qquad (2)$$

where $A$ is the number of sorption sites taking part in equilibrium (1), corresponding to 1 g of dry protein, $h$ is the energy of water adsorption by the solid protein from the medium (kJ $mol^{-1}$), equal to the enthalpy of

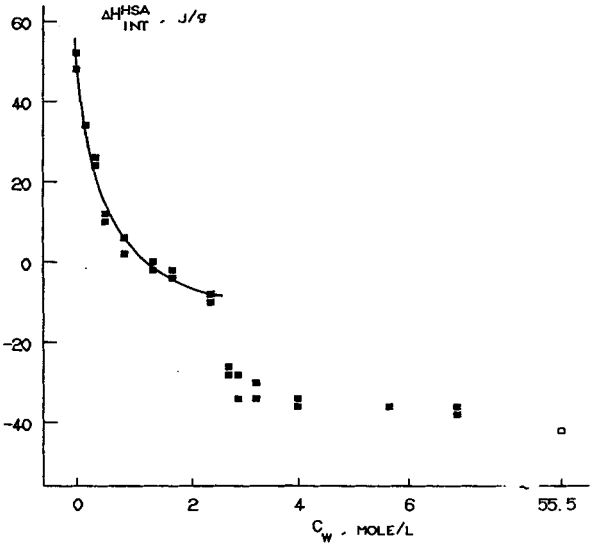


Figure 1. Dependence of HSA interaction enthalpies, $\Delta H_{int}^{HSA}$ on the equilibrium water concentration, $C_w$, in water–dioxane mixtures (■) (298 K). The $C_w$ range is 0·03–7·0 mol $l^{-1}$. The point marked □ corresponds to the solution enthalpy of the HSA in water

reaction (1), $K_c$ is the equilibrium adsorption constant (l $mol^{-1}$), $C_w$ is the equilibrium concentration of water in the solvent and $\theta_0$ is the fraction of the sorption sites occupied by water molecules in the initial HSA preparation.

In conformity with this model, a low water content in the initial solvent ($C_0$) must result in the desorption of water from the protein $[\theta_0 > K_c C_0/(1 + K_c C_0)]$ and in positive values of the interaction enthalpies. An increase in the water concentration in the water–organic mixture must involve the replacement of water desorption by water adsorption from the environment to the solid $[\theta_0 < K_c C_0/(1 + K_c C_0)]$ and a reversal of sign of the interaction enthalpies. This reversal can be observed in Figure 1.

For quantitative verification of equation (2), the transfer enthalpies were calculated as the difference between the interaction enthalpy of the HSA ($\Delta H_{int}^{HSA}$) in a mixture and the interaction enthalpy ($\Delta H_{int}^{HSA*}$) in a mixture where the equilibrium water concentration $C_w^*$ is 0·03 mol $l^{-1}$. Further, equation (2) may be converted into

$$\frac{C_w^* - C_w}{\Delta H_{int}^{HSA} - \Delta H_{int}^{HSA*}} = \frac{1 + K_c C_w^*}{AhK_c} + \frac{1 + K_c C_w^*}{Ah} C_w \qquad (3)$$

Graphical treatment of the experimental data in accordance with equation (3) is shown in Figure 2. In Figure 2 the $\Delta H_{int}^{HSA}$ values estimated at the low water concentrations in the solvent are seen to yield a satisfactory

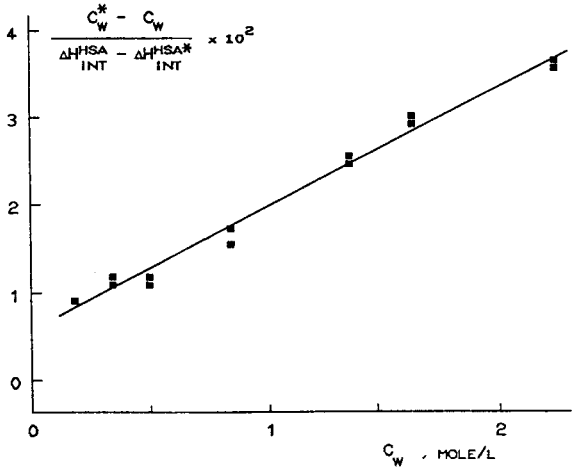


Figure 2. Graphical treatment of data according to equation (3):

$$\frac{C_w^* - C_w}{\Delta H_{int}^{HSA} - \Delta H_{int}^{HSA*}} = (0{\cdot}0060 \pm 0{\cdot}0011) + (0{\cdot}0138 \pm 0{\cdot}0009) C_w$$

Number of points $n = 13$; correlation coefficient $r = 0{\cdot}995$; standard deviation $\sigma = 0{\cdot}0010$

linear dependence. From this linearity it can be concluded that the isotherm observed at the low water contents in the solvent is superficially similar to the Langmuir isotherm for monolayer adsorption. In our opinion, it is of interest that such a simple effect is usually manifested for adsorption by solids which, as distinct from proteins, cannot undergo inter- and intramolecular transformations.

The adsorption equilibrium constant $K_c = 2{\cdot}3 \pm 0{\cdot}7\ \mathrm{l\ mol^{-1}}$ and the energy of monolayer formation $Ah = -77{\cdot}4 \pm 8{\cdot}4\ \mathrm{J\ g^{-1}}$ were evaluated within the framework of the model [equations 1–3] from the intercept and the slope of the line in Figure 2. Subsequently a) value $\theta_0 = 0{\cdot}75 \pm 0{\cdot}17$ was obtained. The curve plotted according to equation (2) on the basis of the evaluated $K_c$, $Ah$ and $\theta_0$ values is shown as a solid line in Figure 1. The calculated curve in Figure 1 is in good agreement with the experimental dependence of the $\Delta H_{int}^{HSA}$ values at low water content.

Let us consider the dependence in Figure 1 at water concentrations above $2\ \mathrm{mol\ l^{-1}}$. The interaction enthalpies of the HSA vary in the concentration range $2–3\ \mathrm{mol\ l^{-1}}$. Further, the interaction enthalpies depend on the water content very slightly and are close to the measured solution enthalpy of HSA in water, which is $-43{\cdot}5 \pm 3{\cdot}8\ \mathrm{J\ g^{-1}}$ (298 K) at an HSA concentration of $1\ \mathrm{mg\ ml^{-1}}$. In spite of this similarity, the protein sample does not dissolve in the water–dioxane mixtures investigated. At present it is difficult to interpret these results clearly. However, it can be assumed that the solvent water content may be sufficient for the intermolecular interactions of HSA suspended in a water–organic mixture to be similar to those in aqueous protein solution.

One can expect that a more detailed calorimetric study would provide some new information concerning the intermolecular interactions of proteins suspended in water–organic mixtures of various water contents.

## REFERENCES

1. A. M. Klibanov, *Chemtech.* **16**, 354–359 (1986).
2. A. M. Klibanov, *Trends Biochem. Sci.* **14**, 141–144 (1989).
3. A. M. Klibanov, *Acc. Chem. Res.* **23**, 114–120 (1990).
4. M. D. Borisover, V. A. Sirotkin and B. N. Solomonov, *Zh. Fiz. Khim.* **66**, 3130–3133 (1992).
5. A. Zaks and A. M. Klibanov, *J. Biol. Chem.* **263**, 8017–8021 (1988).
6. T. Yamane, T. Ichiryu, M. Nagata, A. Ueno and S. Shimizu, *Biotechnol. Bioeng.* **36**, 1063–1069 (1990).
7. P. J. Halling, *Biochim. Biophys. Acta* **1040**, 225–228 (1990).
8. D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals*. Pergamon Press, Oxford (1980).
9. V. A. Medvedev and M. E. Efimov, *Zh. Fiz. Khim.* **49**, 1324–1327 (1975).
10. G. F. Nichugovskiy, *Opredeleniye Vlazhnosti Khimicheskikh Veshchest.* Khimiya, Leningrad (1977).