

PHASE AND STRUCTURAL TRANSITIONS IN VICINAL WATER ON PROTEIN SURFACES BY MEANS OF THE DSC

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Abstract

This paper presents investigations of phase and structural transitions occurring in water adsorbed on the surface of bovine serum albumin (BSA) and on the so-called 'intelligent' or 'smart' silica gel surface covered with a chemically bonded BSA phase. Cyclic changes of heat flow (HF) were observed in the samples studied during cooling and heating of the measuring cell of the differential scanning calorimetry (DSC) apparatus. These cyclic changes reflect structural transitions occurring in the water adsorbed on the surface at subambient and elevated temperatures. This is connected with cyclic changes (decay and reproduction) of ice-like structures existing in the adsorbed water layers. On the basis of quantitative investigations it appears that, depending on the direction of the cooling or heating process of the samples studied, the number of ice-like water structures in the surface film increases or decreases. It has been stated that the observed fluctuations occur spontaneously and suddenly in the whole volume of adsorbed water in different and not regular temperature ranges, especially at the 'paradoxical effect' temperatures.

Keywords: BSA, 'paradoxical' effect, 'smart' surfaces, structural transitions, vicinal water

Introduction

Water is a basic component of biological cells and an indispensable medium of life. It is a very peculiar substance because of some specific properties both in the bulk phase and in layers adsorbed on solid surfaces. Properties of bonded or so-called vicinal water adsorbed on different surfaces of adsorbents and biological systems, i.e. viscosity, density, freezing point are different from those in bulk water and well recognized and documented in numerous papers [1-8]. The main feature of water determining the course of many interface and biological processes occurring in cells is the original structure of adsorbed water layers. However, this property of vicinal water has been rarely recognized, so far. In the bulk water very distinct structures exist, e.g. tetrahedral ice-like structures. Near the solid surface, owing to interactions of surface centers, the amount of such structures increases as a result of adsorbate-adsorbent interactions. In the literature relating to this subject [9-16] there are different schemes of linear and cyclic structures which can be formed by water molecules bonded with solid surfaces as well as the calculations of

interaction energy corresponding to one water molecule. The papers present also models and theories of water structure based on the results of thermal analysis, neutron scattering and total internal reflection Raman spectrometry.

Very great difficulties connected with the elucidation of the structure of bulk and bonded water arise from possibilities of formation of different branched bond structures in both bulk and vicinal water. It is known that one water molecule can form simultaneously 0–4 hydrogen bonds. The lifetime of such bonds is very short and, depending on types of interactions and interfaces, such structures can be reproducible or not [17, 18].

Vicinal water shows a very interesting but scarcely recognized feature consisting in structural transitions near the temperatures of 15, 30 and 45°C [19–21]. Periodic changes in water structure are very advantageous in biological systems. The cell life is characterized by a continuous motion (flow of liquids) and biochemical reactions in water solutions. The temperature range 30–40°C and the processes occurring in this temperature range are optimal for the effective action of most enzymes. The molecular mechanism of these structural changes of water films has not been recognized, so far. Drost-Hansen [19–21] has called this phenomenon observed in both solid surface and biological systems as a 'paradoxical effect'. At temperatures 15, 30 and 45°C maxima of viscosity, disjoining pressure and entropy of water contained between quartz plates [22–24], maxima of selectivity coefficient of Na⁺, K⁺ and Li⁺ ions [25, 26] and many physiological anomalies [27] have been recorded. Moreover, investigations of the 'paradoxical effect' in the vicinal water/rat renal cortex system showed that structural transitions of water are of biological importance [28].

However, the reports on the 'paradoxical effect' and structural transitions of water found in the literature do not fully explain the phenomena. Papers [29–32] present studies on the heat capacity of vicinal water and heavy water in the pores of silica gel and porous glasses. It appears that the heat capacity of water present in micropores is greater (by 25%) than that of bulk water. Significant heat capacity 'spikes' were observed at temperatures which can be related to the structural transitions in vicinal water. Some papers show the results of calorimetric and dielectric studies of the glassy state of water obtained by rapid cooling or by hyperquenching, demonstrate the reversibility of its glass-liquid transitions and consider the implications in our understanding of its other non-crystalline solid forms [33–39]. Studies of glass transitions performed for supercooled organic liquids and aqueous solutions by means of DSC method [40, 41] have shown that the tendency to glass formation increases with the increase of asymmetry of liquids as well as with the increase of fragility. Such behaviour is observed for liquids having a specific structure and showing anomalous deviations from the Debye's relaxation time and Arrhenius viscosity. Moreover, these liquids are capable of forming anti-crystalline clusters in bulk liquid. On the basis of dielectric studies it can be stated that structural transitions of some organic liquids are not simple kinetic phenomena but represent a combination of two processes, i.e. a main process connected with changes in the lattice of hydrogen bonds and a less important one connected with Van der Waals interactions. So far, calorimetric data confirming the above hypothesis have been

lacking. It was stated that the energies of transitions lie in the range from 1 to 20 cal mol⁻¹, only [42].

Some papers [43, 44] present DSC studies of the glass transition of water in hydrogel reflecting the state of the water interaction with polysaccharide. Freezing bound water forms metastable ice on slow cooling and amorphous ice on quenching. Novel results [45] obtained by means of DSC method on phase (freezing, melting, evaporation) and structural transitions taking place from -50 to 100°C in bulk and adsorbed water on different surfaces have been presented. Cyclic changes of heat flow were observed which were associated with those in the properties of water due to changing temperature. The occurrence of structural transitions of the 'paradoxical effect' in the bulk water and in the aqueous adsorption layer at subambient and elevated temperatures was confirmed. They are due to cyclic changes of some properties of water, particularly of a tetrahedral ice-like structure found in bulk and vicinal water.

The lack of quantitative data characterizing the structural changes of water results mainly from experimental difficulties. Such experimental conditions as sensitivity of apparatus, heating rate, purity of water, position of a sample pan in the measuring cell, etc. should be selected very carefully and precisely. The above difficulties result also from anomalous properties of water molecules (small dimensions and little asymmetry), their specific structure and great ability of structural changes (great fragility). For that reason, the structural transitions shown by water are of low energy and sudden but they occur in the whole volume.

In order to explain such unusual phenomena occurring in the structure of adsorbed water layers, the latest investigations of structural transitions were conducted on the surface of pure bovine serum albumin (BSA) as well as on samples of silica gel covered with a chemically bonded BSA phase.

Experimental

Materials

Proteins have often been applied for a variety of analytical purposes [46-54]. The present studies were carried out using bovine serum albumin (BSA) samples containing 15.5% nitrogen (Sigma Chemical CO., USA). BSA consists of a single polypeptide chain of 582 amino acid residues that folds to form a series of alpha-helices which are organized into three domains. It is 'cigar-shaped' with molecular dimensions of 42×141 Å and molecular weight of 66 267. BSA has an isoelectric point of 4.7 and at neutral *pH* a net charge of -18. The three-dimensional structure of proteins is stabilized by 17 disulphide bridges and is capable of withstanding a range of solution *pH* and temperature without denaturing [55-57]. Recently, immobilized proteins have been used as stationary phases in liquid chromatography (HPLC) [58, 59]. Silica-immobilized BSA and a HPLC packing (so-called 'intelligent' or 'smart' surfaces) was used in investigations of phase and structural transitions of adsorbed water films. The details of the sample preparation and properties are given in [55, 56, 59].

Before measurements, the samples were saturated with water vapour in a vacuum desiccator at $p/p_0=1$ for 24 h.

Method

DSC measurements were made using a DSC 2910 (TA Instruments Inc., New Castle, USA) apparatus equipped with subambient accessories. 10 mg samples were placed in aluminium pans, cooled to -50°C and then heated to 100°C . Before measurements the apparatus was calibrated by the two-points method with indium and mercury as standards. The reproducibility of temperatures and sizes of the phase transition process is about 5%. The reproducibility of the fusion enthalpy values at a given temperature is about 10% [45].

Results

Figure 1 presents the DSC curve of cooling and heating of a BSA sample saturated with water vapour in a vacuum desiccator. The complex shape with peaks represents phase transitions of adsorbed water on the surface studied (the arrows show the sequence of cooling and heating processes) in the succession: i/ freezing of water (peak 1), ii/ melting (peak 2) and iii/ evaporation from the surface (peak 3). From the data presented in this Figure it appears that the freezing of supercooled water takes place at a temperature of -5°C . Melting of this water takes place at -2°C and evaporation begins at 15°C . Significant and sharp fluctuations of heat flow (HF) were observed on the DSC curve at some temperatures. The frequency of these sharp HF changes increases with the decrease of temperature of the measuring cell and attains a maximum at 16°C . Figure 2 shows an enlarged part of the DSC curve in Fig. 1 in the temperature range from -2 to 23°C . It is worth noting

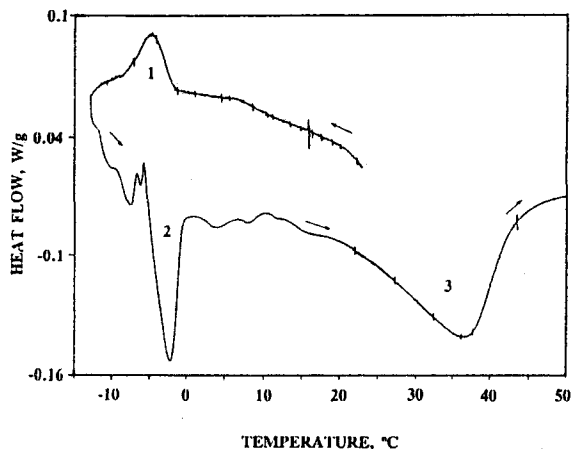


Fig. 1 DSC curve of phase and structural transitions of adsorbed water film during cooling and heating of pure BSA sample: peak 1 – freezing, peak 2 – melting of adsorbed water film and peak 3 – evaporation of water from the surface

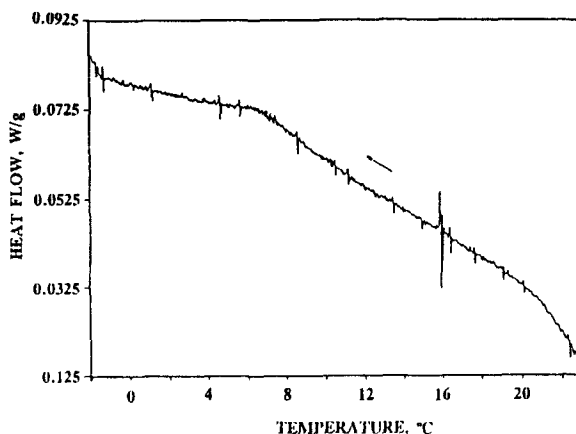


Fig. 2 Structural transitions of water adsorbed on pure BSA sample (the enlargement of the DSC curve in Fig. 1) in the temperature range from -2 to 23°C

that at 16°C multiple HF fluctuations can be observed on the DSC curve. Analogous fluctuations were observed in the shape of the DSC curve in the case of water evaporation from the BSA sample. Figure 3 presents the enlarged peak 3 in Fig. 1 in the temperature range from 21 to 47°C . A great number of HF changes were observed on the DSC curve, especially in the temperature range 25 – 40°C . With further increase in the temperature these HF changes disappear.

Investigations of the cooling and heating processes of benzene and octane have not shown any HF changes in the DSC curves but such changes were observed in the samples of pure water as well as in the water film adsorbed on silica gel and other surfaces, only [45]. Thus, the sudden changes of HF are connected with changes in the lattice of hydrogen bonds i.e. with the changes of water structure. From the data presented in Figs 1–3 it also appears that changes take place in the

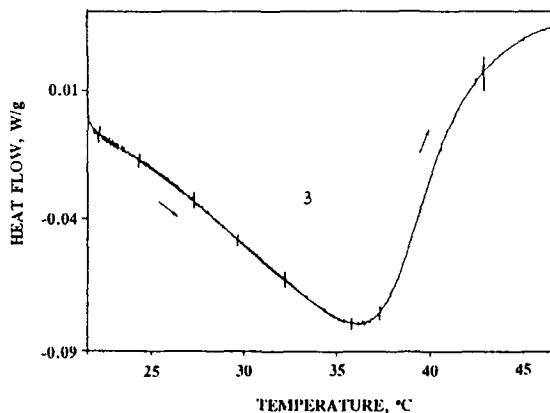


Fig. 3 Structural transitions of water adsorbed on a pure BSA sample (the enlargement of the DSC curve in Fig. 1) in the temperature range from 21 to 47°C

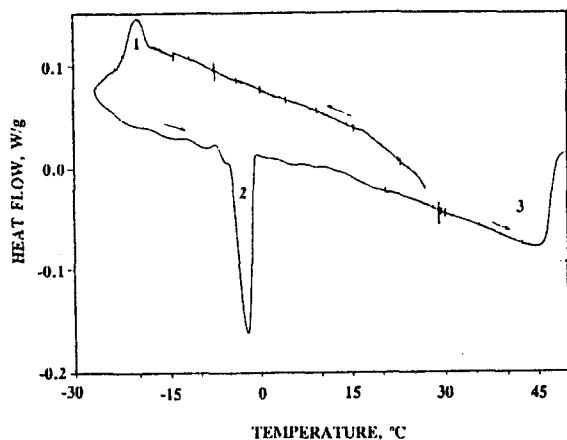


Fig. 4 DSC curve of phase and structural transitions of adsorbed water film during cooling and heating of a silica gel sample covered with chemically bonded BSA phase: peak 1 – freezing, peak 2 – melting of the adsorbed water film and peak 3 – evaporation of water from the surface

temperature regions corresponding to a 'paradoxical effect' observed in water/solid systems as well as in biological systems containing water [19–21, 60, 61].

Figure 4 presents the DSC curve determined during cooling and heating of silica gel covered with BSA and saturated with water vapour in a desiccator. On the DSC curve HF changes similar to those in Fig. 1 were observed, at subambient and elevated temperatures. Figure 5 shows the enlarged part of peak 3 in Fig. 4 of HF changes taking place at 27, 34, 37, 41 and 44°C. It should be emphasized that in the case of the water/silica gel covered with the BSA system the HF changes observed at 34°C are great and multiple in comparison to those observed for the water adsorbed on a pure BSA surface (Fig. 3) but similar to those obtained at 16°C (Fig. 2). It is worth noting that the above multiple HF changes obtained at 16 and 34°C are very close to the 'paradoxical effect' temperatures.

Different changes of HF values observed in Figs 1 and 4 occur during both cooling and heating of the samples in an irregular temperature range every 3–15°C. Figures 6–9 present the enlarged parts of single HF changes in the DSC curves in Figs 1 and 4. In these curves significant changes of HF with temperature are observed. Figures 6 and 7 show in an enlarged scale the HF changes in given temperature ranges during cooling (from –1 to 0°C) and heating (from 41.5 to 42.5°C) of the pure BSA sample containing adsorbed water shown in Fig. 1. From these Figures it results that the cycle of HF changes consists of two stages illustrated by peaks A and B. The first endothermal peaks A are connected with heat absorption and result in 'melting' of some parts of the investigated sample system. The second exothermal peaks B are connected with heat release during 'freezing' of a part of the sample systems. It should be noted that two-stage changes of HF values take place during both cooling and heating of the sample studied. The sequence of both peaks is always the same, i.e. first peaks A come before the second peaks B. The

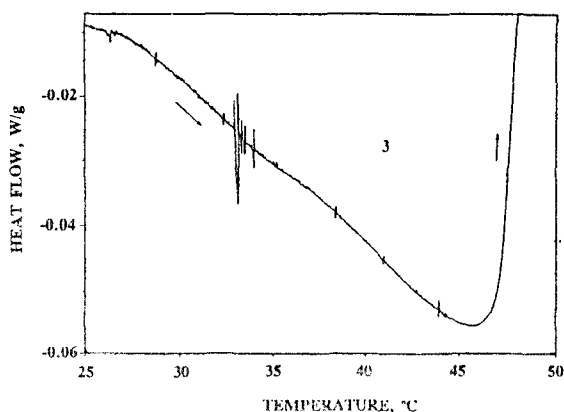


Fig. 5 Structural transitions of water adsorbed on a silica gel sample covered with BSA (the enlargement of the DSC curve in Fig. 4) in the temperature range from 25 to 50°C

first step of the process is connected with destruction of ice-like structures of water existing in the adsorbed film (endothermic peaks below the baseline) and the second stage with reconstruction of these or similar structures (exothermic peaks above the baseline). The change of the process direction, i.e. transition from cooling to heating (Figs 6, 7 and Figs 8, 9) causes a change in HF shape (the change of fluctuation phase illustrating this process) but the above sequence remains unchanged.

The fusion enthalpy for a cooling stage (Fig. 6, peak A) of the first process of destruction of water structure is 0.09106 J g^{-1} and is lower than the fusion enthalpy for reconstruction of these structures (peak B) which is equal to 0.09852 J g^{-1} . From Fig. 7 (sample heating process) it results that during the 'melting' process of water structure (peak A) the system absorbs 0.1076 J g^{-1} , and releases 0.05518 J g^{-1}

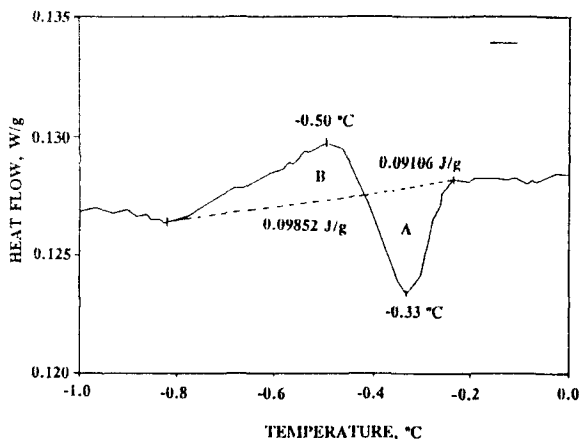


Fig. 6 Structural transitions of water adsorbed on pure BSA during sample cooling (the enlargement of the DSC curve in Fig. 1 in the temperature range from -1 to 0°C): peak A - 'melting' of the water structure, B - 'freezing' of the adsorbed water

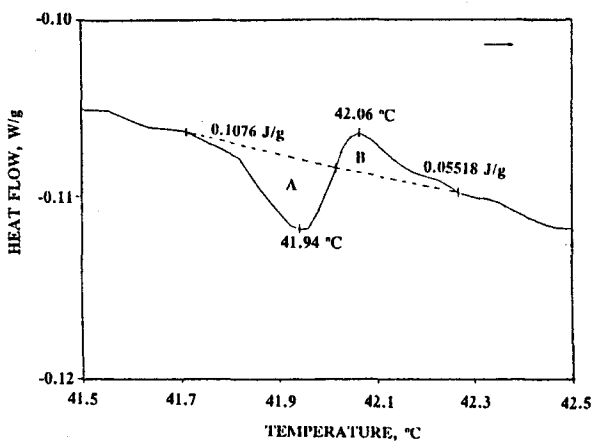


Fig. 7 Structural transitions of water adsorbed on pure BSA during sample heating (the enlargement of the DSC curve in Fig. 1 in the temperature range from 41.5 to 42.5°C): peak A – ‘melting’ of the water structure, B – ‘freezing’ of the adsorbed water

(peak B) in the ‘freezing’ process. Analogous changes of enthalpy corresponding to the ice-like structure transitions of water are observed for silica gel samples covered with chemically bonded BSA phase (Figs 8 and 9). During the cooling process (Fig. 8, peak A) the value of fusion enthalpy for the process of destruction of ice-like structures in water is equal to 0.3702 J g^{-1} whereas during the process of reconstruction of these structures (peak B) the released heat is 0.4683 J g^{-1} . In the process of sample heating (Fig. 9) the corresponding values are 0.01995 J g^{-1} (‘melting’, peak A) and 0.01334 J g^{-1} (reconstruction of ice-like structures, peak B). The quantitative data presented above suggest the increase in number of the recon-

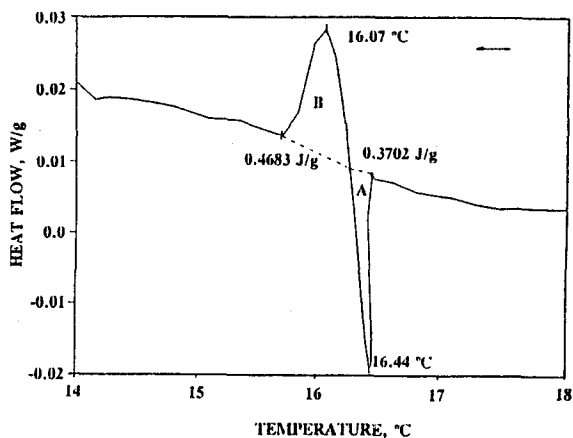


Fig. 8 Structural transitions of water adsorbed on silica gel covered with BSA during sample cooling (the enlargement of the DSC curve in Fig. 4 in the temperature range from 14 to 18°C): peak A – ‘melting’ of the water structure, B – ‘freezing’ of the adsorbed water

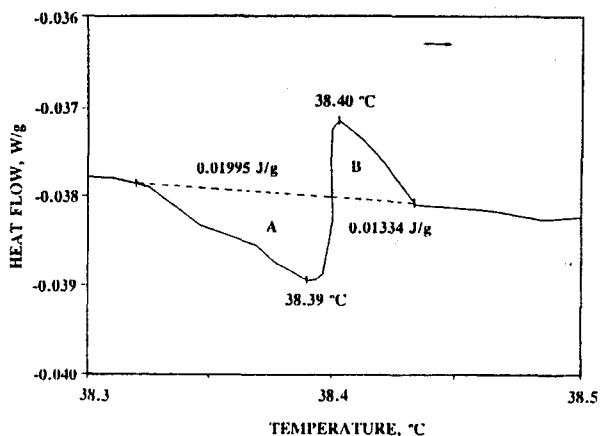


Fig. 9 Structural transitions of water adsorbed on silica gel covered with BSA during sample heating (the enlargement of the DSC curve in Fig. 4 in the temperature range from 38.3 to 38.5°C): peak A – ‘melting’ of the water structure, B – ‘freezing’ of the adsorbed water

structured ice-like structures during the cooling of the sample and disappearance of these structures during the heating process.

The shapes of the curves presented in Figs 6–9 are analogous to those of DSC curves obtained during the investigations of phase transitions occurring in organic substances [40], pure water and silica gel, alumina oxides, active carbon and zeolites containing adsorbed water films [45].

It is very interesting that the observed structural transitions of water occur at so-called biotic temperatures (20–40°C, Figs 3 and 5), i.e. at temperatures at which all cell processes including biochemical reactions are most effective [40]. It is well

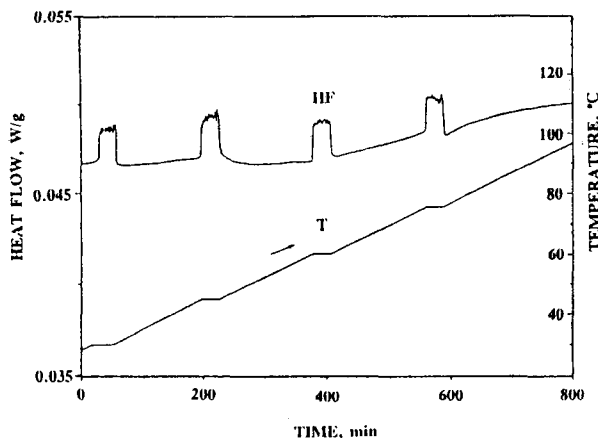


Fig. 10 HF and *T* curves in a DSC run obtained by maintaining the silica gel sample covered with BSA during sample heating in isothermal conditions at constant temperatures of 30, 45, 60 and 75°C for 30 min

known [62, 63] that the processes in biological cells are characterized by both continuous passing of the solution through the membranes and dynamic changes connected with the exchange of ions and compounds. It appears that the cyclic changes in water structure occurring in the range of biotic temperatures influence favourably the proper course of the above processes.

For this reason, a suitable program of the DSC measurements has been chosen to maintain sample studied in so-called isothermal conditions in a DSC cell for 30 min at 30, 45, 60 and 70°C, i.e. at the temperatures corresponding to a 'paradoxical effect' has been treated. Figure 10 shows typical HF and T curves obtained under the above mentioned conditions. In the constant temperature ranges corresponding to a 'paradoxical effect' on the HF curve a number of successive structural transitions of water were recorded. Figure 11 shows the enlarged HF curve corresponding to the successive processes of structural changes of water adsorbed on silica gel covered with BSA at 30°C.

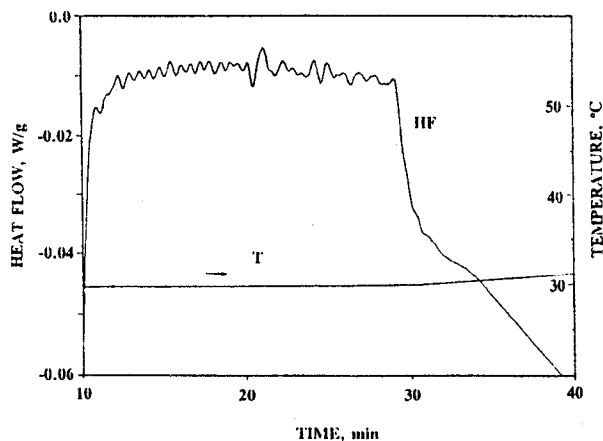


Fig. 11 Structural transitions of water adsorbed on silica gel covered with BSA during sample heating in isothermal conditions (the enlargement of the DSC curve in Fig. 10 at 30°C)

The structural transitions described above can be explained as follows. The observed HF changes are connected with the presence of hydrogen bonds in the system studied. These changes are observed in the water films adsorbed on pure BSA and on silica gel covered with BSA and result from the changes of hydrogen bonds, i.e. its structure on the surface of biological systems [43–45]. Therefore, the HF changes reflect the transitions in the ice-like structure of water on the BSA surface of samples. In the bulk water there exist about 10% of tetrahedral ice-like water structures which are the most stable and characterized by highest energy of hydrogen bonds corresponding to one water molecule [9]. Near the surface of biological systems and different heterogeneous solids, the water films are enriched in these structures and the content of these structures can attain even 40%. Water structures undergo continuous changes because the lifetime of hydrogen bonds in such struc-

tures is 10^{-12} s. This phenomenon can be referred to as a structural transition, too. Thus, the ice-like structures can be reconstructed or undergo destruction leading to formation of other structures, for example amorphous ice type.

Structural transitions of water in the whole volume of an adsorbed film are stimulated mainly by thermal fluctuations. The phenomenon of sudden changes of water structure can be connected also with dislocation of electrical charge (electron) in the water structure via hydrogen bonds. It is known that the electric conductivity of water occurs is due to the motion of electrons but not of H^+ and OH^- ions as in aqueous electrolyte solutions. Such type of conductivity results from the quantum-mechanical tunnel transition of electrons through hydrogen bonds present in water [7, 8].

During cooling of water/solid system structural transition processes are accompanied by formation of a great number of tetrahedral ice-like structures before the phase transition of adsorbed water to ice (higher value of heat of fusion in the sample freezing process, Figs 6 and 8). This process becomes more intense until the freezing of water bonded with the sample surface takes place. In the sample heating and water evaporation, the processes of the cyclic structural transitions are of opposite directions. After the transition of solid ice to liquid adsorbed water films on the surfaces a large number of tetrahedral ice-like structures (being the residue of ice structure) has been created. Due to the heating process these structures undergo decomposition (higher values of 'melting' heat, Figs 7 and 9). The observed process occurs spontaneously, suddenly and in the whole volume of the adsorbed liquid in certain but not regular temperature ranges. From the above investigations it appears that this process becomes more intense in the temperature range 20–40°C.

From the quantitative data listed in Figs 6–9 it results that tetrahedral ice-like structures are reconstructed during the cooling process and disappear during the heating of the water/solid systems. The number of the created ice-like structures is connected with the direction of the course of a given experiment. In other words, it depends on the fact whether the heat is introduced into or removed from the system and reflects the differences in the heats of destruction and reconstruction of ice-like structures. This cyclic process occurs as long as the temperatures of phase transitions of water (freezing or evaporation of the total amount of the adsorbed water) are not attained.

Conclusion

Investigations of phase transition occurring in the water adsorbed on pure BSA and silica gel surface covered with chemically bonded BSA phase confirmed the occurrence of structural changes in water/biological systems. Spontaneous transitions were observed in the water structure in irregular temperature ranges during cooling and heating of samples. Continuous structural transitions were recorded in adsorbed water films under isothermal conditions in temperature ranges corresponding to a 'paradoxical effect'. On the basis of obtained data it appears that the process of HF changes reflects the phenomenon of sudden decomposition of ice-like structures of water (creation of a metastable system) following the reconstruction of

these structures (reversion to a stable ice-like system). Depending on the temperature and direction of the experimental process the number of reconstructed ice-like structures increases (in the cooling process) or decreases (in the heating process). In the cooling process the number of these structures increases as long as the temperature of transformation of water to ice is not attained. During the heating process the gradual decomposition of larger and larger number of ice-like structures of water takes place and proceeds till the end of evaporation of water from solid surface.

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References

- 1 W. Stumm, *Chemistry of the Solid-Water Interface*, John Wiley & Sons, Inc., New York 1992.
- 2 P. F. Low, *Soil Sci. Soc. Am. J.*, 43 (1979) 652.
- 3 B. V. Derjaguin, N. V. Churaev, V. M. Muller, *Surface Forces*, Consultants Bureau, New York 1987.
- 4 G. A. Parks, in: *Mineral-Water Interface Geochemistry*, M. F. Hochella Jr. and A. F. White, Eds, Mineralogical Society of America, 1990, pp. 133-175.
- 5 P. Staszczuk and A. Wakszudzki, *Problemy Agrofizyki, Ossolineum, Wrocław*, 37 (1982) 1.
- 6 J. C. Westall, in: *Aquatic Surface Chemistry*, W. Stumm, Ed., Wiley-Interscience, New York 1987, pp. 3-32.
- 7 F. M. Etzler and W. Drost-Hansen, *Croatica Chemica Acta*, 56 (1983) 563.
- 8 F. M. Etzler, *J. Colloid Interface Sci.*, 92 (1983) 43.
- 9 K. Klier and A. C. Zettlemoyer, *J. Colloid Interface Sci.*, 58 (1977) 216.
- 10 J. W. Halley, J. R. Rustad and A. Rahman, *J. Chem. Phys.* 98 (1993) 4110.
- 11 A. Vegiri and S. C. Farantos, *J. Chem. Phys.*, 98 (1993) 4059.
- 12 S. Sastry, F. Sciortino and H. E. Stanley, *J. Chem. Phys.*, 98 (1993) 9863.
- 13 M.-C. Bellissent-Funel, J. Lal and L. Bosio, *J. Chem. Phys.*, 98 (1993) 4246.
- 14 I. I. Vaisman, L. Perera and M. L. Berkowitz, *J. Chem. Phys.*, 98 (1993) 9859.
- 15 Z. S. Nickolov, J. C. Earnshaw and J. J. McGarvey, *Colloids and Surfaces A: Physicochem. and Engin. Aspects*, 76 (1993) 41.
- 16 L. Jichen and D. K. Ross, *Nature*, 365 (1993) 327.
- 17 P. Staszczuk and B. Biliński, *Thermochim. Acta*, 122 (1987) 363.
- 18 P. Staszczuk and B. Biliński, *J. Thermal Anal.*, 32 (1987) 1457.
- 19 W. Drost-Hansen, *Ind. Eng. Chem.*, 61 (1969) 10.
- 20 W. Drost-Hansen, in *Chemistry of the Cell Interface*, F. Franks, Ed., Wiley, New York 1971.
- 21 W. Drost-Hansen, in: *Biophysics of Water*, F. Franks, Ed., Wiley, New York 1982.
- 22 G. Peschel and K. H. Aldfinger, *Z. Naturforsch.*, 26 (1971) 707.
- 23 G. Peschel and K. H. Aldfinger, *Naturwissenschaften*, 11 (1969) 1.
- 24 G. Peschel and K. H. Aldfinger, *J. Colloid Interface Sci.*, 34 (1970) 505.
- 25 P. M. Wiggins, *Biophys. J.*, 13 (1973) 385.
- 26 P. M. Wiggins, *Clin. Exp. Pharmacol. Physiol.*, 2 (1975) 171.
- 27 J. Nishiyama, *Res. Bull. Mokkaido Natl. Agric. Exp. Stn.*, 10 (1975).
- 28 F. M. Etzler, W. Drost-Hansen, in: *Cell Associated Water*, (W. Drost-Hansen and J. S. Clegg, Eds.), Academic Press, New York 1979, p. 125.
- 29 F. M. Etzler and D. M. Fagundus, *J. Colloid Interface Sci.*, 115 (1987) 513.
- 30 F. M. Etzler and P. White, *J. Colloid Interface Sci.*, 120 (1987) 94.

- 31 F. M. Etzler, *Langmuir*, 4 (1988) 878.
- 32 F. M. Etzler and J. J. Conners, *Langmuir*, 6 (1990) 1250.
- 33 G. P. Johari, A. Hallbrucker and E. Mayer, *Nature*, 330 (1987) 552.
- 34 A. Hallbrucker, E. Mayer and G. P. Johari, *J. Phys. Chem.*, 93 (1989) 4986.
- 35 G. P. Johari, G. Astl and E. Mayer, *J. Chem. Phys.*, 92 (1990) 809.
- 36 G. P. Johari, A. Hallbrucker and E. Mayer, *J. Chem. Phys.*, 92 (1990) 6742.
- 37 G. P. Johari, A. Hallbrucker and E. Mayer, *J. Chem. Phys.*, 95 (1991) 2955.
- 38 G. P. Johari, A. Hallbrucker and E. Mayer, *J. Chem. Phys.*, 97 (1992) 5851.
- 39 G. P. Johari, *J. Chem. Phys.*, 98 (1993) 7324.
- 40 S. S. N. Murthy, S. Gangasharan and S. K. Nayak, *J. Chem. Soc. Faraday Trans.*, 89 (1993) 509.
- 41 M. J. Blandamer, B. Briggs, P. M. Cullis, J. A. Green, M. Waters, G. Soldi, J. B. Engberts and D. Hoekstra, *J. Chem. Soc. Faraday Trans.*, 88 (1992) 3431.
- 42 R. Lumry and S. Fajender, *Biopolymers*, 9 (1970) 1125.
- 43 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *Viscoelasticity of Biomaterials*, (W. G. Glasser and H. Hatakeyama, Eds), American Chemical Society, ACS Symposium Series, 1992, No. 489, chapter 14, pp. 217–230.
- 44 H. Yoshida, T. Hatakeyama and H. Hatakeyama, *J. Thermal Anal.*, 40 (1992) 483.
- 45 P. Staszczuk, *Colloids and Surfaces A: Physicochem. and Engin. Aspects*, 94 (1995) 213.
- 46 C. Pidgeon and U. V. Venkataram, *Anal. Biochem.*, 176 (1989) 36.
- 47 G. K. Bonn, K. Kalghatagi, W. C. Horne and C. Horvath, *Chromatographia*, 30 (1990) 484.
- 48 K. Dabulis and A. M. Klibanov, *Biotechnology and Bioengineering*, 39 (1992) 176.
- 49 K. Kandori, Y. Yamamoto, H. Saito and T. Ishikawa, *Colloids and Surfaces A: Physicochem. and Engin. Aspects*, 80 (1993) 287.
- 50 P. W. Wojciechowski and J. L. Brash, *Colloid and Surfaces B: Biointerfaces*, 1 (1993) 107.
- 51 A. Kondo, A. Oku, F. Murakami and K. Higashitani, *Colloids and Surfaces B: Biointerfaces*, 1 (1993) 197.
- 52 M. C. Wahlgren, M. A. Paulsson and T. Arnebrant, *Colloids and Surfaces A: Physicochem. and Engin. Aspects*, 70 (1993) 139.
- 53 R. Nicholov, R. P. Veregin, A. W. Neumann and F. DiCosmo, *Colloids and Surfaces A: Physicochem. and Engin. Aspects*, 70 (1993) 159.
- 54 O. W. Reif, R. Lausch and R. Freitag, *International Laboratory*, 24 (1994) 10.
- 55 R. K. Gilpin, S. E. Htsham and R. B. Gregory, *Anal. Chem.*, 63 (1991) 2825.
- 56 V. Tittlebach and R. K. Gilpin, *Abstracts: The Pittsburgh Conference*, Atlanta, Georgia (1993) 421.
- 57 T. Peters, Jr., in: *Advances in Protein Chemistry*, (C. B. Anfinsen, J. T. Edsall, F. Richards, Eds) Academic Press, New York, 37 (1985) 161.
- 58 S. G. Allenmark, In: *Chromatographic Enantioseparation Methods and Applications*, (R. A. Chalmers, M. Masson, Eds), Halsted Press: New York 1988.
- 59 R. K. Gilpin, H. Gao and M. Jaroniec, *J. Liquid Chromatogr.*, 15 (1992) 2503.
- 60 F. M. Etzler, R. F. Ross and R. A. Halcomb, *Physica A*, 172 (1991) 161.
- 61 F. M. Etzler and J. J. Conners, *Langmuir*, 7 (1991) 2293.
- 62 R. Glaser, *Introduction to Biophysic*, PZWL, Warszawa 1975.
- 63 T. Ścibor-Rylska, *The Secret of Living Cell Organization*, PAX, Warszawa 1986.