

# Separate Contributions of Large- and Small-scale Dynamics to the Heat Capacity of Proteins. A New Viscosity Approach

Alex Käiväräinen,\* Ludmila Fradkova and Timo Korpela

Finnish–Russian Joint Biotechnology Laboratory, JBL, Department of Biochemistry, University of Turku, SF-20500, Turku, Finland

Käiväräinen, A., Fradkova, L. and Korpela T., 1993. Separate Contributions of Large- and Small-scale Dynamics to the Heat Capacity of Proteins. A New Viscosity Approach. – Acta Chem. Scand. 47: 456–460.

We present here a theoretical description and experimental verification of a novel method of separating large-scale (LS) and small-scale (SS) dynamics contributions to the heat capacities of proteins in solution by using viscosity dependences. It was assumed that the LS dynamics, related to the thermal fluctuations of domains and subunits, is dependent on solvent viscosity within a sucrose concentration of 0–15% w/v, in contrast with the SS dynamics, which are related to fluctuations of atoms and amino acid residues. The results obtained with immunoglobulin G, hemoglobin and cytochrome c were in reasonable accordance with the previous data achieved by a spin-label method: the LS dynamics decrease, whereas the SS dynamics increase within the temperature elevation. The changes are dependent on the stage of the active sites of the proteins. The internal compensation of molecular dynamics between the LS and SS fluctuations may be an important factor in the design of thermostable enzymes and proteins. A new phenomenon concerning the ability of macromolecules to increase free volume in water as a result of ice-like clusters stabilization is revealed.

The role of the equilibrium between large-scale (LS) and small-scale (SS) dynamics in the mechanism of the protein actions and long-order effects such as solvent-mediated interactions between different proteins or between proteins and cells has been under investigation for quite some time.<sup>1–7</sup> For a successful quantitative estimation of the equilibria, a variety of methods, together with an understanding of the dependence of the LS and SS dynamics on different factors, are required. In accordance with our classification<sup>2,7</sup> the equilibrium thermal LS dynamics are subdivided into (1) pulsations represented by translational–rotational motions of the protein domains and subunits leading to **A** ⇌ **B** transitions of the interdomain cavities between less (**A**) and more (**B**) water-accessible states with a frequency of  $10^4$ – $10^7$  s<sup>-1</sup> and (2) librations of the domains and subunits during the lifetime of each **A** and **B** protein conformers with a frequency of  $10^7$ – $10^8$  s<sup>-1</sup>. The latter type of LS dynamics, in contrast with low-frequency pulsations, decreases the correlation time and effective Stokes radius of macromolecules. On the other hand, the SS dynamics reflect the thermal fluctuations of atoms, amino acid residues  $\alpha$ -helix- and  $\beta$ -sheet-forming structures that do not change the effective volume.

The resulting partial heat capacity of proteins in dilute solutions must depend not only on the SS-dynamics but also on the LS-dynamics and the effect on the sur-

rounding water. In accordance with the equations derived by one of us (A.K.),<sup>7</sup> generalized Stokes–Einstein and Eyring–Polanyi equations,<sup>1</sup> the rate constant of LS-dynamics ( $k^{LS}$ ) decreases with increasing solvent viscosity ( $\eta$ ), as shown in eqn. (1), where  $V$  is the effective volume of a Brownian particle (domain or a subunit of a macromolecule), which is relatively independent of the other part of the macromolecule with the probability of independent fluctuations [eqn. (2)],  $G_{st}$  is the activation energy of structural rearrangements in the contact (hinge) region of the molecule, necessary for independent fluctuations of the particle, and  $\tau_M$  is the effective correlation time of diffusion of this particle (LS-translations or librations).

$$k^{LS} = (kT/\eta V) \exp(-G_{st}/RT) = \tau_M^{-1} \quad (1)$$

$$P_{st} = \exp(-G_{st}/RT) \quad (2)$$

The effective volume,  $V$ , may change with temperature owing to the action of perturbing agents or to the specific action of ligands on the macromolecule. At low values of  $(\eta/T)$  of solvents with respect to the viscosity of the protein matrix,  $(\eta/T)_{pr} \geq 10^{-2} P/K$ , the effect of solvent viscosity on the SS dynamics can be neglected. There is experimental evidence from spin- or fluorescence-labelled proteins within the temperature range 15–40°C that the effect of sucrose on the mobilities of the labels and on the surface matrix of protein is negligible up to sucrose concentrations of 35–40%.<sup>1–6</sup> The motions of internal

\* To whom correspondence should be addressed.

residues are even less dependent of the viscosity of the solvent.<sup>8</sup> In our experiments the maximum concentration of sucrose was always less than 20%.

### Theoretical background

Described here is a new quantitative approach for the separate estimation of the contributions of LS and SS dynamics to the resulting heat capacity of proteins in sucrose solutions of different viscosity.

The heat capacity of a macromolecular solution ( $C_p^{SM}$ ) is defined by summing the following three contributions.<sup>9</sup>

(I) The heat capacity of the fraction of bulk solvent in a solution of macromolecules, eqn. (3), where,  $\rho_s$  is the

$$\rho_s(V_s - m_M[V^M] - m_{H_2O}^h[V^h] - \Delta V_f)[C_p^S] \quad (3)$$

density of the solvent;  $[C_p^S]$  is the partial heat capacity of the solvent;  $m_M$  and  $m_{H_2O}^h$  are the masses of macromolecules and their hydration shells;  $[V^M]$  and  $[V^h]$  are the corresponding partial volumes of macromolecules and hydration shells, and,  $[\Delta V_f]$  is the the additional free volume in water stimulated by the surface of the macromolecules,<sup>5,7</sup>  $V_s$  is total volume of solution.

(II) The heat capacity caused by SS dynamics of the macromolecules and their hydration shells, eqn. (4).

$$m_M[C_p^{SS}] + m_{H_2O}^h[C_p^h] \quad (4)$$

(III) The contribution of LS dynamics of macromolecules, eqn. (5).

$$m_M[C_p^{LS}] \quad (5)$$

Hence, the total heat capacity of the solution of macromolecules is

$$C_p^{SM} = (I) + (II) + (III).$$

The heat capacity of the solvent in the reference cell of the measuring instrument is given by eqn. (6).

$$C_p^S = \rho_s V_s [C_p^S] \quad (6)$$

The difference ( $\Delta C_p$ ) registered by the microcalorimeter can be written as eqn. (7).

$$\Delta C_p(t)/m_M = (C_p^S - C_p^{SM})/m_M \quad (7)$$

Taking into account that  $\rho_s = [V^s]^{-1}$ , we obtain eqn. (8).

$$\begin{aligned} \frac{\Delta C_p}{m_M} = & \frac{[V^M][C_p^S]}{[V^S]} + \frac{m_{H_2O}^h[V^h]}{m_M[V^S]} [C_p^S] \\ & + \frac{\Delta V_f[C_p^S]}{m_M[V^S]} - [C_p^{SS}] - \frac{m_{H_2O}^h}{m_M} [C_p^h] - [C_p^{LS}] \quad (8) \end{aligned}$$

By arranging eqn. (8) and assuming that  $[V^h]/[V^s] \cong 1$ , the resulting partial capacity  $[C_p^{Res}]$  of

the macromolecules, determined by SS and LS dynamics, can be written as eqn. (9)

$$[C_p^{Res}]_T = [C_p^{SS}]_T^* + [C_p^{LS}]_{(T/\eta)} = A - \Delta C_p/m_M \quad (9)$$

where

$$\begin{aligned} [C_p^{SS}]_T^* & \equiv [C_p^{SS}] + \frac{m_{H_2O}^h}{m_M} \{ [C_p^h] - [C_p^S] \} \\ & = [C_p^{Res}]_{(T/\eta) \rightarrow 0} \quad (10) \end{aligned}$$

and

$$A \equiv \frac{[C_p^S]}{[V^S]} \left\{ [V^M] + \frac{\Delta V_f}{m_M} \right\}_{P,V} \cong \text{const.} \quad (11)$$

The value of  $A$  is obtained by using eqn. (9) and the extrapolated viscosity dependence of  $[C_p^{Res}]$  from  $T/\eta$ . We assume that at  $(T/\eta) \rightarrow 0$ ,  $[C_p^{LS}] \rightarrow 0$  and  $A = [C_p^{SS}]^* + (\Delta C_p/m)_{\max}$ . Simultaneously we obtain eqn. (12).

$$[C_p^{Res}]_{(T/\eta) \rightarrow 0} = [C_p^{SS}]_T^* = A - [(\Delta C_p)_{\max}/m] \quad (12)$$

For the determination of the constant parameter  $A$  in eqn. (12), it is necessary to set the initial value of  $[C_p^{SS}]_T$  at an initial temperature (20°C). We took it to be equal to the partial heat capacity of rubber,<sup>10</sup> eqn. (13).

$$[C_p] = 0.4 \text{ cal g}^{-1} \text{ K}^{-1}; T = 20^\circ\text{C} \quad (13)$$

The contribution  $[C_p^{LS}]$  at temperatures of interest can be determined by using the data of the corresponding isotherm (see Fig. 2), eqn. (14).

$$[C_p^{LS}]_T = [C_p^{Res}]_T - [C_p^{SS}]_{(T/\eta) \rightarrow 0} \quad (14)$$

The value of  $[C_p^{LS}]_{st}$ , adjusted to standard conditions, can also be obtained from eqn. (9) and tangent of the isotherms  $[C_p^{Res}]_T = f(T/\eta)_T$  and of the angles ( $\alpha$ )<sub>T</sub>, eqn. (15), from which we obtain eqn. (16) where

$$\text{tg } \alpha = \Delta[C_p^{Res}]/\Delta(T/\eta)_T = \Delta[C_p^{LS}]/\Delta(T/\eta)_T \quad (15)$$

$(T/\eta)_{st} = 3 \times 10^5 \text{ K/P}$ , corresponding to standard conditions (water, 25°C).

$$[C_p^{LS}]_{st} = \text{tg } \alpha(T/\eta)_{st} \quad (16)$$

It can be shown that the theory includes the traditional approach as a limiting case. After following simplified assumptions such as eqn. (17), we obtain eqn. (18) from

$$[V^h] = [V^s], [C_p^h] = [C_p^{LS}], [C_p^{LS}] = 0, \Delta V_f = 0 \quad (17)$$

$$[C_p^{SS}] = \frac{[V^M][C_p^S]}{[V^S]} - \frac{\Delta C_p}{m} \quad (18)$$

This formula has traditionally been used for the analysis of differential scanning calorimetric data.<sup>11</sup>

Calculations based on eqn. (18) without obtaining the viscosity dependences of  $[C_p^{\text{Res}}]$  on  $(T/\eta)$  will consequently yield only the effective values of  $[C_p^{\text{SS}}] = [C_p^{\text{M}}]$  when the conditions of eqn. (17) are postulated. Eqns. (9)–(12) make it possible to achieve more detailed and valuable information about biopolymers in their native state than previously. The additional (cavitation) free volume ( $\Delta V_f$ ) in ice-like water stimulated by proteins was calculated from eqns. (9) and (11) with experimental isotherms. The contributions  $[C_p^{\text{SS}}]$  and  $[C_p^{\text{LS}}]$  were also calculated based on the experiments and were compared with previous data obtained by a modified spin-label method.<sup>1,2</sup>

### Experimental

The heat capacities of protein solutions were investigated with a DASM-1M differential adiabatic scanning microcalorimeter. During the runs, the rate of warming was  $1^\circ\text{C min}^{-1}$  and the volume of the measuring cell was 1 ml. So that the effect of solvent viscosity could be studied, sucrose was employed at concentrations ranging from 0 to 15% by weight. The same amount of sucrose, dissolved in 0.01 M phosphate buffer, pH 7.3, containing 150 mM NaCl was placed in the reference cell. The protein solutions were prepared not less than 12 h before the measurements were taken. The concentration of sucrose was controlled using an IRF-454B refractometer of the Abbe type.

The solvent viscosity within the given temperature range was determined using the standard nomogram. The

$$\Delta C_p(t) = C_p^{\text{S}}(t) - C_p^{\text{SM}}(t) \quad (19)$$

differential dependences at different sucrose concentrations were adjusted to the same base line (Fig. 1). In eqn. (19)  $C_p^{\text{S}}(t)$  is the heat capacity of solvent in the reference cell,  $C_p^{\text{SM}}(t)$  is the heat capacity of macro-

molecular solutions and  $\Delta C_p(t)$ , is the difference in heat capacities of the solvent and protein solution reflecting the total contribution of protein SS and LS dynamics and water structure change, induced by macromolecules to the heat capacity of the solution.

### Results and discussion

The results of the primary data calculations (Fig. 1) for solutions of human Ig G using eqns. (9)–(11) are presented in the form of the isothermal dependences of  $[C_p^{\text{Res}}]$  on  $(T/\eta)$  (Fig. 2).

The intercepts of each extrapolated isotherm at  $(T/\eta) \rightarrow 0$  give  $[C_p^{\text{SS}}]_T$ .  $C_p^{\text{LS}}$  was determined as the difference between the resulting heat capacity at a given temperature  $[C_p^{\text{Res}}]_T$  and  $[C_p^{\text{LS}}]_T$  in accordance with eqn. (14).

The temperature dependences of  $[C_p^{\text{SS}}]_T$  and  $[C_p^{\text{LS}}]_T$  in the interval  $20\text{--}40^\circ\text{C}$  are presented in Fig. 3. It is seen that the contribution of SS dynamics  $[C_p^{\text{SS}}]$  increases with the temperature while the contribution of LS dynamics decreases. Comparison of Figs. 3(a) and 3(b) shows that the rise in  $[C_p^{\text{SS}}]$  correlates with the rise of  $\tau_R^{-1}$  which defines the increasing mobility of spin-label with respect to the protein surface.<sup>1</sup> Both of the parameters describe the thermoactivation of SS dynamics of proteins which provide new degrees of freedom due to a decrease in the protein microviscosity.

Parameter  $\tau_M^{-1}$  reflects the relative mobility of the domains and subunits of the spin-labeled protein as well as  $[C_p^{\text{LS}}]$ . Both parameters diminish with rise in temperature. These processes seem to be the result of a shift in the  $A \rightleftharpoons B$  equilibrium of the protein cavities to the left, due to destabilization of the B-state and stabilization of the A-state. The interdomain van der Waals interactions increase with increased temperature owing to partial 'melting' of the water cluster in the open B-state cavities.<sup>1,12</sup> Analogous data were obtained for human

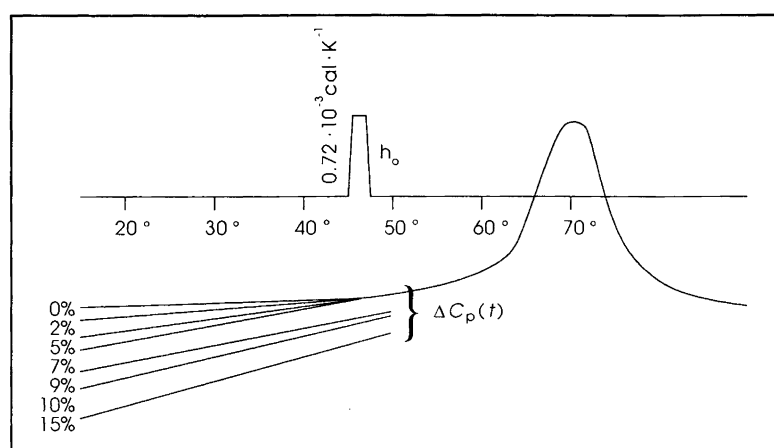


Fig. 1. Experimental differential temperature dependences of heat capacities of human Ig G solutions at different sucrose concentrations (w/v).  $\Delta C_p(t) = C_p^{\text{S}}(t) - C_p^{\text{SM}}(t)$ , where  $C_p^{\text{S}}$  is the solvent heat capacity,  $C_p^{\text{SM}}$  is the heat capacity of the protein solution. The sucrose concentrations both in the cell containing protein and in the control cell were equal. Ig G was dissolved in 0.01 M phosphate buffer, pH 7.3, containing 150 mM NaCl to a concentration of  $3 \text{ mg ml}^{-1}$ .

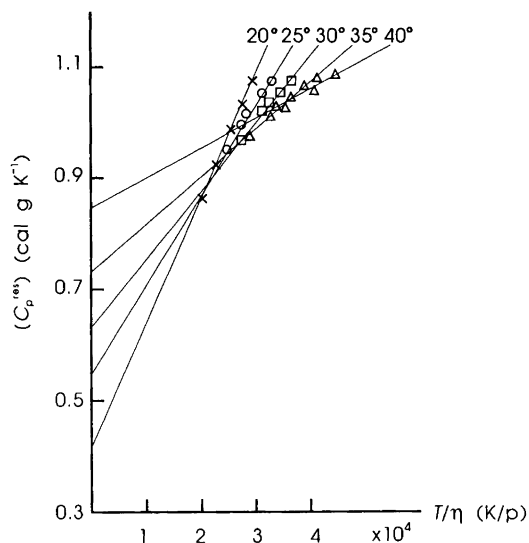


Fig. 2. Isothermal dependences of resulting partial heat capacities for Ig G.  $[C_p^{\text{res}}] = C_p^{\text{SS}} + [C_p^{\text{LS}}]$  was plotted against  $(T/\eta)$  obtained using sucrose dependences of  $C_p(t)$  in accordance with eqn. (9).

oxy- and met-hemoglobins (Hb) as well as for reduced and oxidized cytochrome c (Fig. 4).

The values of  $[C_p^{\text{LS}}]$  for oxy-Hb, met-Hb and cytochrome c were lower than for Ig G (Fig. 4). LS dynamics are interrelated with the flexibility of hinge regions of proteins and with the dynamics of the hydration shell. The results of the modified spin-label method<sup>1</sup> also indicated more intensive LS dynamics of Ig G compared with oxy- and met-Hb. At the same time, the LS dynamics of oxy-Hb were more significant than those of met-Hb<sup>1</sup>. In both cases there is a tendency towards damping of LS dynamics and activation of SS dynamics with increased temperature.

The ability of temperature to alter the LS and SS dynamics of proteins (Fig. 4) and the resulting heat capacity may play an important biological role. Such effects may be responsible for the primary mechanism of thermoadaptation on a molecular level, i.e., widening the temperature range of the functional activity of macromolecules.

The data from <sup>1</sup>H NMR spectroscopy,<sup>13</sup> IR spectroscopy<sup>1</sup> and light scattering<sup>2</sup> point to an ability of pulsating proteins to destabilize the structure of the solvent by the generation of acoustic impulses.<sup>5,7,13</sup> On the other

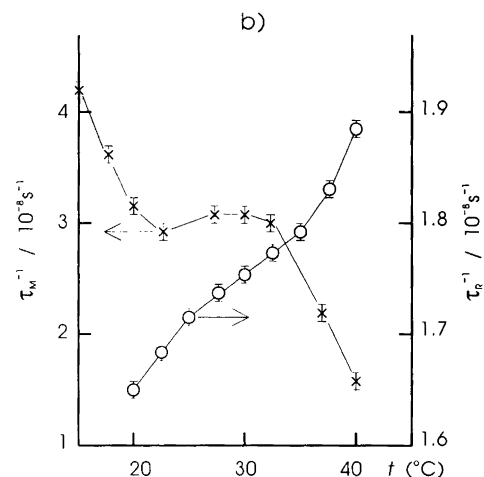
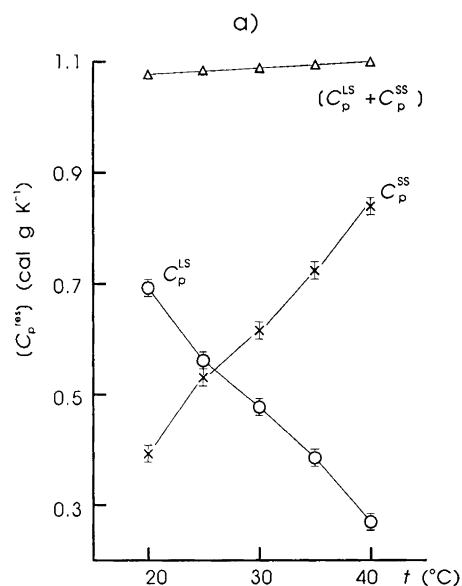


Fig. 3. (a) Temperature dependences of the resulting partial heat capacity for Ig G solution  $[C_p^{\text{res}}] = [C_p^{\text{SS}}] + [C_p^{\text{LS}}]$  and the contribution of  $[C_p^{\text{SS}}]$  and  $[C_p^{\text{LS}}]$  caused by small-scale (SS) and large-scale (LS) dynamics of Ig G molecules  $[\text{cal g K}^{-1}]$ . (b) Temperature dependences of the inverse values of the reduced correlation times for protein:  $(\tau_M^{-1}) \sim C_p^{\text{LS}}$  and those for the labels bound to this protein:  $(\tau_R^{-1}) \sim C_p^{\text{SS}}$  obtained previously by using the modified spin-label method.<sup>1,2</sup>

Table 1. Summary of the additional free volumes in water ( $\Delta V_f$ ) stimulated by the presence of Ig G and the volume occupied by proteins themselves ( $V_M$ ) at 20°C for two different assumptions, concerning properties of the hydration shell.<sup>a</sup>

Concentration of Ig G/mg ml <sup>-1</sup>	$(m_{\text{H}_2\text{O}}^h/m_M) = 0.5; [C_p^h] = 1$			$(m_{\text{H}_2\text{O}}^h/m_M) = 0.2; [C_p^h] = 1.3$		
	$V_M/\text{cm}^3$	$\Delta V_f/\text{cm}^3$	$\Delta V_f/V_M$	$V_M/\text{cm}^3$	$\Delta V_f/\text{cm}^3$	$\Delta V_f/V_M$
1.2	$8.9 \times 10^{-4}$	$7.9 \times 10^{-4}$	0.89	$8.9 \times 10^{-4}$	$8.6 \times 10^{-4}$	0.97
3.0	$2.2 \times 10^{-3}$	$2.1 \times 10^{-3}$	0.95	$2.2 \times 10^{-3}$	$2.2 \times 10^{-3}$	1.0

<sup>a</sup> Protein was dissolved in 0.01 M phosphate buffer (pH 7.3) containing 150 mM NaCl.

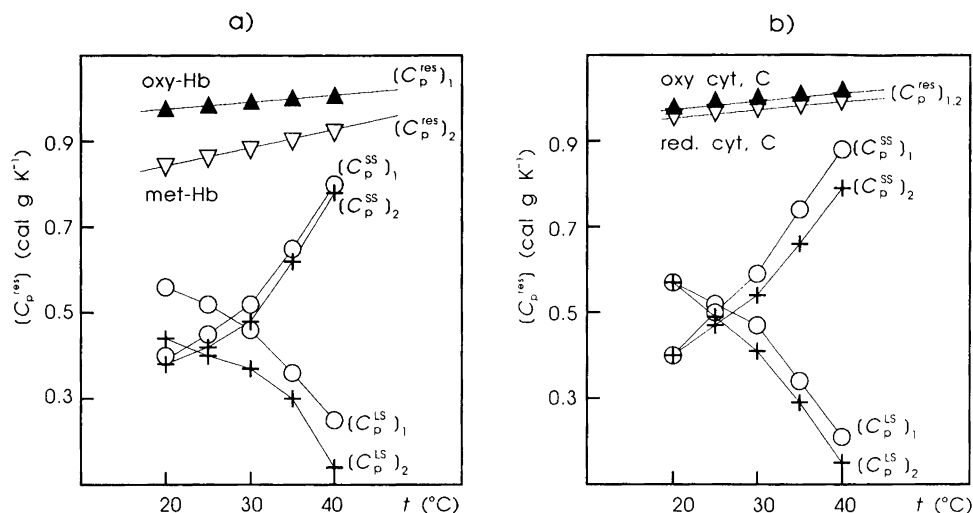


Fig. 4. (a) Temperature dependences of the resulting partial heat capacities  $[C_p^{Res}] = [C_p^{SS}] + [C_p^{LS}]$  of the oxy Hb (1), met-Hb (2) and corresponding separate contributions of small-scale  $[C_p^{SS}]_{1,2}$  and large-scale  $[C_p^{LS}]_{1,2}$  dynamics of these proteins. Concentrations of oxy- and met-Hb were 3.5 mg ml<sup>-1</sup>. (b) Temperature dependences of resulting partial heat capacities of cytochrome c in oxidized  $[C_p^{Res}]_1$  and reduced  $[C_p^{Res}]_2$  forms and the corresponding separate contribution of small-scale  $[C_p^{SS}]_{1,2}$  and large-scale  $[C_p^{LS}]_{1,2}$  dynamics of these proteins.

hand, the stabilization of the expanded solvent structure (ice-like clusters) in water medium by the protein surface must lead to the appearance of an extra free volume in protein solutions ( $\Delta V_f$ ). The results of our calculations of  $\Delta V_f$  for two Ig G concentrations (1.2 and 3.0 mg ml<sup>-1</sup>), for hemoglobin and cytochrome c in different states of activity, and of their ratio to the volume occupied by the macromolecules themselves are presented in Tables 1 and 2. The experimental data available imply that, in some cases, the thermocapacity of the hydration shell is about 30% higher than that of pure water, i.e.,  $[C_p^h] = 1.3$ .<sup>14</sup> This indicates greater cooperativity in the hydration shell compared with bulk water.

From Tables 1 and 2, two major conclusions can be drawn: the additional free volume ( $\Delta V_f$ ) is proportional to the number of dissolved macromolecules, and the additional free volume ( $\Delta V_f$ ) stimulated by the surface effects of proteins is close to the volume of the macromolecules themselves. The effects are in accordance with our mesoscopic model of thixotropic-like structure self-organization in water-macromolecules systems,<sup>15</sup>

Table 2. Volume occupied by macromolecules ( $V_M$ ) and additional free volume ( $\Delta V_f$ ) in the water, caused by the presence of proteins at 20°C for conditions  $m_{H_2O}^h/m_M = 0.5$  and  $[C_p^h] = 1$  cal g<sup>-1</sup> K<sup>-1</sup>.

Protein	$V_M/10^{-3}$ cm <sup>3</sup>	$\Delta V_f/10^{-3}$ cm <sup>3</sup>	$\Delta V_f/V_M$
Oxy Hb (3.5 mg ml <sup>-1</sup> )	2.9	2.0	0.68
Met Hb (3.5 mg ml <sup>-1</sup> )	2.6	1.4	0.54
Oxidized cytochrome c (3 mg ml <sup>-1</sup> )	2.12	1.89	0.89
Reduced cytochrome c (3 mg ml <sup>-1</sup> )	2.12	1.96	0.92

experimentally revealed in Italy.<sup>16</sup> The results in Table 2 can be explained in terms of large-scale pulsations of proteins which decrease their ability to stimulate the growth of coherent ice-like clusters and which represent the most probable 3D standing de Broglie waves of water molecules, related to their librations.<sup>17</sup>

## References

- Käiväräinen, A. I. *Solvent-dependent Flexibility of Proteins and Principles of their Functions*, Reidel, Lancaster, UK 1985.
- Käiväräinen, A. I. *Ann. Immunol. Hung.* 26 (1986) 425.
- Käiväräinen, A. I., Rozhkov, S. P., Sykulev, Yu. K., and Franck, F. *Immunol. Lett.* 3 (1981) 5.
- Käiväräinen, A. I. and Rozhkov, S. P. *Biophysica* 30 (1985) 777.
- Käiväräinen, A. I. *Biophysica* 33 (1988) 549.
- Käiväräinen, A. I. In: Lichtenstein, G. I. and Zhdanov, R., Eds., *Biomacromolecules in the Method of Spin-labels and Probes*, Nauka, Moscow 1988.
- Käiväräinen, A. I. *Large-scale Dynamics of Protein Molecules and their Interaction with Water*. Thesis, Scientific and Technical Union of the USSR Academy of Sciences, Leningrad 1989.
- Eftink, M. R. and Hagaman, K. A. *Biophys. Chem.* 25 (1986) 277.
- Käiväräinen, A. I. and Golikova, L. I. *Biophysica* 34 (1989) 168.
- Tables of Physical Parameters*, Atomizdat, Moscow 1976.
- Privalov, P. L. *FEBS Lett.* 40 (1974) 140.
- Ben-Naim, A. *Hydrophobic Interactions*, Plenum Press, London 1980.
- Käiväräinen, A. I., Goryunov, A. S. and Sukhanova, G. A. *Biophysica* 35 (1990) 174.
- Suurkuusk, J. *Acta Chem. Scand.* 28 (1984) 409.
- Käiväräinen, A. *Mesoscopic Theory of Matter and its Interactions with Light*, University of Turku, Finland 1992, p. 213.
- Giordano, R., Salvetti, G., Wanderlingh, F. and Wanderlingh, U. *Phys. Rev. A* 41 (1990) 689.
- Käiväräinen, A. *J. Mol. Liquids* 41 (1989) 53.

Received April 27, 1992.