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Glass Transition of Hydration Water and Structural Flexibility of Myoglobin

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The structure of proteins can be characterized by a few conformational states (R and T structure of hemoglobin). Fluctuations around these states have been termed "conformational substates" (1). The original definition of substates was based on motions which freeze near 200 K leading to static disorder at low temperatures (2,3). A more precise description requires information about the type of motion and the interactions involved. One particular aspect of this problem is the role of hydration water. We studied the O-H stretching band and the specific heat of hydration water in myoglobin films and crystals as a function of temperature and hydration. The ir spectra reveal that the surface water is amorphous and solid at low temperatures. Fig.1 displays the glass transition between the solid and the liquid phase at various water concentrations. No transition is found below 0.24 g H_2O/g protein. The spectrum is that of liquid bound water even at low temperatures. Even though all charged and polar sites are hydrated at this concentration, the water still forms isolated patches on the protein surface. The contribution of the temperature independent component decreases with increasing water content. The spectrum of amorphous ice appears between 0.25 and 0.3 g/g. The glass transition thus involves fluctuations of connected water clusters including water around nonpolar sites. Melting starts above 190 K.

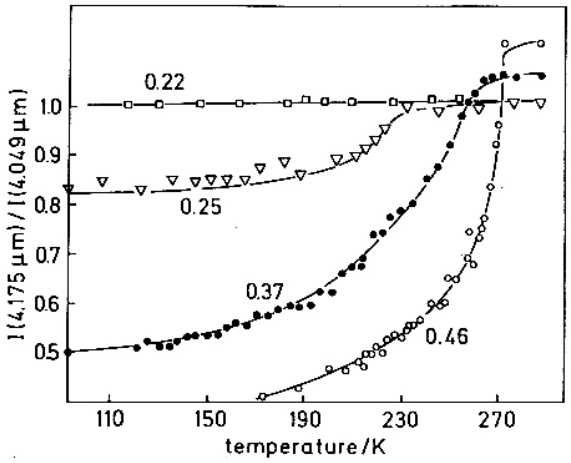

FIG. 1: Transmitted light intensity at the amorphous ice band (4.175 µm). The numbers indicate hydration of Mb films in g H_2O/g protein (6).

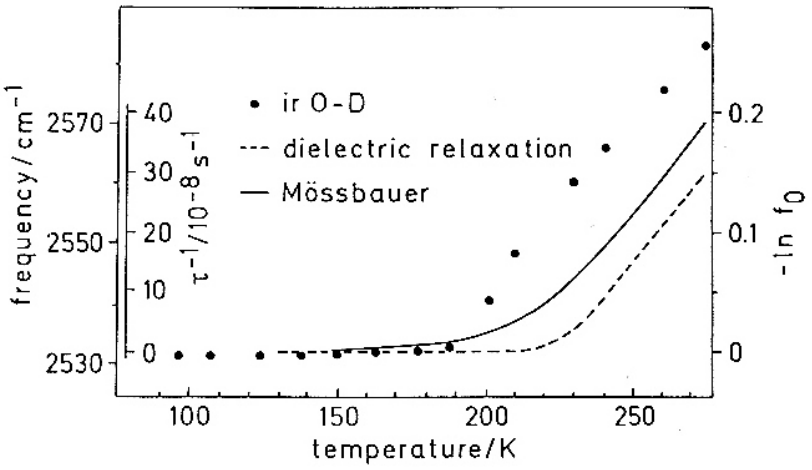

FIG. 2: Peak position of the O-D stretch, dielectric relaxation rate of water and the Lamb-Mössbauer factor f_0 of the heme iron in myoglobin crystals (6).

Similar results were obtained for water in myoglobin crystals. The position of the O-D absorption band of partially deuterated crystals is constant below 190 K and shifts to higher frequencies above this temperature as shown in fig. 2. The peak position is related to the average hydrogen bond length. The slope is proportional to the thermal expansion coefficient. The discontinuity of the expansion coefficient at 190 K is a characteristic feature of a glass transition. The dielectric relaxation rate of water and the Lamb-Mössbauer factor of the heme iron (4,5) display the same type of temperature dependence suggesting a common mechanism. The different apparent transition temperatures may be the result of different experimental time scales: ir spectroscopy provides an ensemble average. The Lamb-Mössbauer factor is insensitive to motions which are slower than 100 ns and the dielectric experiment has an upper limit of 10 ns. Thus, $T^G_{diel} > T^G_{f_O} > T^G_{ir}$.
The specific heat of crystal water, shown in fig. 3, fits well into this picture:

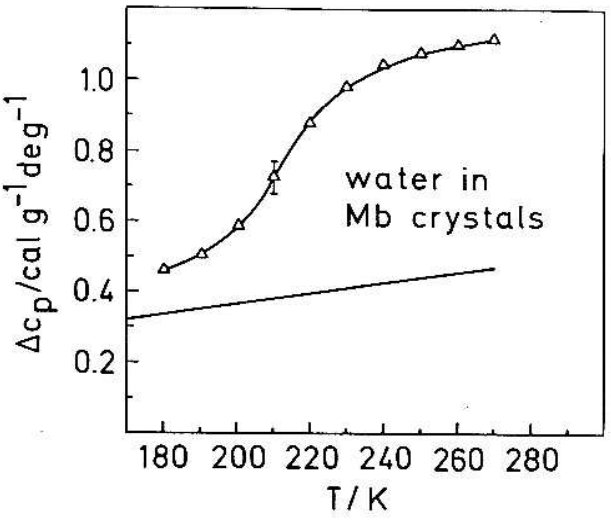

FIG. 3: Specific heat of water in Mb crystals and of ice (solid line). The data represent the difference between intact and dried crystals, h = 0.35 g H_2O/g protein and salt (6).

The heat capacity approaches the ice value at the low temperature end and is slightly larger than in pure water at high temperatures. The glass transition is extremely broad (40 K) suggesting a broad distribution of relaxation times.

The corresponding distribution of activation energies can be derived from a simple model:
The specific heat is proportional to the number of available number of degrees of freedom, which can relax (t_R) during the experimental time t_E: $t_R < t_E$. We define an availability function $F(t_E,t_R)$:

$$F(t_E,t_R) = \begin{cases} 1 & t_E > t_R \\ 0 & t_E \not> t_R \end{cases}$$

t_R and the activation enthalpy H are related by:

$$t_R = t_o \exp(H/RT)$$

c_p is then proportional to the following average:

$$c_p \propto \int_{H_{min}}^{\infty} F(t_E,t_R)\, g(H)\, dH$$

where g(H) is the activation enthalpy distribution. H_{min} is given by $RT_o \ln t_E/t_o$. The following g(H) fits the data very well above $T_o \simeq 190$ K:

$$g(H) \propto \begin{cases} \exp(-\alpha(H-H_{min})) & H \geq H_{min} \\ 0 & H < H_{min} \end{cases}$$

One obtains:

$$c_p = c_p^{ice} + \Delta c_p(1 - \exp(-\alpha(T-T_o)\ln t_E/t_o))$$

For t_E = 1s, $t_o = 10^{-13}$ s one finds: T_o = 194 K, α^{-1} = 5.1 kJ/mol. A similar distribution was derived from ligand recombination data below 200 K (1).

The data presented above indicate that the exchange within one class of conformational substates is governed by hydrogen bond dynamics. The freezing of hydrogen bonds imposes severe constraints on conformational relaxation. Photolysis of the ligand below 200 K may thus lead to partial relaxation and intermediate states between the oxy and deoxy structure.

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