Until $\vec{Q}$-domain structure can be experimentally controlled, it will not be possible to make detailed comparisons between theory and data. We illustrate this in Fig. 4, where three spectra are shown for $\vec{H}$ in a (211) plane. Only the texture parameters have been changed. Finally, the dashed curve in Fig. 4(c) shows how open-orbit spectra can be suppressed if metallurgical preparation has caused too small a domain size.

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Role of Adsorbed Water in the Dynamics of Metmyoglobin

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By microwave measurements we have determined the dielectric relaxation rate for adsorbed water in metmyoglobin crystals in the temperature range from 100 to 300 K. The temperature dependence of the dielectric relaxation rate of the adsorbed water is nearly identical to the temperature dependence of the conformational fluctuation rate of the protein as measured by the Mössbauer effect. This surprising correlation may be understood in terms of the mechanical and electrical interactions between the adsorbed water and the protein.

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The physiological function of proteins cannot be understood from their static structure since dynamical aspects are of great importance. Recently x-ray[1] and Mössbauer[2-4] studies on myoglobin crystals and frozen solutions have revealed that the atoms in the protein molecule undergo unusually large displacements. For example, the average displacement of the Fe atom in myoglobin is as large as 0.24 Å at room temperature. Such anomalously large values can be ascribed to the fluctuations between conformational substates of the protein molecule.[1] These substates arise from a large number of slightly different structural configurations which protein molecules can adopt. In Mössbauer experiments it was found that the characteristic time of these large-amplitude motions is about 10^{-7} s at room temperature.[4] On cooling, however, these fluctuations slow down, and below 200 K, they are too slow to be seen by Mössbauer experiments.

Mössbauer experiments on proteins[5,6] and Rayleigh scattering experiments on myoglobin[7] powders with controlled water content have already shown that the large displacements of the iron atom disappear if the amount of water adsorbed on the protein is reduced. Therefore to understand the role played by water, we have studied the dynamical dielectric properties of water in metmyoglobin crystals. We find that the temperature dependence of the dielectric relaxation rate of the adsorbed water in metmyoglobin crystals is strongly correlated to the temperature dependence of the displacement of the iron atom as seen by Mössbauer measurements. In this paper we discuss how the dynamic dielectric properties of the adsorbed water may influence the conformational fluctuations inside the protein.

Dielectric measurements at microwave frequen-

cies as a function of temperature are a very suitable tool for investigating the dynamics of adsorbed water. Previous dielectric studies on several proteins have shown that adsorbed water layers on proteins are the principle source of dielectric loss at 10 GHz.[8] Protein side chains contribute to the dielectric loss mainly at lower frequencies (10–100 MHz), but hardly at 10 GHz. Our measurements were carried out at 10 GHz using the cavity perturbation technique.[9] From the observed quality factor of the microwave resonance cavity loaded with the sample and the known filling factor, we deduce ϵ'' the imaginary part of the dielectric constant.[10] To deduce ϵ'', we assumed, following Ref. 11, that only one third of the crystal volume is occupied by water. We used sperm-whale myoglobin crystals grown from $(NH_4)_2SO_4$ solution at pH 6.1 following the procedure of Ref. 12. The sample contained a large number of small crystals (typical size, 0.1 mm). Under optical inspection, we carefully removed the excess mother liquid, making sure that crystals were neither dry nor had any excess $(NH_4)_2SO_4$ solution on their surfaces. To estimate the effect, if any, of excess mother liquid, experiments were repeated in the absence of any protein with the 3.75-M $(NH_4)_2SO_4$ solution alone.

The imaginary part of the dielectric constant at a frequency ω for a dielectric medium having only one relaxation time τ may be written as

$$\epsilon'' = (\epsilon_0 - \epsilon_\infty)\frac{\omega\tau}{1+\omega^2\tau^2}. \quad (1)$$

Here ϵ_0 and ϵ_∞ are the static and the high-frequency limits of the dielectric constant, respectively. For water adsorbed on several proteins[8] at 10 GHz, $\omega\tau \gg 1$. As will be seen below the same is true for metmyoglobin; we can therefore simplify Eq. (1):

$$\epsilon'' = \frac{\epsilon_0 - \epsilon_\infty}{\omega\tau}. \quad (2)$$

In order to deduce the temperature dependence of τ^{-1} from our measured value of ϵ'' at 10 GHz, we need the value of $\epsilon_0 - \epsilon_\infty$ for water in metmyoglobin crystals. For this we measured the temperature dependence of ϵ'' at a lower frequency of 1 GHz. At 1 GHz, ϵ'' showed a maximum at $T = 265$ K, indicating that at $T = 265$ K, $\omega\tau = 1$ [see Eq. (1)]. Using this information and the value of ϵ''(10 GHz) at $T = 265$ K, we find from Eq. (2), $\epsilon_0 - \epsilon_\infty = 90 \pm 10$, for water in the metmyoglobin crystals. For comparison Pennock and Schwan[13] found that for water adsorbed on horse hemoglobin $\epsilon_0 - \epsilon_\infty$ lies in the range 80-100.

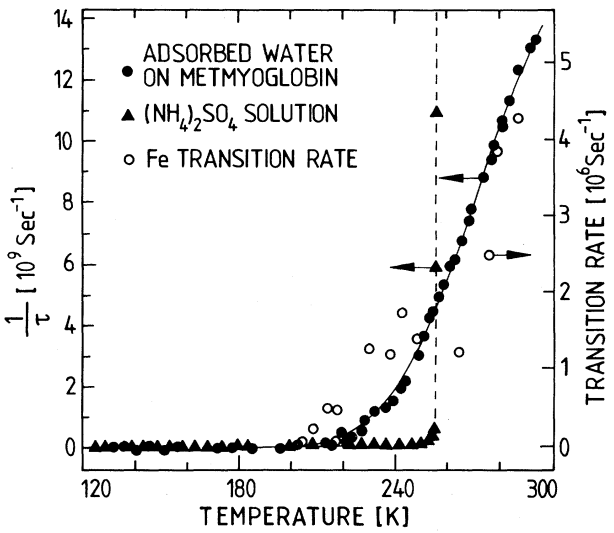

FIG. 1. The temperature dependence of the dielectric relaxation rate of water in metmyoglobin (full circles, left scale) and of $(NH_4)_2SO_4$ solution (triangles, left scale). The conformational transition rate of Fe atoms (open circles, right scale) is plotted for comparison.

In Fig. 1 we show the dielectric relaxation rate τ^{-1} as deduced from our 10-GHz data for water in metmyoglobin crystals (full circles, left scale) and also for the $(NH_4)_2SO_4$ solution (triangles, left scale). We have assumed $\epsilon_0 - \epsilon_\infty = 90$ as well for the $(NH_4)_2SO_4$ solution. This may not be correct, but a possible error does not affect the measured temperature dependence. It is clearly seen that τ^{-1} for the $(NH_4)_2SO_4$ solution alone exhibits a discontinuity at its freezing point, $T = 253$ K. No such discontinuity is found for the water in metmyoglobin crystals. The absence of a discontinuity and the relatively slow relaxation rate in comparison with free water (see Fig. 2) indicate that practically no free water exists in the crystals and that all water molecules (nearly 400 per protein molecule) are adsorbed.

This fast increase of the relaxation rate with temperature between 200 and 300 K is not unique for the adsorbed water. As already mentioned above, the average displacement of the iron atom in the protein also increases rapidly above 200 K as a result of fluctuations of the molecule between different conformational substates. In addition, the transition rate of the Fe atom as determined from Mössbauer linewidth data on deoxymyoglobin crystals[4] also rapidly rises with temperature and is shown for comparison in Fig. 1

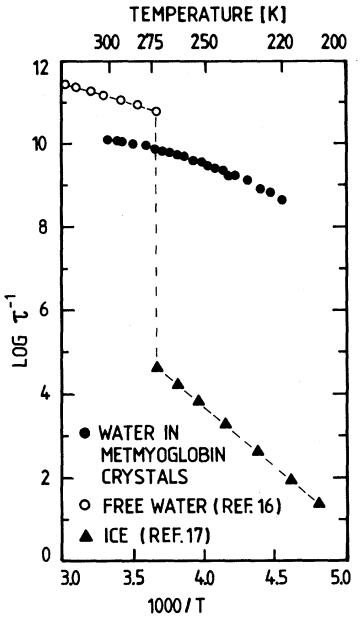

FIG. 2. Dielectric relaxation rates of water in metmyoglobin, free water, and ice.

(open circles, right scale). These values have been obtained under the assumption that the residual linewidth at 200 K is determined only by the source and the inhomogeneous broadening of the myoglobin samples. As is seen from Fig. 1, the fluctuation rate inside the protein is roughly three orders of magnitude slower than the relaxation rate we found for the adsorbed water. The important point is, however, that both sets of data (open and full circles) follow nearly the same temperature dependence.

The temperature dependence of the dielectric relaxation rate found for water adsorbed in metmyoglobin crystals is very similar to that of water adsorbed on a variety of other substrates.[14] Furthermore, at a given temperature the dielectric relaxation rate of adsorbed water is known to decrease with decreasing thickness of the water film.[15] Considering these facts, the Mössbauer experiments on dried proteins[5,6] (i.e., proteins with only a few water molecules adsorbed) and on metmyoglobin crystals appear consistent with the view that it is the adsorbed water which imposes its dynamics on the protein. We propose here two mechanisms of coupling between the protein and the adsorbed water, which may explain the observed phenomena. We restrict ourselves to a qualitative discussion as it seems difficult to offer a quantitative model at this early stage.

In Fig. 2 we compare the dielectric relaxation rates of free water,[16] of ice,[17] and of water in metmyoglobin crystals. In free water the dielectric relaxation arises mainly from reorientation of water molecules and is very fast. This process also determines the viscosity of water and in fact the activation energy of both, the viscosity and the dielectric relaxation time, is the same.[18] In ice, however, the molecules are no longer able to reorient themselves, and therefore the viscosity of ice is many orders of magnitude higher than that of free water. As shown in Fig. 2 the relaxation rate of the water in metmyoglobin crystals is considerably higher than that of ice. This indicates that the water adsorbed inside the crystal retains its ability for molecular reorientation down to temperatures far below 273 K. In view of this it appears reasonable to assume that the mechanical damping that the adsorbed water may produce for the motion of protein side groups increases with an increasing dielectric relaxation time of the adsorbed water. Therefore the correlation between the dynamics of the protein and of the adsorbed water may be understood as follows. At room temperature the water molecules can reorient at a fast rate and therefore hardly exert any frictional force on the motion of protein, which can therefore fluctuate between the conformational substates at a fast rate. With decreasing temperature the decreasing reorientation rate of the water molecule leads to a stronger damping of the protein motion resulting in a decreasing fluctuation rate of the protein.

We consider now another mechanism which involves a dynamic electrical coupling between the protein and the water around it, as opposed to a purely mechanical interaction assumed in the model discussed above.

In myoglobin and several other proteins the distribution of charges is well known. As investigated by Friend and Gurd,[19] the electrostatic interaction between the charged polar amino acids on the surface of a protein provides an important contribution to the stabilization energy of the tertiary structure of myoglobin. Consequently any disturbance of the charges or dielectric properties of the environment of the molecules is likely to have a strong influence on the structure and dynamics of the protein.

Usually the potential barrier between the conformational substates of the protein is high in comparison with the thermal energy, and therefore transitions between these substates are rare. The reorientation of the electrical dipole mo-

ments of the water molecules leads to local fluctuations of the electric field, which interacts with the polar groups located at the surface of the protein molecule and causes them to move. Thus the potential barrier between the conformational substates of the protein is modulated by the fluctuating dipole moments of the adsorbed water. The mean amplitude of these potential modulations is small and they occur at an average rate given by the dielectric relaxation rate. Occasionally, however, because of statistical correlation among the fluctuating dipoles, these potential modulations may reach large amplitudes. In this case the barrier height can be lowered to such an extent that the system undergoes a transition from one substate to another. The probability for such sufficiently large fluctuations required to explain the relatively slow fluctuation rate of proteins in comparison with the fast relaxation rate in adsorbed water is about a part in a thousand. Since the rate of dipole fluctuations in adsorbed water increases with increasing temperature, the rate of conformational transitions in protein is also expected to increase proportionally as observed experimentally. The effect of electrical coupling may also be viewed as the shielding of the motion of the charged side groups at the protein surface by the high dielectric constant of the adsorbed water. This shielding is effective only for those conformational fluctuations of the protein which are slower than the dielectric relaxation rate of the adsorbed water. Consequently the conformation rate decreases with decreasing dielectric relaxation rate of adsorbed water, as the temperature is lowered.

Although both these mechanisms appear plausible, further experiments on proteins not having electrically charged groups on the protein surface are desirable, before a more quantitative model along these lines can be developed.

In conclusion, our experiment demonstrates the important role of adsorbed water for the dynamics of the protein. The observed temperature dependence of the dielectric relaxation rate of water in metmyoglobin crystals is found to be nearly identical to the temperature dependence of the conformational fluctuation rate of the protein as determined from Mössbauer linewidths.[4] The protein molecule and the water around it form a strongly coupled system. Two mechanisms, one the mechanical damping of the protein motion by the adsorbed water and second a dynamic electrical coupling between the fluctuating electric dipoles of the adsorbed water and the polar side groups of the protein, appear to provide a qualitative understanding of the observed phenomena.

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