Proteins and Glasses: A Relaxation Study in the Millikelvin Range

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Comparative spectral diffusion studies in the millikelvin range between a protein and a glass point to a strong shielding of the chromophore from the strain and/or electric fields of the host and to quite specific features of the energy landscape.

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The spectroscopy of chromoproteins offers quite interesting aspects of the physics of the glassy state: Proteins are mesoscopic particles with typical dimensions of some tens of angstroms. They are highly organized; their building blocks are spatially correlated. Yet, they have glasslike properties too: For example, a specific heat linear with temperature below 3 K points to the presence of TLS-like disorder modes [1,2]. (TLS stands for two level systems; for a review of glasslike properties of proteins see [3].) From the viewpoint of solid state physics the most interesting aspect of frozen solutions of chromoproteins is the fact that space, as seen from a chromophore, is not any more homogeneous: Each chromophore sees the same organized nearby environment of the protein in addition to the distant amorphous environment of the glass. This gives rise to interesting questions as to the solid state physics of mesoscopic particles embedded in a host glass: To what extent is the chromophore in the protein shielded against the strain and/or electric fields of relaxing TLS in the host glass? Is relaxation in the protein frozen out, or is it still going on, even in the millikelvin range? Is it possible to get insight into the specific features of the energy landscape of a protein as compared to a glass? To answer these questions we performed a comparative relaxation study between a protein and a glass in the millikelvin range.

Our experimental approach is spectral hole burning [4] (for reviews see [5]). The chromophore is protoporphyrin-IX (Fig. 1). The two inner ring protons of free base porphyrins can be photochemically converted into a series of configurational states [6–8]. The resulting tautomers differ in their absorption energies, typically by some tens to hundred wave numbers. These proton transfer reactions are used to burn narrow photochemical holes into the inhomogeneously broadened long-wavelength absorption band. At 100 mK the respective width of the holes is on the order of 50 MHz (Fig. 2). The protein is myoglobin whose natural prosthetic group was replaced by protoporphyrin-IX. Its mean diameter is about 35 Å. It was dissolved in a glycerol/water glass (2.5/1 volume per volume) at a concentration of about 85 mg/ml. For comparison, protoporphyrin-IX was also directly dissolved in a glass. In this case the glass was glycerol with a 30% addition (volume per volume) of dimethylformamide to ensure solubility of the chromophore.

The quantity which we measured is the so-called spectral diffusion kernel [9–13]. The basic idea of the experiment is the following: The system is marked at a temperature of 100 mK with a narrow spectral hole. Then, we performed either one of two different kinds of experiments: In the first one the hole is observed as a function of time [14–17]. Since frozen proteins are, like glasses, characterized by quasistatic disorder, they may undergo structural relaxation either by tunneling or, if the conformational barriers are low enough, even by activated processes. Such processes change the microscopic fields (strain and/or electric fields) which act on a dye probe. As a consequence, the chromophores adjust their spectral energies. The hole broadens. The difference in the width of the broadened and the initial hole is called the spectral diffusion kernel. It is the quantity plotted in Fig. 1. Note that in all our experiments the shapes of the holes are very close to Lorentzians [9,18].

In the second type of experiment, it is the temperature which is varied. We changed it in a cyclic fashion [19–22]. We call the highest temperature in a cycle

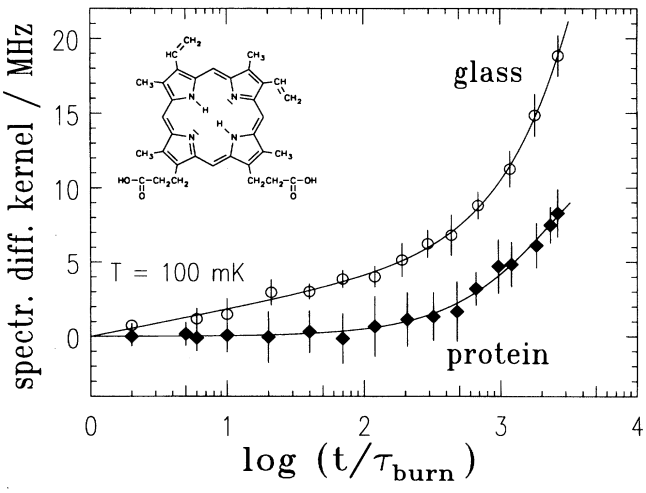

FIG. 1. Spectral diffusion broadening of a photochemical hole in a protein and a glass sample as a function of time. The fitted curve for the glass data is based on a logarithmic broadening pattern superimposed by an exponential, whereas the fitted curve for the protein data is a pure exponential. The spectral diffusion kernel for the glass is arbitrarily set to zero at $t = \tau_{\text{burn}}$.

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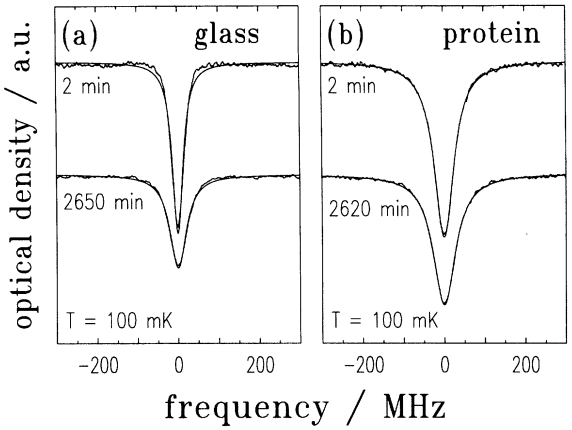

FIG. 2. Hole traces at the beginning and at the end of the experiment in Fig. 1. Fitted curves are Lorentzians. τ_{burn}: about 45 s. Intensity of burning laser: 10 μW/cm^2.

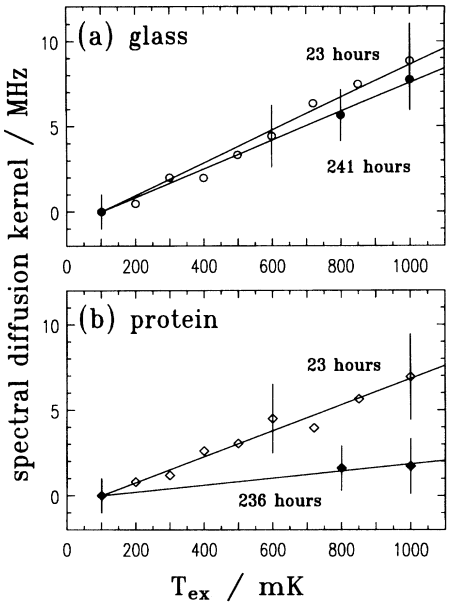

FIG. 3. Spectral diffusion broadening induced by cyclic temperature variations. T_{ex} is the excursion temperature, i.e., the highest temperature in a cycle. Note that all the data are corrected for time dependent spectral diffusion broadening.

the excursion temperature T_{ex}. It is the parameter of this second type of experiment. When the temperature is raised from the burn temperature (100 mK) to the excursion temperature, relaxation processes may be induced. Upon recooling, the system may be trapped in a different structural state. Again the microscopic fields will change and, again, the hole will broaden. The difference between the increased and the initial hole width is plotted in Fig. 3 as a function of the excursion temperature T_{ex}. Since a temperature cycle to be performed in the millikelvin regime needs quite a lot of time, time dependent relaxation occurs simultaneously with the temperature-induced relaxation. However, we measured the respective time dependence after each temperature cycling experiment. Hence, we were able to correct the temperature induced relaxation data in an appropriate way. In all experiments hole burning was performed in the wave-number range 16 021 $\mp$ 1 cm^{-1}. For both samples, this is close to the maximum of the long-wavelength band.

Figure 1 shows the time dependent relaxation features. Note that these data were obtained after letting both the glass and the protein relax for 147 h in a temperature range between 100 mK and 1 K before hole burning. There are a few remarkable things: (i) The overall relaxation pattern in both systems as measured over 44 h is nonlogarithmic in time, quite in contrast to what is known from many experiments on glasses [11,12,14–17]. (ii) The protein behaves significantly different from the glass: Spectral diffusion broadening is always less. Within the first 3 h, there is practically no relaxation. In the glass, on the other hand, relaxation does occur in this time regime. We note that the respective relaxation pattern of the glass is in line with a logarithmic time dependence. (iii) After about 3 h there is a strong increase in the line broadening on the logarithmic time scale for both samples.

Figure 2 shows the holes at the beginning and at the end of the time dependent spectral diffusion experiment together with Lorentzian fit curves.

Figure 3 shows the broadening of the burnt-in holes under thermal cycling conditions. We performed a whole series of such experiments as a function of waiting time. The figure just shows the first (after a waiting time of 23 h) and the very last (after a waiting time of about 240 h) of these experiments for the glass (a) and for the protein (b). We note that in the temperature range of the experiment the spectral diffusion kernel increases linearly with excursion temperature. The slope of the respective straight line is determined by the number of structural states which can be reached by the system upon changing the excursion temperature by a certain interval. The noteworthy features of these results are the following: (i) The slope is nearly independent of waiting time in the glass sample. This means that the number of accessible structural states per interval of the excursion temperature does not change with time. (ii) In the protein, this slope changes significantly with time. After a waiting time of 236 h, the slope is close to zero. This means that a temperature cycle cannot induce structural changes anymore.

Note that the slope is, apart from the accessible number of states, also influenced by the respective coupling matrix element. However, our argumentation is focused on the relative changes of the slope which is not influenced at all by the coupling matrix element.

Figure 4 summarizes the data of the thermocyclic linewidth changes. Plotted is the slope (from the data

in Fig. 3) as a function of waiting time. The difference between glass and protein is quite obvious. The slope seems to relax to zero in the protein whereas in the glass it does not seem to change significantly with time.

What can we learn from these experiments about the glass, the protein, and the respective similarities and differences? Let us start with the discussion of the time evolution of the spectral diffusion kernel (Fig. 1). The onset of the strong increase in the broadening occurs in both samples after about the same time. Hence, it seems reasonable to assume that this increase comes from the same sort of structural state(s). We argue that the host matrix behaves in the usual fashion, i.e., causes logarithmic spectral diffusion broadening patterns in time. Yet, superimposed to the usual TLS is a distinguished structural state. By distinguished we mean that this special TLS is spatially strongly correlated with the chromophore, hence, is not subject to the TLS statistics. In this case an exponential broadening would be superimposed to the logarithmic spectral diffusion pattern. The fitted curves in Fig. 1 are based on such an assumption. Note that a logarithmic evolution term is needed to fit the data of the glass but is not needed to fit the protein data. A strong correlation of this distinguished TLS with the chromophore is suggested by the results because the respective relaxation features occur in both samples. The associated configurational barrier must be sufficiently high because this TLS does not show up in the thermal cycling experiments. We note that such distinguished structural states have been observed in spectral diffusion experiments with other proteins [23]. It should also be noted that various classes of TLS, although all of them with broad distributions of relaxation times, have been observed in quite a series of glasses with accumulated photon echo techniques [24–26].

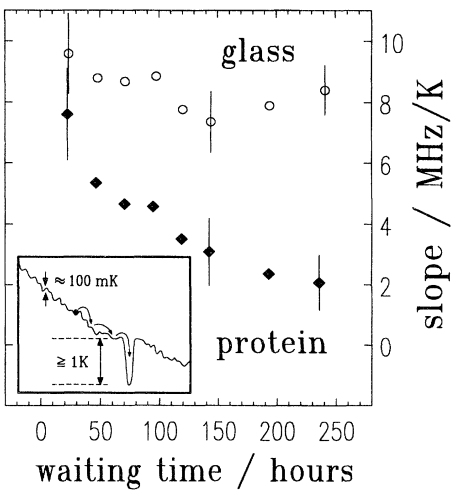

FIG. 4. The slopes (of Fig. 3) as a function of waiting time. The inset shows a sketch of the energy landscape of a protein as inferred from our experiments: A fine-structured roughness interrupted by rather deep valleys.

The experiments further show that the chromophore is rather effectively shielded through the apoprotein against the changing strain and/or electric fields created by the relaxing host matrix. This follows immediately from the absence of any relaxation broadening at 100 mK in the first 3 h, quite in contrast to the glass. Obviously the chromophore in the protein is rather insensitive to the matrix TLS which cause the quasilogarithmic line broadening in the glass. This observation has an important implication: Whenever spectral diffusion is observed, as is the case, for example, in the thermal cycling experiments, it must correspond with relaxation processes occurring within the protein.

There is another interesting conclusion: The rugged energy landscape seems to have threshold energies which cannot be passed anymore at temperatures in the 100 mK range. These threshold energies seem to be related to valleys rather than to barriers. These valleys act as structural traps from where the protein cannot escape anymore. In the glass, on the other hand, there is no indication for such threshold energies because spectral diffusion is not inhibited.

Do the thermal cycling experiments corroborate these conclusions? We think answer is yes. Figure 3 clearly characterizes the situation: In the glass there obviously are sufficiently shallow TLS which can be flipped by a thermal cycle between 100 mK and 1 K, even after letting the system relax for 241 h. The protein, on the other hand, also has sufficiently shallow structural states which can be changed with a temperature cycle. However, this only holds during the starting phase of the experiment [Fig. 3(b)]. After letting the protein relax for 236 h, thermal cycling induces only little changes in the linewidth. This is quite a strong indication for rather deep structural traps on the scale of k_BT (with $T = 1$ K). The energy needed to kick the protein out of the shallowest trap is too large as compared to the thermal energies of the temperature cycle. Figures 3 and 4 beautifully demonstrate that this type of spectral diffusion experiment is quite sensitive to the protein: If the chromophore in the protein saw the relaxing glass TLS, we could not understand the strong decrease of the respective slope (Fig. 4). This specific insensitivity obviously comes from the efficient shielding of the matrix fields through the apoprotein. The interaction in spectral diffusion broadening itself is of dipolar nature and of long range. Hence, from the nature of this interaction one would not expect that the dye probe is shielded through a protein shell with a thickness of some 10 Å only. At present, the reason for this shielding is not quite clear, considering the fact that the differences in the mean compressibilities between protein and host glass are rather low [27,28].

These results even allow for a qualitative picture of the energy landscape appropriate for the temperature range of our experiment (100 mK–1 K); during its downward relaxation to the structural trap, the respective barriers

cannot exceed a few 100 mK, because a change of the excursion temperature on this scale causes broadening. Hence, there obviously is a rather fine-structured roughness. This fine-structured roughness is interrupted by comparatively deep energy valleys from where escape is not possible anymore . It is also clear that the threshold energies must be related to valleys and not to barriers because an energy landscape with a fine surface roughness interrupted by high barriers would never prohibit thermally induced spectral diffusion: On one side of the barrier thermal access to shallow structural states would always be possible. In conclusion, the energy landscape on the temperature scale of our experiment must look as sketched in the inset of Fig. 4. This picture is quite similar to the one published on the basis of Mössbauer spectroscopy [29,30]. It seems that the structure is repeating itself on a different scale [31,32].

There is another interesting aspect of the energy landscape of the protein: The respective slope seems to decrease to zero (Fig. 4). Hence, *all* protein molecules must end up in a deep structural trap. In other words, for all protein molecules a deep structural trap must be within a reachable distance in conformation space. The conclusion is that the energy landscapes have some similarity. They cannot vary on a random scale as one goes from one protein to another. There must be a correlation. The glass does not show anything like this. We attribute this energetic correlation to the finite size of the protein and to the spatial correlation of its building blocks. If the size of a system becomes sufficiently small, the number of accessible deep structural trap states may shrink dramatically as temperature goes to zero [33]. Spatial correlation even further limits this number. As a consequence, there may be just a few accessible trap states.

In summary, our comparative relaxation experiments in the millikelvin range demonstrate that proteins do have strong similarities with glasses, yet they have also very specific features mainly due to their finite size and their spatial correlation.

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[1] G. P. Singh, H. J. Schink, H. von Löhneysen, F. Parak, and S. Hunklinger, Z. Phys. B **55**, 23 (1984).
[2] I-.S. Yang and A. C. Anderson, Phys. Rev. B **34**, 2942 (1986).
[3] H. Frauenfelder, F. Parak, and R. D. Young, Annu. Rev. Biophys. Chem. **17**, 451 (1988).
[4] J. Friedrich and D. Haarer, Ang. Chem., Int. Ed. Engl. **23**, 113 (1984).
[5] *Persistent Spectral Hole Burning: Science and Application*, edited by W. E. Moerner, Topics in Current Physics Vol. 44 (Springer-Verlag, Berlin, 1988).
[6] J. Fidy, J. M. Vanderkooi, J. Zollfrank, and J. Friedrich, Biophys. J. **61**, 381 (1992).
[7] J. Zollfrank, J. Friedrich, and P. Parak, Biophys. J. **61**, 716 (1992).
[8] S. Völker, in *Relaxation Processes in Molecular Excited States,* edited by J. Fünfschilling (Kluwer, Dordrecht, 1989), p. 113.
[9] J. L. Black and B. I. Halperin, Phys. Rev. B **16**, 2879 (1977).
[10] T. L. Reinecke, Solid State Commun. **32**, 1103 (1979).
[11] L. A. Narasimhan, K. A. Littau, D. W. Pack, Y. S. Bai, A. Elschner, and M. D. Fayer, Chem. Rev. **90**, 439 (1990).
[12] K. A. Littau, Y. S. Bai, and M. D. Fayer, J. Chem. Phys. **92**, 145 (1990).
[13] P. D. Reilly and J. L. Skinner, Phys. Rev. Lett. **71**, 4257 (1993).
[14] W. Breinl, J. Friedrich, and D. Haarer, J. Chem. Phys. **81**, 3915 (1984).
[15] J. Friedrich and D. Haarer, in *Optical Spectroscopy of Glasses*, edited by I. Zschokke-Gränacher (Reidel, Dordrecht, 1986), p. 149.
[16] W. Köhler, J. Meiler, and J. Friedrich, Phys. Rev. B **35**, 4031 (1987).
[17] S. Jahn, K. P. Müller, and D. Haarer, J. Opt. Soc. Am. B **9**, 925 (1992).
[18] A. M. Stoneham, Rev. Mod. Phys. **41**, 82 (1969).
[19] J. Friedrich, H. Wolfrum, and D. Haarer, J. Chem. Phys. **77**, 2309 (1982).
[20] G. Schulte, W. Grond, D. Haarer, and R. Silbey, J. Chem. Phys. **88**, 679 (1988).
[21] W. Köhler and J. Friedrich, Europhys. Lett. **7**, 517 (1988).
[22] W. Köhler, J. Zollfrank, and J. Friedrich, Phys. Rev. B **39**, 5414 (1989).
[23] J. Zollfrank, J. Friedrich, J. M. Vanderkooi, and J. Fidy, J. Chem. Phys. **95**, 3134 (1991).
[24] H. C. Meijers and D. A. Wiersma, Phys. Rev. Lett. **68**, 381 (1992).
[25] H. C. Meijers and D. A. Wiersma, J. Lumin. **53**, 80 (1992).
[26] H. C. Meijers, thesis, University of Groningen, 1994.
[27] J. Gafert, J. Friedrich, and F. Parak, J. Chem. Phys. **99**, 2478 (1993).
[28] T. Yamato, J. Higo, Y. Seno, and N. Go, Proteins **16**, 327 (1993).
[29] F. Parak, Phys. Bl. **41**, 396 (1985).
[30] F. Parak, M. Hartmann, and G. Nienhaus, in *Protein Structure: Molecular and Electronic Reactivity,* edited by R. Austin *et al.* (Springer-Verlag, New York, 1987), p. 65.
[31] H. Frauenfelder, in *Structure and Dynamics of Nucleic Acids Proteins and Membranes*, edited by E. Clementi and S. Chin (Plenum, New York, 1986), p. 169.
[32] H. Frauenfelder, S. G. Sligar, and P. Wolynes, Science **254**, 598 (1991).
[33] H. Frauenfelder and P. Wolyner, Phys. Today **47**, No. 2, 58 (1994).