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Conformational barriers in low-temperature proteins and glasses

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We measured the distribution of conformational barriers in a chromoprotein by using a spectral hole as a probe. The results can be interpreted by a superposition of glasslike states and a discrete feature with a Gaussian distribution.

We showed in several recent papers that temperature cycling experiments of low-temperature glasses allow for a precise sampling of the distribution of conformational barriers.[1,2] The basic idea is the following: A spectral hole is burnt at low temperatures (T_b). The burning reaction creates some product which is stabilized by a barrier against the educt. There are many reactions where the height of the barriers is determined by the local structure of the host-guest system. Examples are the so-called "photophysical" hole-burning reactions[3-5] or all types of reactions where the matrix is involved, such as quinizarin in alcohol glass.[5] In all these cases the barriers between the product and the educt state are subject to a broad distribution due to the disorder of the glass or polymer matrix. Recently, we tried to find out if the distribution of conformational barriers in glasses follows some general characteristic pattern and if such a distribution could be measured. We could indeed show that the barrier heights V follow a distribution of the form[1]

$$P_g(V) \sim (1/V)^{1/2} , \tag{1}$$

where the index g indicates that this distribution is characteristic for the glassy state. Equation (1) is a direct outcome of the well-known tunneling model.[6] We normalize the above distribution by introducing cutoff values $V_{\max}$ and $V_{\min}$, respectively.

Temperature cycling experiments[1,2] allowed for an experimental verification of Eq. (1): The change in area which the hole experienced by cycling the temperature between the burning temperature T_b and, what we call the excursion temperature T, is a measure of the number of molecular centers which returned from the product to the educt state during the temperature cycle. In these experiments T_b is usually a very low temperature of a few K and T is always higher than T_b. The quantity we measure is the area of the hole as a function of the excursion temperature. The area is directly related to the above distribution function [Eq. (1)]. To each value of the excursion temperature there is a related, well-defined, barrier height V_T in the sense that all centers with barriers

$$V < V_T$$

relax during the cycle ($T_b \rightarrow T \rightarrow T_b$) to the educt state while those with

$$V > V_T$$

stay unaffected. V_T can be easily calculated: We assume that the temperature cycle leads to an activated barrier crossing with a rate

$$R = R_0 e^{-V/kT} . \tag{2}$$

If the experimental time for driving the system through the cycle is τ, then roughly all those centers can relax having

$$R^{-1} \leqq \tau . \tag{3}$$

The barrier which can just be crossed in a specific cycle ($T_b \rightarrow T \rightarrow T_b$) is obtained by the condition

$$R^{-1} = \tau . \tag{4}$$

Hence, from Eqs. (2) and (4) we get

$$V_T = kT \ln R_0 \tau . \tag{5}$$

The factor $\ln R_0 \tau$ is on the order of 30 and is, due to its logarithmic form, very insensitive to changes in R_0 or τ. Hence, V_T is indeed well defined. The area of the hole as a function of T is then easily calculated by integrating the distribution Eq. (1) from $V = V_T$ to the maximum barrier height $V_{\max}$. The result is

$$A(T)/A_0 = 1 - \left(\frac{k \ln R_0 \tau}{V_{\max}} \right)^{1/2} T^{1/2} . \tag{6}$$

Here we have normalized the area to the value extrapolated to $T = 0$ K. We also assumed that $V_{\min}$ can be neglected compared to $V_{\max}$ and is smaller than kT, the thermal energies of the experiment. Equation (6) tells us that the area of a hole reduces with the excursion temperature T proportional to the square root of T in case the barriers are indeed distributed according to Eq. (1).

Equation (6) shows scaling properties in case the temperature is measured in units of $T^* = V_{\max}/k \ln R_0 \tau$. It is a universal law and data points from different samples should fall on the same plot irrespective of the difference in $V_{\max}$. Figure 1 shows data for a series of quite different glasses and dopand molecules. Equation (6) is indeed extremely well fulfilled. From these results we conclude that

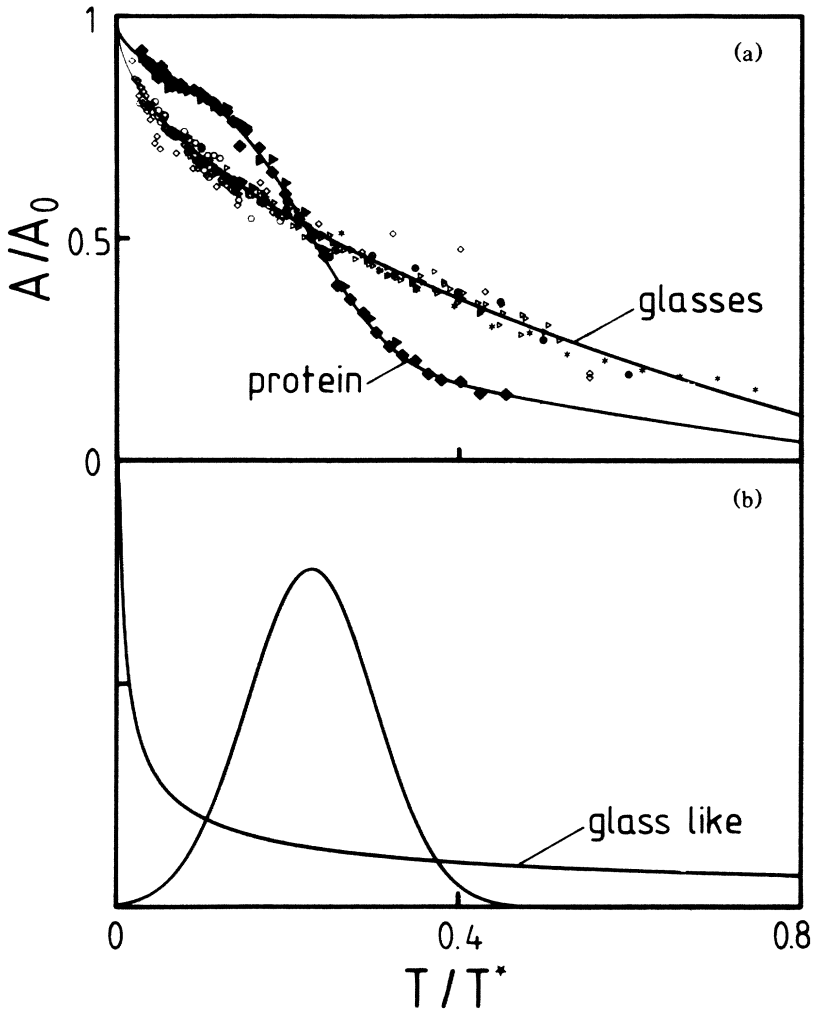

FIG. 1 (a) Temperature cycling experiments of a spectral hole burnt in a frozen protein solution (filled symbols) and in a series of doped glasses (open symbols). Plotted is the relative area A/A_0 as a function of a reduced temperature scale T/T^*. The solvent is saccharose saturated/phosphate buffer. (b) Probability distribution of conformational barriers of the investigated protein as derived from the experimental results of (a). The fit to the protein data in (a) is made by the superposition of a glasslike distribution $(1/\sqrt{V})$ and a Gaussian distributed, discrete feature.

(i) the barrier heights in polymers and glasses are indeed distributed according to $(1/V)^{1/2}$ and (ii) the distribution is continuous within the resolution of the experiment which is roughly 1 K.

It is an interesting problem to compare the above results for polymers and glasses with proteins: Proteins have many features in common with glasses. For instance, they show a linear specific heat at temperatures below 1 K (Refs. 7 and 8) and they also show a dielectric response which is characteristic for two-level systems (TLS). The TLS are the basic disorder modes of the glassy state.[6]

Their configurational space is immense and, hence, it is not surprising that the probability distribution of conformational states in a protein is smooth and may be similar to that of a glass. These glasslike states are due to local rearrangements of certain atoms or group of atoms. Examples are the rearrangement of hydrogene bonds and rotation of methyl or similar groups, etc. However, unlike glasses, proteins may, in addition, show discrete features in the probability distribution of conformational states which are superimposed the smooth glasslike distribution. There are conformational states which have a collective character in the sense that a transition into such a state requires the simultaneous rearrangement of many local groups. A transition of this kind could, for instance, be related to a rearrangement of a large part of a peptid chain or, generally speaking, to conformational changes in the secondary or tertiary structures of the proteins considered. These latter transitions are classified as "functionally important."[9,10] Whenever states of this sort are involved we expect pronounced changes in the density of states distribution because the corresponding barriers are expected to have rather well defined heights.

In Fig. 1, we compare the distribution of barrier heights for a protein with the glass data. The protein used are phycobilisomes from the cyanobacterium *Mastigocladus laminosus,* e.g., a chromoprotein complex composed of

many subunits functioning as light-harvesting antenna in photosynthesis. It contains several chromophores which absorb in the wavelength range between 530 and 680 nm. These chromophores can serve as intrinsic reporter groups within the native complex. Their absorption spectrum is shown in Fig. 2. The arrow marks the hole-burning position (15 267 cm^{-1}). The chromophore affected belongs to allophycocyanin. It is worthwhile to stress that in phycobilisomes the chromophores are well buried within the protein environment and much less accessible to solvent molecules than in individual biliproteins. The burning temperature T_b was 4 K. Burning was achieved with a cw laser of 300 $\mu W/cm^2$ within 45 min. Cycling experiments could be done up to temperatures of 60 K. Note that the holes in our experiments are always measured at the same temperature, namely, T_b, so that there is no influence on the parameters of the hole from dynamic effects such as phonon-scattering processes, etc. It is only the irreversible change of the matrix due to conformational transitions induced by the temperature cycle which shows up in the features of the hole.

From Fig. 1, two things are obvious: (i) The distribution of barriers for the protein sample is definitely different from the glass distribution, and (ii) there is sort of a step in this distribution which occurs around 30 K.

It is very interesting that the data of the protein can be almost perfectly fitted by assuming a glasslike distribution of barrier heights [Eq. (1)]

$$P_g(V) \sim (1/V)^{1/2}$$

to which a rather discrete feature with a Gaussian (G) distribution

$$P_G(V) \sim \exp[(V-V_0)^2/2\sigma^2]$$

is superimposed. Both distributions are shown in Fig. 1(b). The fit to the experimental data is shown in Fig. 1(a). From the fit the interesting parameters of the system can be determined. These are V_{max}, the maximum barrier height of the glasslike states, which is on the order of 2800 cm^{-1}; V_0, the average barrier height of the so-called discrete feature which might be related to a functionally important mode and which is on the order of 650 cm^{-1}; and σ, the width of the corresponding distribution which is 210 cm^{-1}. The values for V_{max} and V_0 fit into the scenery known from similar investigations on glasses and proteins.[1,2,9,10]

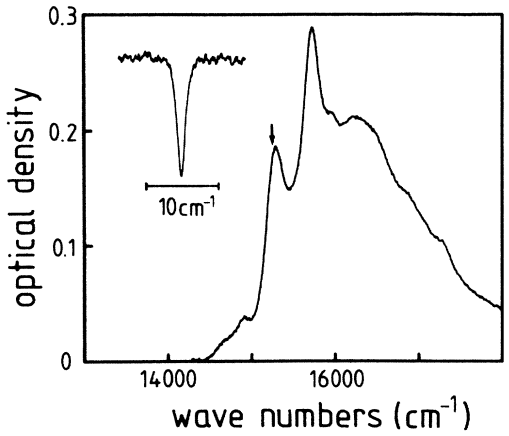

FIG. 2. Absorption spectrum of phycobilisomes of the algae *Mastigocladus laminosus*. Temperature: 4.2 K. The hole-burning position is marked with an arrow. The hole investigated is shown on an enlarged scale.

The origin of the width σ seems to be clear: Since a protein has so many glasslike conformational states, the barrier for the discrete feature depends on the actual substate the protein occupies.

We conclude this Rapid Communication by stressing that the conformational states were, in our case, populated by "photoreactive events." We do not know how the reaction proceeds but it is not unlikely that it occurs in the ground state mediated by radiationless energy release of the excited chromophores. We also treated the dissolved protein as sort of an independent state of matter decoupled from the glassy solvent. Regarding the size of the protein investigated and the fact that the burnt chromophores are buried within the protein,[11] this approximation seems to be justified.

In summary, we have shown that the distribution of conformational barriers, in proteins follows a glasslike behavior, i.e., a $1/\sqrt{V}$ distribution, but there are discrete structures superimposed.

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