Trehalose Effect on Low Temperature Protein Dynamics: Fluctuation and Relaxation Phenomena

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ABSTRACT We performed spectral diffusion experiments in trehalose-enriched glycerol/buffer-glass on horseradish peroxidase where the heme was replaced by metal-free mesoporphyrin IX, and compared them with the respective behavior in a pure glycerol/buffer-glass (Schlichter et al., *J. Chem. Phys.* 2000, 112:3045–3050). Trehalose has a significant influence: spectral diffusion broadening speeds up compared to the trehalose-free glass. This speeding up is attributed to a shortening of the correlation time of the frequency fluctuations most probably by preventing water molecules from leaving the protein interior. Superimposed to the frequency fluctuation dynamics is a relaxation dynamics that manifests itself as an aging process in the spectral diffusion broadening. Although the trehalose environment speeds up the fluctuations, it does not have any influence on the relaxation. Both relaxation and fluctuations are governed by power laws in time. The respective exponents do not seem to change with the protein environment. From the spectral dynamics, the mean square displacement in conformation space can be determined. It is governed by anomalous diffusion. The associated frequency correlation time is incredibly long, demonstrating that proteins at low temperatures are truly nonergodic systems.

INTRODUCTION

A central problem of spectral diffusion experiments on chromoproteins concerns the question of to what extent the chromophore, which serves as a probe for the conformational dynamics, is also sensitive to processes that occur close to the surface of the protein or in the host matrix (Fritsch et al., 1996, 1998; Thorn-Leeson et al., 1997). A straightforward way to solve this problem are comparative experiments between solutions of the same protein in different host glasses.

A glass-forming material with a very specific interaction with proteins is trehalose (Branca et al., 1999; Crowe et al., 1994, 1998; Green and Angell, 1989). Trehalose is a disaccharide that has a high H-bonding affinity, thus may replace the water molecules close to the protein surface. It has a rather high glass-transition temperature, much higher, for instance, than a glycerol/water glass (Green and Angell, 1989; Miller et al., 1999), which we use as a reference matrix. Hence, the naive expectation is that the spectral diffusion dynamics of a protein may slow down in a trehalose environment because the rigidity of the protein is increased, thus the number of conformational degrees of freedom is reduced.

To check this idea, we performed a series of low-temperature spectral diffusion experiments with a modified horseradish peroxidase protein in a trehalose environment. The native heme chromophore of the protein was replaced by mesoporphyrin IX, whose inner ring protons undergo a light-induced proton transfer reaction. This reaction is exploited for spectral hole burning. Horseradish peroxidase was selected because of its stability, known crystal structure (Gajhede et al., 1997) and central heme position. We have recently investigated this protein in a glycerol/buffer glass (Schlichter et al., 2000a). Thus, a comparison of its spectral diffusion behavior in glycerol/buffer and in the respective trehalose-enriched glass became possible.

The influence of trehalose on protein dynamics has been investigated by different groups (Gottfried et al., 1996; Hagen et al., 1995, 1996; Sastry and Agmon, 1997). Hagen et al. investigated CO-rebinding in myoglobin after flash photolysis. The kinetics in trehalose was found to be faster than in glycerol. This result was attributed to the high viscosity of trehalose, which prevents protein relaxation and thus keeps the barriers for rebinding low. Another interpretation was offered by Sastry and Agmon (1997), who argued that the sugar matrix preserves water in the heme pocket. Water molecules in the protein interior may retain a high internal flexibility and thus speed up the rebinding kinetics. Relaxation of the protein, in contrast, may not be hampered at all through the trehalose environment. As we will show, this latter view is also favored by our experiments.

EXPERIMENTAL

Mesoporphyrin IX substituted horseradish peroxidase was prepared and purified as described elsewhere (Paul and Stigbrand, 1970; Teale, 1959). The protein was dissolved in a saturated trehalose/buffer mixture at pH 8, which, in turn, was mixed with glycerol. Glycerol was necessary to ensure a sufficiently good glass quality. We investigated two samples to check a possible dependence of the results on the concentration of trehalose. In sample 1, the concentration of

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saturated trehalose/glycerol/buffer in the respective mixture was 40/50/10% v/v. In sample 2, the respective concentrations were 60/33/7% v/v. In the comparative experiment without trehalose, a glycerol/buffer mixture of 50/50% v/v was used (Schlichter et al., 2000a). The samples were quickly frozen to 4.2 K by plunging them into liquid Helium.

Hole burning was performed with a dye ring laser pumped by a frequency-doubled Nd:vanadate laser. Typical burn intensities were of the order of a few μW. During hole reading, the laser power was reduced by a factor of ~5000. We measured the spectral diffusion broadening σ of burnt-in holes as a function of waiting time t_w and aging time t_a. We define t_a as the time elapsed after the sample has reached its final temperature but before it is labeled with a hole. The first waiting/aging time experiment (Fig. 1 *A*) ran for about two weeks. During this time period, seven holes were burnt in a very narrow frequency range at different aging times reaching from 40 min to about 264 h. The second waiting time experiment (sample 2, Fig. 1 *B*) ran for about four days. In addition, we measured the so-called quasi homogeneous line width γ at 4.2 K.

The quantity measured in a spectral diffusion waiting-time hole-burning experiment is the change σ of the hole width as a function of time (Friedrich and Haarer, 1986; Breinl et al., 1984; Schlichter et al., 1999). σ is extracted from a line shape which is a convolution of the initial hole with the so-called spectral diffusion kernel which is the time-dependent part. To extract σ from the measured holes, a model is needed. Our data evaluation is based on the assumption that the diffusion kernel is a Gaussian (Skinner et al., 1999) with a variance σ^2. This assumption is quite in contrast to the TLS-model used to describe spectral diffusion in glasses, which predicts a Lorentzian diffusion kernel (Reinecke, 1979; Pack et al., 1990). The data evaluation procedure is described elsewhere (Schlichter et al., 2000a).

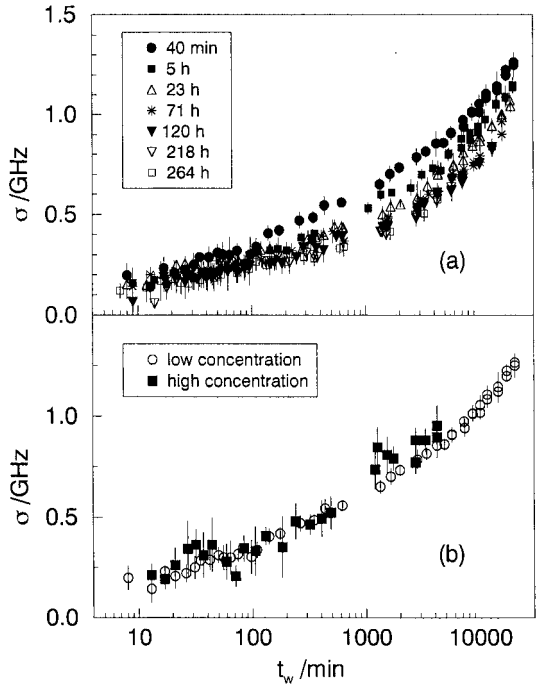

FIGURE 1. (*A*) Spectral diffusion width σ (square root of variance of the Gaussian diffusion kernel) as a function of waiting time t_w for various aging times (*insert*) in a semilogarithmic representation. Temperature 4.2 K. Sample: Mesoporphyrin IX-substituted horseradish peroxidase in a trehalose/glycerol/water glass. The overall width of the holes after burning ($t_w = 0$) was ~3 GHz. (*B*) A comparative experiment with a higher trehalose concentration.

RESULTS

Figure 1 *A* shows the width σ of the spectral diffusion kernel as a function of waiting time t_w for a series of aging times t_a at 4.2 K. Figure 1 *B* shows the comparative experiment where the trehalose concentration was changed from 40% to 60%. Three features should be stressed: First, in the representation of σ over log t_w, it is immediately obvious that $\sigma(t_w)$ does not increase proportional to log t_w. Otherwise, the data points should fall on straight lines. Second, spectral diffusion broadening is clearly subject to aging: as the aging times increase, σ becomes progressively smaller. However, the change of σ with aging time is much smaller than the respective change with waiting time. Third, an increase of the concentration of trehalose does not have a significant influence on spectral diffusion broadening: the data points for sample 1 and sample 2 (40% versus 60% trehalose concentration) measured at the same aging time fall on top of each other (Fig. 1 *B*).

Figure 2 shows the aging-time dependence of spectral diffusion broadening for the trehalose-enriched glass in comparison with the trehalose-free glass. In this experiment, the waiting time t_w was kept fixed at 10^4 min, and σ was

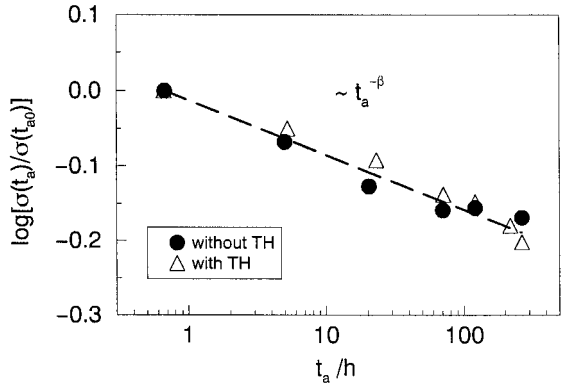

FIGURE 2 The decay of the spectral diffusion broadening with aging time t_a in a log-log representation measured at a waiting time $t_w = 10^4$ min. The data sets for the trehalose-enriched (*TH*) and for the trehalose-free glass follow the same power law. The respective coefficient $\beta = 0.07 \pm 0.01$. Note that the data points at the beginning of the aging experiments (t_{a0}) are arbitrarily normalized to 1.