

## Protein dynamics at low temperatures

J. Schlichter and J. Friedrich

*Lehrstuhl für Physik Weihenstephan, Technische Universität München, D-85350 Freising, Germany*

L. Herenyi and J. Fidy

*Institute of Biophysics, Semmelweis Medical University, Budapest, Hungary*

(Received 13 September 1999; accepted 10 November 1999)

The low temperature conformational dynamics of the heme type protein mesoporphyrin-IX-substituted horseradish peroxidase is investigated by spectral diffusion waiting time/aging experiments. Spectral diffusion broadening is governed by a power law in time. There is a small but significant aging effect. It is assumed that the conformational dynamics of the protein which leads to the spectral broadening of the burnt-in holes is governed by a diffusion type equation. In this case the shape of the spectral diffusion kernel is Gaussian. This model is contrasted with spectral diffusion phenomena as described by the TLS-model (TLS, two level system). © 2000 American Institute of Physics. [S0021-9606(00)50306-7]

### I. INTRODUCTION

Understanding the basic laws which govern the dynamics of a protein in its conformation space constitutes an important aspect in protein physics because conformational dynamics determines everything from folding to functioning. Most experimental methods for measuring conformational dynamics require the preparation of a specific nonequilibrium state of the protein under investigation. Conformational dynamics is then monitored via its influence on the relaxation of this nonequilibrium state back to equilibrium.

One of the best known examples is carbonmonoxy myoglobin.<sup>1-3</sup> After flashing off the CO-ligand from the prosthetic group, the resulting state of the protein is far from equilibrium. The back relaxation is highly nonexponential. This nonexponential relaxation feature is associated with the motion of the apoprotein in conformation space. The conformational motion changes the barrier between the photoproduct state and the ground state and, as a consequence, there is an accompanying change of the rate constant of the back reaction which modifies the relaxation of the prepared initial state.<sup>4,5</sup>

The most straightforward way to study conformational relaxation and fluctuations is spectral diffusion.<sup>6-8</sup> In spectral diffusion experiments the frequency fluctuations of absorptive or emissive transitions are measured. These fluctuations are directly associated with conformational motions.<sup>9</sup> In some cases, for instance in single molecule experiments, it is possible to measure frequency fluctuations directly and to determine the respective frequency autocorrelation function.<sup>10,11</sup> In ensemble averaged experiments, like hole burning, it is the width of the conditional probability distribution that a molecule absorbs at frequency  $\nu$  at time  $t$  given it absorbed at  $\nu_0$  at time zero, which is measured. This width is also directly related to the frequency autocorrelation function. The respective relation is obtained from measuring the width of the hole as a function of time (in the following referred to as “waiting time”  $t_w$ ). However, in order to extract the conditional probability from the measured hole

width, a model is needed for the basic physics which governs the conformational motion and the associated frequency fluctuations.

A well known model which has been widely used for describing structural changes and the associated frequency fluctuations in glasses and polymers<sup>12-15</sup> but also in proteins,<sup>15-20</sup> is the so-called TLS-model<sup>21,22</sup> (TLS stands for “two level system”). The model is based on the assumption that structural relaxation occurs in local double well potentials (the TLSs) associated with a rather small number of atoms. Under certain restrictive assumptions as to the coupling of the relaxation to the monitoring transition and the density of the TLS, the model makes precise predictions on the time evolution of the conditional probability which are accessible to experimental checks. The TLS-model leads to a Lorentzian line shape. Another, quite different approach, is the diffusion model.<sup>9,23,24</sup> In the diffusion model it is assumed that conformational dynamics is governed by diffusion processes. In this case, the appropriate equation for the time evolution of the conditional probability in conformation space is a Fokker-Planck-type equation. The implication on the shape of the conditional probability in frequency space which is the quantity measured (the so-called spectral diffusion kernel), is quite severe; this shape is Gaussian.

In this paper we report on low temperature spectral diffusion experiments with a modified heme type protein, namely mesoporphyrin IX-substituted horseradish peroxidase. We demonstrate that the TLS model which has been quite successful in explaining structural changes in glasses and polymers at sufficiently low temperatures, seems to be less appropriate for describing conformational dynamics in proteins.

### II. SPECTRAL DIFFUSION MODELS AND THEIR CHARACTERISTIC FEATURES

#### A. General aspects

The purpose of our spectral diffusion experiments is to characterize the specific features of conformational dynamics

of proteins. To this end it is important to make sure that the features observed are related to the protein under study and not to the host glass. We note that in horseradish peroxidase the chromophore is deeply buried in the protein, hence, is decoupled from the host glass to a high degree. We have verified the decoupling from the host glass experimentally for smaller heme-type proteins than horseradish peroxidase by comparing the spectral diffusion behavior of the chromoprotein with the isolated chromophore in similar host glasses.<sup>23,25,26</sup> We found qualitative differences. Similar results were obtained from comparative Stark-effect experiments.<sup>27,28</sup> If the chromophore is well decoupled in the smaller heme-type proteins, decoupling in larger proteins of this type, like horseradish peroxidase, is even better. As a result we can conclude that the dynamics probed by the present spectral diffusion experiments essentially reflects features of the protein under consideration and not of the host glass.

## B. The TLS-model

The TLS-model is based on a few essential assumptions; a homogeneously distributed spatial density of double wells, where only the two lowest states play a significant role. Two independent characteristic parameters, namely the energy splitting and the so-called tunneling parameter govern structural dynamics in the double wells. The density of states with respect to both parameters is constant.<sup>21,22</sup> The coupling of the double wells to the monitoring transition occurs via dipolar forces. If so, then the spectral diffusion line shape (i.e., the conditional probability distribution in frequency space) is Lorentzian<sup>29–32</sup> and the respective width broadens with time proportional to  $\log t_w$ .<sup>6,7,31,32</sup> It is especially the  $\log t_w$ -dependence which has been verified experimentally in many glassy systems<sup>6,7,32,33</sup> and which is considered as the signature of the tunneling model. Recently, deviations from the  $\log t_w$ -behavior has been found.<sup>13,14,34</sup> In one case it could be shown that these deviations were due to the fact that the TLS were not in thermal equilibrium.<sup>34</sup> In other cases they were related to TLS–TLS coupling.<sup>13,14,35</sup> TLS–TLS-coupling was predicted to play a role only at temperatures as low as millikelvin.<sup>35,36</sup> We note that even if the double well barriers are crossed via activated processes, the time dependence of the spectral diffusion width would not change significantly.

Clearly, some of the basic ingredients of the TLS-model are never met by proteins, for instance the homogeneous spatial distribution. Yet, this would only influence the line shape but not the time evolution of the width which is solely determined by the distribution of the relaxation rates.

## C. The diffusion model

The concept of the diffusion model is very different as compared to the TLS-model. It is assumed that motion in conformation phase space is a diffusive process where the whole protein is involved. If the protein is not in equilibrium, potential forces are superimposed on the diffusive forces, so that the appropriate equation for the conditional probability in conformation space is a Fokker–Planck-type equation. As

has been shown quite generally by Stephens *et al.*,<sup>37</sup> diffusive motion in conformation space can be directly related to the fluctuations in frequency space via the frequency auto-correlation function  $C(t)$ .  $C(t)$  gives rise to a time dependence in the line width  $\sigma(t)$  of the spectral diffusion kernel,

$$\sigma^2(t) = \sigma_0^2 [1 - C^2(t, \tau)], \quad (1)$$

where  $\sigma_0$  is the final width which the spectral diffusion kernel would reach after an infinite waiting time. As long as the conformational dynamics is sufficiently slow, the associated line shape is Gaussian.

$C(t)$  contains all the physics of the respective conformational diffusion processes. In the simplest case  $C(t)$  is an exponential implying a Gauss–Markov process for the frequency fluctuations. However, proteins are not that simple. Instead, as our experiments show, the correlation function is nonexponential. In this case, an appropriate ansatz for  $C(t)$  is a stretched exponential,

$$C(t) = \exp[-(t/\tau)^\alpha]. \quad (2)$$

$\tau$  is the frequency correlation time. The stretching parameter  $\alpha$  is a measure for the complexity of the energy landscape in which the protein is moving.

We stress that within the frame of the diffusion model the nonexponential correlation function is a homogeneous property of the system quite in contrast to the TLS-model, where nonexponential features arise from the heterogeneity of the TLS-ensemble.

## D. Aging effects

A very interesting aspect of spectral diffusion phenomena concerns sample aging. Whenever aging effects are present, one time parameter (e.g., the waiting time  $t_w$ ) is not enough to describe the phenomena. In addition to  $t_w$ , a so-called aging time  $t_a$  is introduced.  $t_a$  is the time elapsed after the sample has reached its final temperature but before it is labeled with a hole.

In the TLS-model, aging occurs whenever the two lowest states of the double well ensemble are not in thermal equilibrium. This yields an excess contribution to the spectral diffusion width which ceases as the ensemble comes closer to equilibrium.<sup>34</sup> In the diffusion model aging may occur as well. If the protein is far from equilibrium (at low temperatures this is almost always the case), there is, in addition to the frequency fluctuations, also relaxation. Relaxation may lead to a time dependence of the parameters involved, i.e., the correlation time  $\tau$ , the final width  $\sigma_0$  and the stretching parameter  $\alpha$ . However, since  $t_a$  is the same for all members of the ensemble of protein molecules, aging does not seem to change the shape of the spectral diffusion kernel. It should also be stressed that aging does not necessarily have to occur, even if the protein is far from equilibrium. Diffusion in conformational space can also attain a stationary character, as has been found, for instance, in cytochrome c.<sup>9</sup>

## III. EXPERIMENT

Holes were burnt with a CW dye ring laser in sulforhodamine to a relative depth of about 30%. The hole burning

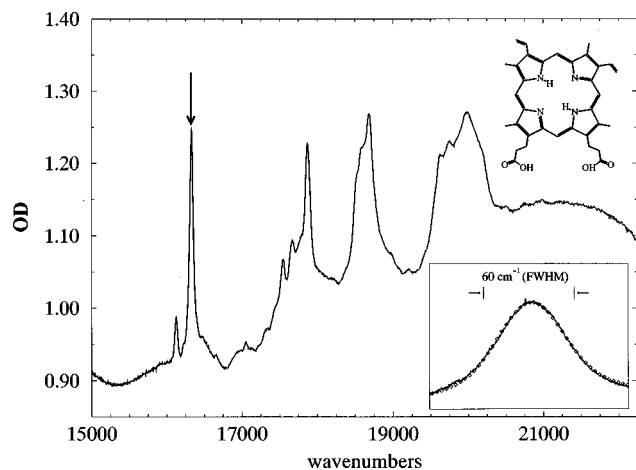


FIG. 1. Absorption spectrum of mesoporphyrin-IX-substituted horseradish peroxidase at 4.2 K. Hole burning was performed close to the maximum of the main origin (arrow). The insert shows that the shape of this band is well described by a Gaussian with a width (FWHM) of 60 wave numbers. Also shown is the chromophore, free base mesoporphyrin-IX.

reaction is a light induced transfer of the inner ring protons of the chromophore mesoporphyrin-IX.<sup>38</sup> Burning times and powers were of the order of 16 s and 1  $\mu$ W, respectively. Reading of the holes was performed in the transmission mode. The respective power was reduced by more than three orders of magnitude. The temperature of the experiment was 4.2 K. The sample was immersed in liquid helium and kept in a glass cryostat with a stand-by time of the order of 20 h. At 4.2 K, the homogeneous line width is about 1 GHz. The bandwidth of the laser light (<1 MHz) and even the respective frequency jitter are orders of magnitude less and can be neglected.

Data evaluation was performed in the following way: The holes were fit to Lorentzians. Then a Voigtian profile was calculated with the same width, whose Lorentzian component was taken from the very first hole. The Gaussian kernel of this Voigtian profile was taken as the spectral diffusion kernel. This procedure ensured an efficient data evaluation. We checked the accuracy of this procedure for a few holes by comparing the respective Gaussian kernels with those obtained from a direct fit of the hole to a Voigtian line shape. Agreement was always excellent. Apart from fitting the line shape also the hole depth was treated as a fit parameter to account for the significant decay of the holes due to the back reaction.

#### IV. RESULTS

Figure 1 shows the inhomogeneously broadened absorption spectrum of mesoporphyrin-IX-substituted horseradish peroxidase at 4.2 K. Hole burning was performed close to the maximum of the main peak at about 16 300 wave numbers (arrow). In order to estimate the inhomogeneous width, this peak is fit to a Gaussian (insert). The respective width (FWHM) is  $60 \pm 15$  wave numbers. The rather large uncertainty in the inhomogeneous width comes from the uncertainty in the baseline.

Figure 2 shows a log-log representation of the time evolution of the linewidth at 4.2 K for a series of spectral holes

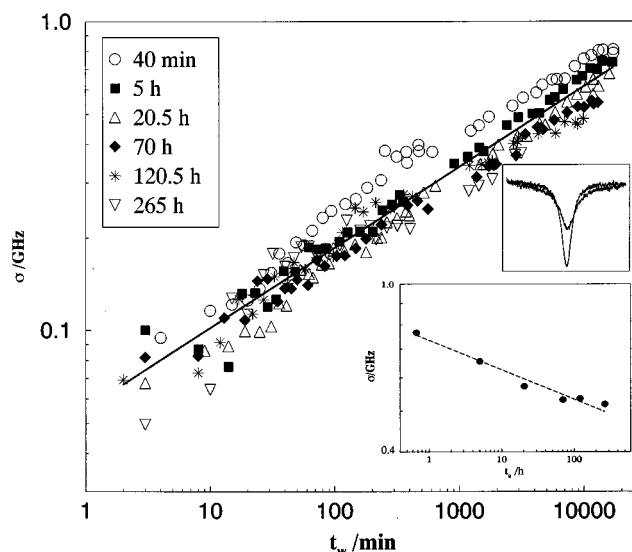


FIG. 2. Log-log representation of the spectral diffusion broadening  $\sigma$  as a function of waiting time  $t_w$  at 4.2 K for various aging times  $t_a$  as indicated by the different symbols. The solid line represents an average straight line fit. The respective slope is  $0.27 \pm 0.02$ . Holes are shown at the beginning and close to the end of a waiting time experiment. Another insert shows the aging behavior of the spectral diffusion width  $\sigma$  at a waiting time of  $10^4$  min, also in a log-log-representation.

burnt at different aging times  $t_a$ . Plotted is the square root of the variance  $\sigma(t_w)$  of the spectral diffusion kernel over the waiting time  $t_w$ . The span of the aging times covered more than two orders of magnitude. The actual numbers are shown in the insert. There are two clear-cut results: First,  $\sigma(t)$  does definitely not follow a  $\log t_w$ -law. Instead, within the error limit of the experiment, it seems to be governed by a power law in time. This result is independent of whether the spectral diffusion kernel is assumed to have a Lorentzian or a Gaussian line shape. If the data analysis is based on a Gaussian line shape, then the respective exponents is  $0.27 \pm 0.02$ .

Second, spectral diffusion broadening is subject to aging, although the respective amount is rather moderate. In the insert it is shown how the spectral diffusion width at a waiting time of  $10^4$  min decays as a function of  $t_a$ . Within about 200 h,  $\sigma$  decays by about 20%. The data suggest that this decay may also be governed by a power law.

#### V. DISCUSSION

##### A. Failure of the TLS-model

Disorder phenomena in proteins show up in almost all experimental investigations despite their high level of organization. Kinetic experiments show strong nonexponential behavior,<sup>1,2,3</sup> thermodynamic experiments show a low temperature specific heat linear in temperature,<sup>16,17</sup> x-ray scattering experiments show unusually large mean square displacements,<sup>1</sup> optical experiments show rather strong inhomogeneous line broadening, spectral diffusion broadening and thermal line broadening reminiscent to the respective behavior of glasses.<sup>39</sup> Several of these findings were interpreted on the basis of models which were quite successful in describing properties of glasses. For instance, the TLS model was used to describe the low temperature properties of pro-

teins such as the specific heat, dielectric relaxation as well as some of the spectral diffusion phenomena. A general result of this interpretation was that, if there are TLS in a protein at all, their number is small, namely on the order of one per protein.<sup>16,19</sup> As mentioned above, the signature of TLS-dynamics is a  $\log t_w$ -dependence of the spectral diffusion broadening. As can be inferred from Fig. 2, this is clearly not the case. The TLS model offers a few possibilities to account for a nonlogarithmic spectral diffusion behavior: The most straightforward possibility is to adapt the distribution function of relaxation rates in a proper way. This is almost always possible.

It has been argued in the literature<sup>40</sup> that a dye probe may itself be a perturbation of the lattice into which it is doped, hence, may create TLS-like excitations in its immediate neighborhood which follow a Gaussian distribution. Although this view may be correct in the case of glasses, it does not seem to be justified for proteins. There, the chromophore is not a dopant but is a natural constituent of the protein. Hence, adapting the distribution functions in this case has a touch of a fit routine.

Another way to account for the non-logarithmic behavior is considering a non-Boltzmann population of the TLS-levels.<sup>34</sup> In this case the time evolution would have two branches, an equilibrium branch and a nonequilibrium branch, both of which evolve logarithmically with time, yet with different slopes. The nonequilibrium branch would be subject to aging. As the aging time goes on, the onset of the nonequilibrium branch would shift with waiting time to  $t_w \approx t_a$ . For  $t_w < t_a$ , all data points would fall on the same plot because this time window characterizes the equilibrium branch. For  $t_w > t_a$ , there would be a bunch of parallel lines shifted along the  $\log t_w$ -axis by an amount determined by the aging times.

The data in Fig. 2 do not seem to support this view either. First, aging is a small effect, and second, at least the data series for the first three aging times do not seem to have a common equilibrium branch.

Finally, TLS–TLS coupling was shown to change the distribution of relaxation rates in a way that spectral diffusion broadening is governed by a power law instead of a  $\log t_w$ -law.<sup>13,14</sup> There are two reasons why we do not consider TLS–TLS coupling to play any role in the conformational dynamics of proteins. First, as mentioned above, the number of TLS, if present at all, would be of the order of one. Hence, there is no coupling as long as we assume that the proteins do not interact with each other. Second, TLS–TLS coupling is of a coherent nature and breaks down as the temperature increases beyond the milliKelvin range.<sup>36</sup>

Summarizing the problems with a TLS-like description of proteins, one could adopt two views, which we call the ‘‘one TLS-view’’ and the ‘‘many TLS-view.’’ The ‘‘one TLS-view’’ is based on the assumption that there are just a few TLS per protein, say one, but the parameters of this TLS differ from protein to protein. Hence, as the waiting time goes on, the proteins with the progressively slower TLS would come into play. This situation would be perfectly equivalent with a non-Boltzmann populated TLS-model which is not supported by our experiments as stated above.

The many TLS-view is based on a large manifold of the TLS per protein. If so, however, their rates cannot be independent because a protein is structurally highly correlated and, in addition, is quite small. These structural constraints would put constraints onto the dynamics of the TLS which would eventually be best described by a diffusive motion.

The conclusion is that the TLS-model does not seem to describe conformational dynamics in proteins in a proper way.

## B. The origin of the power law in the diffusion model

Our view of the basic physics of spectral diffusion in a protein is the following: At low temperatures the protein is far from equilibrium. This is already obvious from the ratio of  $kT$  to the inhomogeneous width, which covers in our case approximately one order of magnitude. In addition, within the experimental time scale of the order of  $10^7$  s, there is no observable tendency that spectral diffusion levels off. According to our view, spectral diffusion in deeply frozen proteins is governed by a diffusive motion in a rather complex energy landscape towards equilibrium. The time law which governs this diffusion process must somehow reflect this complex structure. We take care of the complex nature of the energy landscape by choosing a stretched exponential as an ansatz for the frequency autocorrelation function [Eq. (2)]. Since we argue that the protein is far from equilibrium, the experimental time has to be much smaller than the correlation time  $\tau$ . Hence, a short time expansion of  $C(t)$  is a good approximation. From Eq. (1), we get

$$\sigma_{SD}(t) = 2^{1/2} \sigma_0 \cdot (t/\tau)^{\alpha/2}. \quad (3)$$

This is the desired power law for the spectral diffusion width.<sup>24</sup> The present experiments are well described by  $\alpha/2 = 0.27 \pm 0.02$ . An important question concerns the final width  $\sigma_0$  which the spectral diffusion width would eventually reach after an infinitely long waiting time. A straightforward possibility is to identify  $\sigma_0$  with the inhomogeneous width (i.e., with the square root of the variance) of the transition considered. This identification complies with the diffusion model because the energy landscape which is sampled by the diffusing particle, is built from all kind of relevant interactions. In this case  $\sigma_0$  can be taken from the spectrum. It is  $60 \text{ cm}^{-1}/\sqrt{8 \ln 2}$ . Inserting this value into the above power law [Eq. (3), Fig. 2], we can calculate the frequency correlation time  $\tau$ .

$\tau$  is of the order of  $10^{16}$  min. Considering such a long correlation time there is no need to take into account that diffusion occurs in a finite system. As to  $\tau$ , it depends of course strongly on temperature. In cytochrome c, for instance, we assumed a power law  $\sigma_{SD} \propto T^\beta$  for spectral diffusion broadening and determined  $\beta$  from experiments at two different temperatures, namely, 100 mK and 4 K.<sup>9</sup>  $\beta$  turned out to be close to 1. Provided the same temperature dependence holds for horseradish peroxidase, we estimate from Eq. (3) a temperature dependence for  $\tau$  of the form,

$$\tau \propto T^{-\xi}, \quad (4)$$

with  $\xi$  being of the order of 3. It is clear that the dependence of  $\tau$  on temperature becomes much steeper at higher tem-

peratures. From many experiments it is well known that proteins undergo a dynamic transition at about 200 K, where large amplitude motions start to dominate the dynamics.<sup>41,42,43</sup> These large amplitude motions will certainly change the temperature dependence of  $\tau$  quite severely.  $\tau$  is related to the diffusion coefficient. In rough landscapes, at sufficiently high temperatures, the diffusion coefficient is quite often governed by an Arrhenius Law or by Ferry's Law,<sup>44,45</sup> which show very steep temperature dependencies. In both cases the individual diffusive step is activated. Recently, the frequency correlation time at room temperature could be measured in a single protein experiment. It is of the order of seconds.<sup>46</sup>

Finally we stress that within the frame of the diffusion model, the observed time law is a property of each individual protein and not of an ensemble. Hence, the associated dynamics is homogeneous in nature and not heterogenous.

### C. Aging

Within the frame of the TLS model there must always be aging whenever the waiting time exceeds the aging time and spectral diffusion broadening has not yet entered the saturation regime. In the diffusion model, there is no aging, provided that the parameters which determine the spectral diffusion width do not depend on aging time. This situation was, for instance, encountered in the case of cytochrome *c*.<sup>9</sup> However, in the present case, we observe aging, although the effect is rather moderate. Consequently, within the frame of the diffusion model, the respective parameters must depend on aging time. In principle, all three parameters in Eq. (3),  $\sigma_0$ ,  $\tau$ , and  $\alpha$  could depend on  $t_a$ . A time dependence of  $\alpha$  would imply a breakdown of the power law. The data in Fig. 2, however, suggest that the power law behavior is rather well documented. On the other hand, a time dependence of  $\sigma_0$  and/or  $\tau$  would affect the intercept in Fig. 2, only. In this case, aging would show up as a series of parallel lines in reasonable agreement with the experimental results.

### D. Microscopic aspects

The diffusion model is a phenomenological model. It does neither offer any insight into the nature of the microscopic processes which determines the frequency correlation time  $\tau$ , nor does it offer any explanation for the stretching parameter  $\alpha$ . Nevertheless, one can speculate about the physics which leads to the observed power law and the associated aging processes. We are considering diffusion in conformation space with many degrees of freedom. This space is for sure nonhomogeneous. Some of the atoms may easily change their position, others are quite tightly packed, so that diffusive motion is hindered. We may translate this situation into a diffusive motion of a fictive particle (with which we associate with protein as a whole) in a complex energy landscape. The structure of this landscape, i.e., the distribution of its features of roughness, determines the motion of the protein, i.e., determines the associated parameters  $\alpha$ ,  $\sigma_0$ , and  $\tau$ .  $\alpha$  is the "landscape parameter," which measures the devia-

tion of this landscape from homogeneity. Also  $\sigma_0$  is related to the irregular features of the energy landscape as they are reflected in inhomogeneous line broadening.

As compared to cytochrome *c*,<sup>9</sup> the present experiments are more complicated because we observe aging. We associated the occurrence of aging with a time dependence of  $\sigma_0$  and  $\tau$ . This implies that, in addition to the fluctuation processes, there are also relaxation processes. Both types of processes can occur on different time scales. One possibility of how this can be interpreted is via an energy landscape in a reduced coordinate space which itself changes with time. A time dependent energy landscape is based on the picture of an effective mean field of the local environment.

We introduce two time scales for the two processes which show up in our experiments, namely, aging and spectral diffusion. In a first approximation we consider the diffusing atoms or groups of atoms (or residues) in configuration space as moving independently in their local effective mean field which is determined by the neighboring groups of atoms. Note that as long as the particles are moving independently, this landscape is constant in time, implying a stationary spectral diffusion behavior. This view complies with x-ray scattering experiments,<sup>1,43</sup> where the individual residues are characterized by individual mean square displacements. The respective motion shows up in the frequency fluctuations which are observed in the spectral diffusion waiting time experiment. However, as time goes on, the motion of certain atoms may eventually trigger the motion of other "resting" atoms. Thus the effective energy landscape becomes time dependent.

That the coupled motion of the diffusing particles is necessarily correlated with a ceasing of the related fluctuations is obvious. The protein is moving towards its most stable structure. When the atoms get closer and closer to their optimum structural position the respective conformational barriers steadily increase until the optimum position is reached. Higher conformational barriers correspond with a smaller average amplitude of the frequency fluctuations, i.e., with a smaller  $\sigma_0$ , and with larger frequency correlation times  $\tau$ . In the optimum structural position, structural relaxation of an atom cannot anymore be triggered by another one, thus the effective energy landscape is fixed in time and steady state behavior has been reached. In other words, the extra contribution to the spectral diffusion broadening due to coupled motion dies away, as the sample ages.

We conclude this discussion with addressing possible relations of our low temperature experiments, to relaxation experiments at higher temperatures. Ansari *et al.*<sup>47</sup> studies the viscosity dependence of CO rebinding in MbCO and found a stretched exponential with a stretching parameter close to what we measured at 4 K. An experiment conceptually similar to our experiment was performed by Shibata *et al.*<sup>48</sup> on Zn-substituted myoglobin. They performed time resolved hole burning experiments measuring the line broadening and the line shift as a function of time between  $10^{-8}$  and  $10^{-2}$  s. They, too, described their results by a stretched exponential. In both cases it was found that the dynamics was controlled by the viscosity of the solvent which obviously puts constraints onto the motion of the protein. The

origin of the stretched exponential was attributed to a hierarchy of sequential cooperative motions similar to the model developed by Palmer *et al.*<sup>49</sup>

For our experiments the appropriate starting picture is that of a static energy landscape as discussed above. We attributed the origin of the stretched exponential and the concomitant anomalous diffusion to the inhomogeneity of conformation space. In a complex inhomogeneous space relaxation and fluctuation seem to be naturally governed by a hierarchy of processes. They can be viewed as a search problem of finding the optimum way to the final state. This optimization procedure sets the hierarchy scale. Easy steps are carried out first before the more difficult steps are tackled. As time goes on the fast processes (i.e., the easy steps) eventually contribute via entropy only,<sup>49</sup> but do not have a severe influence on the dynamics anymore. The occurrence of aging supports such a view even more, because, in this case the slower processes are triggered by the faster processes until they become so slow that aging eventually stops. Although the distance traveled by the protein in conformation space is extremely small at low temperatures, the respective diffusion physics shows parallels to the high temperature relaxation as it is also encountered in the final steps of protein folding.<sup>50</sup>

## VI. SUMMARY

Spectral diffusion broadening of optical holes burnt into the spectrum of free base mesoporphyrin-IX-substituted horseradish peroxidase was investigated over four orders of magnitude in time in waiting time experiments combined with aging time experiments. It does not follow a  $\log t_w$ -law. Instead, it seems to be governed by a power law, and there is a rather small but definite aging process going on. These results were interpreted in terms of a diffusion model quite in contrast to spectral diffusion phenomena in glasses which seem to be well described by the popular TLS-model. Some microscopic aspects as to where the observed anomalous diffusion and aging might come from are discussed.

## ACKNOWLEDGMENTS

The authors acknowledge support from the DFG (SFB 533), from the Hungarian-German collaboration program (UNG 005 97), from the Hungarian grants OTKA T25545 and MKM FKFP 1191/1997, and from the Fonds der Chemischen Industrie.

<sup>1</sup>H. Frauenfelder, F. Parak, and R. D. Young, *Annu. Rev. Biophys. Biochem. Phys. Chem.* **17**, 451 (1988).

<sup>2</sup>A. Ansari, J. Berendzen, D. Braunstein, B. R. Cowen, H. Frauenfelder, M. K. Hong, I. E. T. Iben, J. B. Johnson, P. Ormos, T. B. Sauke, R. Scholl, A. Schulte, P. J. Steibach, J. Vittitow, and R. D. Young, *Biophys. Chem.* **26**, 337 (1987).

<sup>3</sup>H. Frauenfelder, G. U. Nienhaus, and J. B. Johnson, *Ber. Bunsenges. Phys. Chem.* **95**, 272 (1991).

<sup>4</sup>N. Agmon and J. Hopfield, *J. Chem. Phys.* **79**, 2042 (1983).

<sup>5</sup>G. M. Sastry and N. Agmon, *Biochemistry* **36**, 7079 (1997).

<sup>6</sup>J. Friedrich and D. Haarer, in *Optical Spectroscopy of Glasses*, edited by I. Zschokke (Reidel, Dordrecht, 1986), p. 149.

<sup>7</sup>W. Breinl, J. Friedrich, and D. Haarer, *J. Chem. Phys.* **81**, 3915 (1985).

<sup>8</sup>W. Köhler and J. Friedrich, *J. Chem. Phys.* **90**, 1270 (1989).

<sup>9</sup>J. Schlichter, K.-D. Fritsch, J. Friedrich, and J. M. Vanderkooi, *J. Chem. Phys.* **110**, 3229 (1999).

<sup>10</sup>W. P. Ambrose, Th. Basche, and W. E. Moerner, *J. Chem. Phys.* **95**, 7150 (1991).

<sup>11</sup>P. D. Reilly and J. L. Skinner, *Phys. Rev. Lett.* **71**, 4257 (1993).

<sup>12</sup>S. Hunklinger and A. K. Raychaudury, in *Progress in Low Temperature Physics*, edited by D. F. Brewer (Elsevier, New York, 1986), p. 9.

<sup>13</sup>H. Maier, B. M. Kharlamov, and D. Haarer, *Phys. Rev. Lett.* **76**, 2085 (1996).

<sup>14</sup>H. Maier, B. M. Kharlamov, and D. Haarer, in *Tunneling Systems in Crystalline and Amorphous Solids*, edited by P. Esquinazi (Springer-Verlag, Berlin, 1998), Chap. 6.

<sup>15</sup>R. J. Silbey, J. M. A. Koedijk, and S. Völker, *J. Chem. Phys.* **105**, 901 (1996).

<sup>16</sup>G. P. Singh, H. J. Schink, H. v. Löhneysen, F. Parak, and S. Hunklinger, *Z. Phys. B* **55**, 23 (1984).

<sup>17</sup>I.-S. Yang and A. C. Anderson, *Phys. Rev. B* **34**, 2942 (1986).

<sup>18</sup>A. Heuer and P. Neu, *J. Chem. Phys.* **107**, 8686 (1997).

<sup>19</sup>A. Kurita, Y. Shibata, and T. Kushida, *Phys. Rev. Lett.* **74**, 4349 (1995).

<sup>20</sup>C. W. Rella, A. S. Kwok, K. D. Rector, J. R. Hill, H. A. Schwetman, D. D. Dlott, and M. D. Fayer, *Phys. Rev. Lett.* **77**, 1648 (1996).

<sup>21</sup>P. W. Anderson, B. I. Halperin, and C. M. Varma, *Philos. Mag.* **25**, 1 (1972).

<sup>22</sup>W. A. Phillips, *J. Low Temp. Phys.* **7**, 351 (1972).

<sup>23</sup>K.-D. Fritsch, A. Eicker, J. Friedrich, B. M. Kharlamov, and J. M. Vanderkooi, *Europhys. Lett.* **41**, 339 (1998).

<sup>24</sup>J. L. Skinner, J. Friedrich, and J. Schlichter, *J. Phys. Chem. A* **103**, 2310 (1999).

<sup>25</sup>K.-D. Fritsch, J. Friedrich, F. Parak, and J. L. Skinner, *Proc. Natl. Acad. Sci. USA* **93**, 15 141 (1996).

<sup>26</sup>D. Thorn-Leeson, D. A. Wiersma, K.-D. Fritsch, and J. Friedrich, *J. Phys. Chem. B* **101**, 6331 (1997).

<sup>27</sup>J. Gafert, J. Friedrich, and F. Parak, *Proc. Natl. Acad. Sci. USA* **92**, 2116 (1995).

<sup>28</sup>J. Gafert, J. Friedrich, J. M. Vanderkooi, and J. Fidy, *J. Phys. Chem.* **99**, 5223 (1995).

<sup>29</sup>A. M. Stoneham, *Rev. Mod. Phys.* **41**, 82 (1969).

<sup>30</sup>T. L. Reinecke, *Solid State Commun.* **32**, 1103 (1979).

<sup>31</sup>Y. S. Bai and M. D. Fayer, *Phys. Rev. B* **39**, 11 066 (1989).

<sup>32</sup>L. R. Narasimhan, K. A. Littau, D. W. Pack, Y. S. Bai, A. Elschner, and M. D. Fayer, *Chem. Rev.* **90**, 439 (1990).

<sup>33</sup>H. C. Meijers and D. A. Wiersma, *Phys. Rev. Lett.* **68**, 381 (1992); *J. Lumin.* **53**, 80 (1992).

<sup>34</sup>K.-D. Fritsch, J. Friedrich, and B. M. Kharlamov, *J. Chem. Phys.* **105**, 1798 (1996).

<sup>35</sup>P. Neu, D. R. Reichman, and R. J. Silbey, *Phys. Rev. B* **56**, 5250 (1997).

<sup>36</sup>A. L. Burin and Y. Kagan, *JETP* **80**, 761 (1995).

<sup>37</sup>M. D. Stephens, J. G. Sevian, and J. L. Skinner, *J. Chem. Phys.* **106**, 2129 (1997).

<sup>38</sup>J. Fidy, J. M. Vanderkooi, J. Zollfrank, and J. Friedrich, *Biophys. J.* **61**, 381 (1992).

<sup>39</sup>J. Friedrich, in *Methods of Enzymology*, edited by K. Sauer (Academic, San Diego, 1995), Vol. 246, p. 226.

<sup>40</sup>A. Heuer and D. Neu, *J. Chem. Phys.* **107**, 8686 (1997).

<sup>41</sup>F. Parak and G. Nienhaus, *J. Non-Cryst. Solids* **131**, 362 (1991).

<sup>42</sup>W. Doster, S. Cusack, and W. Petry, *Nature (London)* **337**, 754 (1989); *Phys. Rev. Lett.* **65**, 1080 (1990).

<sup>43</sup>B. F. Rasmussen, A. M. Stock, D. Ringe, and G. A. Petsko, *Nature (London)* **357**, 423 (1992).

<sup>44</sup>H. Frauenfelder and P. Wolynes, *Phys. Today* **47**, 58 (1994).

<sup>45</sup>R. Zwanzig, *Proc. Natl. Acad. Sci. USA* **85**, 2029 (1998).

<sup>46</sup>H. P. Lu, L. Xun, and X. S. Xie, *Science* **282**, 1877 (1998).

<sup>47</sup>A. Ansari, C. M. Jones, E. R. Henry, J. Hofrichter, and W. A. Eaton, *Science* **256**, 1796 (1992).

<sup>48</sup>Y. Shibata, A. Kurita, and T. Kushida, *Biochemistry* **38**, 1789 (1999).

<sup>49</sup>R. G. Palmer, D. L. Stein, E. Abrams, and P. W. Anderson, *Phys. Rev. Lett.* **53**, 958 (1984).

<sup>50</sup>J. D. Bryngelson, J. N. Onuchic, N. D. Socci, and P. G. Wolynes, *Proteins: Struct., Funct., Genet.* **21**, 167 (1995).