EVIDENCE FOR A CORRELATION BETWEEN THE PHOTOINDUCED ELECTRON TRANSFER AND DYNAMIC PROPERTIES OF THE CHROMATOPHORE MEMBRANES FROM *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Besides the study of the static structure of biologically important macromolecules, investigation of the dynamic properties has gained much interest in the last few years. X-ray diffraction on myoglobin crystals [1] revealed structural dynamics. Evidence of a large number of conformational substates of a molecule was found. Molecules in different conformational substates have the same gross structure but differ in local configurations.

To separate the static from the dynamic disorder in the myoglobin crystals, X-ray data and Mössbauer measurements on the heme iron of myoglobin were compared [1]. It was shown that these Mössbauer experiments themselves gave valuable information on the dynamic properties of the system [2]. The iron labels the motions of the molecule in a specific important place.

In contrast to the study of the dynamic properties on a well-known molecule like myoglobin, the investigations on membrane-bound proteins are much more complicated. Many systems are not described completely even with respect to their biochemical properties. Energy accumulation by photosynthesis demonstrates the importance of these molecules in nature. A large number of membrane-bound proteins involved in the electron transport after light absorption contain iron. The Mössbauer spectroscopy is, therefore, a valuable tool in the study of such systems, especially with respect to dynamic properties. X-ray investigations, as in [1], cannot be applied because the whole system cannot be crystallized. Despite the large complexity of such systems an interpretation of Mössbauer data can be given if one uses the results obtained on the dynamics of myoglobin. We have, therefore, investigated the Lamb-Mössbauer factor f' of ^{57}Fe incorporated in the membrane protein of chromatophores of the photosynthetic bacterium *Rhodospirillum rubrum*. The cells were grown on a medium enriched in ^{57}Fe and chromatophores were isolated according to a common procedure [3]. On samples derived from the same preparation dark reduction experiments on the photosynthetic apparatus have been performed.

2. Experiments and results

Fig.1 shows a nuclear γ resonance spectrum of the ^{57}Fe contained in chromatophores. Fig.2 gives $-\ln f'$ as a function of T. The Lamb-Mössbauer factor is $f' = \exp(-4\pi^2 < x^2 > /\lambda^2)$, where $< x^2 >$ is the mean square displacement of the absorbing nucleus, usually due to vibrations, and λ is the wavelength of the γ radiation employed. If the motions are fast compared with the decay time of the nuclear resonance level, $< x^2 >$ depends on the temperature. Except for very low temperatures the slope $d(\ln f')/dT$, is, in most cases, practically independent of T if no drastic changes of the system, like phase transitions, occur. This results in a linear dependence of $< x^2 >$ on T. Since the iron content of the sample was not determined, f' is obtained in relative units. As shown [4] f' is typically 0.8 at 4.2 K for iron-containing proteins. In order to get an estimate of the absolute $< x^2 >$ values of these data we have normalized our values to $f' = 0.8$ at 4.2 K and assumed a linear temperature

dependence between 4.2–160 K. This procedure neglects a non-linear temperature dependence in the region at <10 K, but it is, nevertheless, good enough to give the correct order of magnitude. At <170 K, the slope of $-\ln f'$, and hence the $<x^2>$-values, are of the same order of magnitude as observed in solids. But unlike the usual behaviour, the slope of the $-\ln f'$ curve increases drastically at >170 K in our case. This fact indicates that above this temperature a new channel of motions starts to contribute to the $<x^2>$-values.

Following [1] we separate $<x^2>$ into different contributions:

$$<x^2> = <x^2>_v + <x^2>_d + <x^2>_c \qquad (1)$$

the indices stand for vibrations (v), diffusion (d) and fluctuations between conformational substates (c). The onset of diffusion of the membrane system at low temperatures is very unlikely because of the large molecular mass of chromatophores. We may, therefore, neglect $<x^2>_d$ and attribute the new motional degree of freedom of the ^{57}Fe atoms to the term $<x^2>_c$. How can we visualize fluctuations between conformational substates? According to [1] one has strong arguments to suppose that macromolecules can exist in a large number of states having the same gross structure, but differ with respect to their local configurations. All conformational substates presumably have nearly the same energy. Here, fluctuations may occur between conformational substates of the membrane proteins and/or the membrane. The iron atoms serve as labels for these motions. As indicated by the unmodified nature of the nuclear resonance absorption spectra, the nearest neighbours of each iron atom are not influenced by the fluctuation process. A complex composed of the iron, together with its environment, moves in unison.

We had found a close correlation between the conformational mobility of the constituents of the bac-

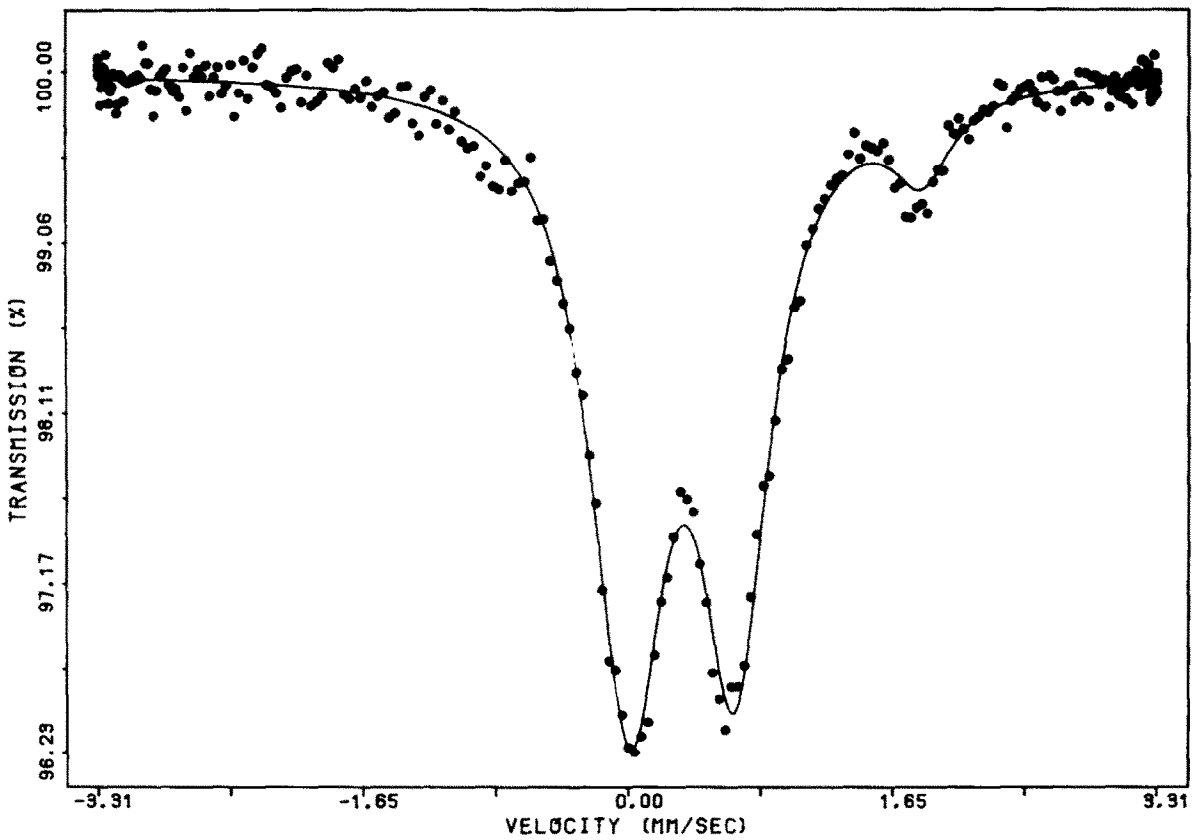

Fig.1. Nuclear γ resonance absorption spectrum of chromatophores of *Rhodospirillum rubrum* at $T = 250$ K.

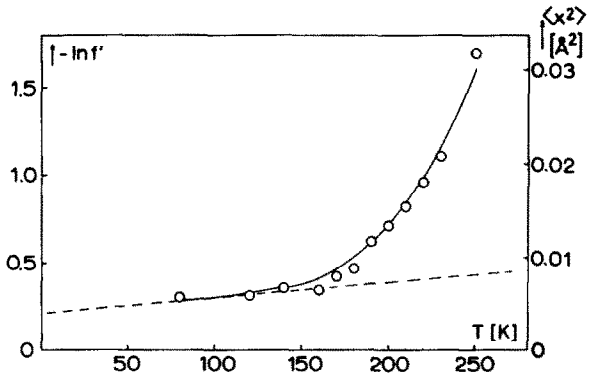

Fig.2. –ln of the Lamb-Mössbauer factor f' of Fe containing parts of the chromatophores of *R. rubrum* as a function of temperature. The values are normalized to $-\ln f' = 0.2231$ at 4.2 K: dashed line, assumed temperature dependence of $< x^2 >_V$; solid line, result of the least squares fit of $< x^2 >_C$. For details see the text.

terial photosynthetic membranes and their functional reaction capacity [5]. The latter can be easily followed by photo-induced optical absorbance change measurements. We now compare the efficiency of the stabilization of photo-separated electric charges between primary reactants in *R. rubrum* chromatophores and the dynamic properties determined from the Lamb-Mössbauer factor f'. The samples for the optical and the nuclear γ resonance experiments come from the same preparation.

Light activation of the photosynthetic apparatus in chromatophores of purple bacteria leads to the rapid separation of charges between protein-bound bacteriochlorophyll dimer (P) and the primary non-porphyrine acceptor (ferroquinone, A_1) in the reaction centers. The mobilized electron then passes to the secondary acceptor (A_2) also of a quinone type [6]. In the dark, the reversal of the photo-initiated electron transfer occurs if the constitutive donors for P^+ are chemically oxidized or lost during chromatophore isolation. These facts can be described by a simple scheme:

$$P \underset{\tau_{-1}}{\overset{\tau_1}{\rightleftarrows}} A_1 \xrightarrow{\tau_2} A_2 \qquad \tau_{-2} \qquad (2)$$

In *R. rubrum* chromatophores at a redox potential of the reaction medium of 350–400 mV and at 293 K, the slow P^+ reduction by A_2^- is observed (τ_{-2} = 4–6 s) after cessation of continuous actinic light of saturating intensity (λ_L = 700–1100 nm, 0.24 mcal $cm^{-1} . s^{-1}$). On lowering the temperature, the fast component in the dark recovery appears at ~230 K due to the back-

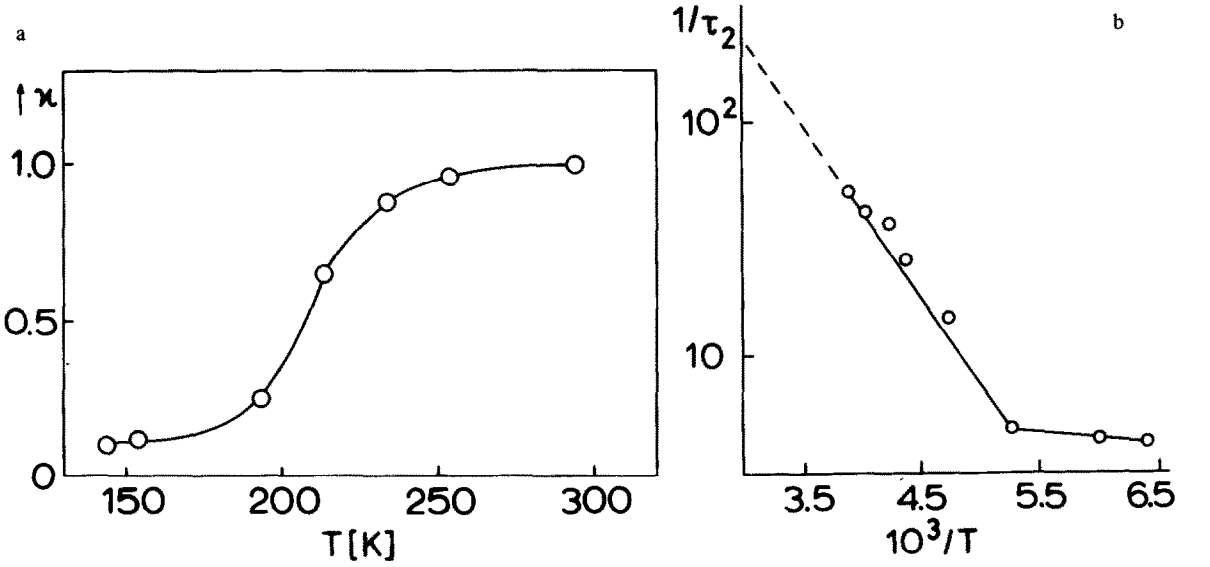

Fig.3. (a) Temperature dependence of the efficiency of the photo-induced electron transfer (κ) from the primary (A_1) to the secondary (A_2) quinone acceptors of the photosynthetic reaction centre in *R. rubrum* chromatophores. (b) Arrhenius plot for the rate constant $1/\tau_2$ of an electron transfer from A_1^- to A_2, calculated from the analysis of P^+ reduction kinetics by quinone acceptors after single turnover laser pulse activation of the chromatophores.

ward reaction of P^+ with A_1^- ($\tau_{-1} \approx 90$ ms). This is obviously the result of the inhibition of the direct electron transfer from A_1^- to A_2^- at cryogenic temperatures. The constants, τ_{-2} and τ_{-1} are hardly dependent on temperature; the contributions of the slow component, $I(\tau_{-2})$ and the fast component, $I(\tau_{-1})$, to the dark reduction of P^+, however, depend strongly on temperature. All these parameters can be obtained in optical experiments with great accuracy. The ratio $\kappa = I(\tau_{-2})/I((\tau_{-1}) + I(\tau_{-2}))$ reflects the efficiency of the charge photo-separation in the forward direction. Its temperature dependence is given in fig.3a. Based on these data, which are measured in the experiment with single turnover laser pulse activation of the reaction centers in chromatophores, one can also estimate the absolute values of $1/\tau_2$, calculating rate constants for the direct electron transfer from A_1^- to A_2 ($1/\tau_2$) by a method proposed [7] and now widely accepted [8]. The temperature dependence for $1/\tau_2$ thus obtained is given in fig.3b as an Arrhenius plot. One can see that the efficiency of the electron transfer in forward direction does not show any appreciable temperature dependence at <170 K; the $1/\tau_2$ values are rather low. Therefore, at low temperatures the removal of an electron from the P–A_1 pair in the direction of the secondary acceptor A_2 is significantly blocked. At >170 K, the electron transport efficiency rises remarkably with temperature. Under natural conditions, the rate constant $1/\tau_2$ of the direct electron transfer from A_1^- to A_2 becomes more than one order of magnitude higher than the rate constant for P^+–A_1^- recombination. Such an accordance of the rates of the elementary electron transfer reactions in the ordered chain of the intermediates enables the photosynthetic system to accumulate light energy with great efficiency.

A comparison between fig.2 and 3 shows a clear correlation between the temperature dependence of the mobility of the system and the efficiency of the electron transfer. Both increase dramatically at >170 K. To gain more insight into the physical nature of the mobility of the system, one may employ a very simple model which was already successfully used for the description of conformational substates in myoglobin crystals [2]. Let us suppose that the iron-containing system has only two conformational substates. The system has to overcome an energy barrier E going from substate 1 to substate 2. The iron moves during this process over a distance d. Substate 2 may differ from substate 1 by an energy Q. According to a proposal in [9], one can describe the term $<x^2>_c$ obtained from nuclear resonance absorption experiments by:

$$<x^2>_c = P_1 \cdot P_2\, d^2\, [1 - \exp(-(k_{12} + k_{21})\tau_N)] \quad (3)$$

P_1 and P_2 are the probabilities that this system is in substate 1 or 2, respectively. $P_2 = P_1 \exp(-Q/(k_B T)$ where k_B is the Boltzmann constant and T the temperature. τ_N equals 1.44×10^{-7} s; $k_{12} = 10^{13} \times \exp(-E/(k_B T))$ and $k_{21} = 10^{13} \times \exp(-(E-Q)/(k_B T))$. For details compare [2]. In order to separate $<x^2>_v$ and $<x^2>_c$, one may assume that $<x^2>_c$ is practically zero at <150 K and extrapolate the temperature dependence of $<x^2>_v$ from <150 K to higher temperatures. We can now perform a least squares fit of (3) to our experimental data. The free parameters are E, Q and d.

We can use the correlation of the temperature dependence of κ and of $<x^2>$ in order to restrict the set of free parameters. Even without a detailed knowledge of the physical nature of this correlation, it is reasonable to assume that the energy E of the potential barrier between the two substates 1 and 2 is approximately the energy which one obtaines from the Arrhenius plot of $1/\tau_2$. From fig.3b one obtains E = 0.14 eV. Using this value, a least squares fit of the $<x^2>_c$ values yields: $Q = 0.1$ eV and $d \approx 1.8$ Å. The fitted $<x^2>$-values are given by the solid line of fig.2. Although the two state model is certainly an oversimplification it illustrates the basic features of the process. The energies obtained from the fit seem reasonable. The characteristic distance d of the fluctuations is quite large, possibly reflecting a strong influence of the membrane on the molecule since for an isolated protein one should obtain much lower values [2].

3. Conclusions

The electron tunneling accompanied by conformational relaxation of macromolecular components results in the stabilization of the photo-mobilized electron. The intramolecular mobility is essential to provide transitions between different conformational states in the macromolecular structure.

The observed temperature dependence of the reduction process suggests the possibility of the electron

tunneling between photosynthetic electron carriers. Theories of electron transfer in biological systems have been widely discussed [11–14]. The mechanisms proposed involve the coupling of electronic transitions to vibrational motions of the reacting molecular complex. In our case the reduction of A_2 by such electron tunneling [7] evidently proceeds in two steps [15]:

(1) A_1 accepts the photo-mobilized electron and the reaction centre undergoes structural rearrangements to the new state preferable for the subsequent electron transfer to A_2. This transition is accompanied by an energy dissipation of ~0.3–0.4 eV, making the backward electron tunneling from A_1^- to P^+ rather improbable.

(2) The electron tunnels from A_1^- to A_2 with an energy loss of 0.1–0.2 eV.

Therefore, a total loss of ~0.6 eV should take place due to the difference between equilibrium redox potentials of P and A_2 [6]. Such relaxation losses in macromolecules are believed to proceed through several non-equilibrium states to the final conformation of the reaction centre with reduced A_2 [16,17].

It is not possible yet to correlate our f' measurements to a specific iron-containing protein of the membrane. In *R. rubrum* the candidates are: cytochromes *c* and *b*; iron–sulfur centres with ESR *g*-factors of 1.90 and 1.94; and the A_1 ferroquinone itself [10]. Even if we assume that the cytochrome components were washed out during the isolation procedure [7], we are left with the iron–sulfur centres and the ferroquinone. Thus the ^{57}Fe label, which was used to indicate the mobility within the whole chromatophore system, cannot be allocated. It may partly belong to the membrane-bound iron sulfur centres and partly to the ferroquinone in the photosynthetic reaction centre complex.

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