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The mobility of chromatophore membranes from *Ectothiorhodospira Shaposhnikovii* revealed by Rayleigh scattering of Mössbauer radiation (RSMR) experiments

Yu. F. Krupyanskii[1], D. Bade[2], I. V. Sharkevich[1], N. Ya. Uspenskaya[3], A. A. Kononenko[3], I. P. Suzdalev[1], F. Parak[4]*, V. I. Goldanskii[1], R. L. Mössbauer[5], and A. B. Rubin[3]

[1] Institute of Chemical Physics of the Academy of Sciences of the USSR, Moscow
[2] Max-Planck-Institut für Biochemie, Am Klopferspitz, D-8033 Martinsried, Federal Republic of Germany
[3] Biology Department, Moscow State University, USSR
[4] Institut für Physikalische Chemie der Universität Münster, Schloßplatz 4–7, D-4400 Münster, Federal Republic of Germany
[5] Physikdepartment E15 der TU-München, James-Franck-Straße, D-8046 Garching, Federal Republic of Germany

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Abstract. Chromatophores from *Ectothiorhodospira Shaposhnikovii* in solvents of different viscosity were investigated by RSMR experiments in the temperature range between 112 K and room temperature. Additional RSMR-experiments were done on solvents only. The mobility of the molecules and within the molecules is then given by the Debye-Waller factor which yields the mean square displacement, $\overline{\langle x^2 \rangle}$, averaged over the atoms in the system. The mobility of the atoms of the chromatophores roughly follows the mobility of the atoms of the solvents. At low temperatures the mobility of the chromatophores remains slightly larger than the mobility of the frozen solvent. At room temperature, however, $\overline{\langle x^2 \rangle}$ of the chromatophores remains significantly smaller.

Chromatophores in a glycerol-water mixture (0.001 M *Tris*-HCl buffer) and in water (0.05 M *Tris*-HCl buffer) show a different dynamic behaviour. A region with enhanced mobility near $T = 180$ K was indicated for the chromatophores in the glycerol-water mixture.

A correlation has been suggested between the rate of electron transfer from the primary to the secondary quinone and the increase of the conformational mobility of the chromatophores in glycerol-water mixture.

Key words: Chromatophores, *Ectothiorhodospira Shaposhnikovii*, RSMR

* To whom offprint requests should be sent
Abbreviations: Mb, Myoglobin; Met-Mb, Metmyoglobin

Introduction

Recently a large influence of the external viscosity on the binding rate of several ligands to the active site of myoglobin (Mb) molecules was observed by Frauenfelder and coworkers (Beece et al. 1980). This cannot be explained by the conventional transition state theory. Therefore, a dynamical model was suggested in which ligand binding is governed by ligand diffusion through the semi-liquid part of the protein. The surrounding medium governs the dynamics of a biomolecule, which in turn drives the kinetics of the ligand binding. The experiments described in this paper were done in order to show direct evidence that the viscosity of the medium influences the dynamics of a biomolecule.

The dynamic properties of biological systems can be investigated on a very detailed level. Information on the mean square displacement, $\langle x^2 \rangle$, of all non-hydrogen atoms has been obtained from X-ray experiments on myoglobin single crystals at various temperatures (Frauenfelder et al. 1979; Hartmann et al. 1982). Mössbauer absorption experiments have given information on the dynamic behaviour of single resonant nuclei in biological systems like myoglobin (Parak et al. 1981, 1982), hemoglobin (Mayo et al. 1981) or chromatophore membranes (Parak et al. 1980). Rayleigh scattering of Mössbauer radiation (RSMR) is not restricted to crystalline materials or single resonant nuclei. In fact, from RSMR measurements an average value for the mean square displacement, $\langle x^2 \rangle$, of atoms in very large systems can be obtained. The method takes advantage of the small energy width of Möss-

bauer radiation and allows one to separate the elastic fraction of the scattered radiation from single crystals (Butt and O'Connor 1967; Albanese and Ghezzi 1971) as well as from viscous solutions (Champeney and Dean 1975). In the case of myoglobin, RSMR data have been obtained from single crystals at various temperatures and from lyophilized material at different humidities (Krupyanskii et al. 1980a, 1982). The present paper reports RSMR experiments on much larger systems, chromatophores from Ectothiorhodospira Shaposhnikovii with a weight of about 10^7 Daltons.

Chromatophores are fragments of the functional membranes from photosynthetic bacteria. They are closed vesicles ($d \approx 600$ Å) which contain all necessary components to absorb light and convert it into a form of energy that is suitable to drive the photosynthetic process (Dutton et al. 1979). Kinetic data on electron transfer are usually obtained from experiments on chromatophores in glycerol-water solutions. Our work deals with the dynamics of chromatophore membranes and particularly takes into account the influence of the external viscosity. The dynamical behaviour of the chromatophores can then be compared with the kinetics of electron transfer processes.

Material and methods

Purple sulfur bacteria, *Ectothiorhodospira Shaposhnikovii,* were anaerobically grown under visible light at 30 °C on a Larsen medium. The photosynthetic membranes (chromatophores) were prepared as described by Samuilov and Kondrat'eva (1969). Solutions of chromatophores were used. The sample CH1 used water as solvent (0.05 M *Tris*-HCl buffer, pH 8.0). The solvent of sample CH2 was a 40% glycerol – 60% water mixtures (weight percent; 0.001 M *Tris*-HCl buffer, pH 7.5). After the experiments the samples were carefully dialyzed against water and freeze dried. The weight of the chromatophores in CH1 and CH2 was calculated from the weight of the dry samples: CH1 contained 267 mg and CH2 contained 375.2 mg chromatophores. Additionally, samples from different suspension media were investigated: BF1 contained pure 0.05 M *Tris*-HCl buffer, BF2 contained 40% glycerol and 60% water (weight percent) and GLY contained pure glycerol. The sample holder volume was about 1.5 ml. The size of the chromatophores was within the range 500 to 1000 Å. The composition of the chromatophores can be found in the literature (Luria 1960; Kondrat'eva 1968).

The experiments were performed with a 50 mCi ^{57}Co*Cr* source and a circular NaJ(Tl) scintillation counter adjusted to a scattering angle, $2\theta = 12°$ (Gaubman et al. 1981). The acceptance angle was $\Delta\theta = 5°$. The elastic fraction, f^{exp}, of the Rayleigh scattered intensity was determined by means of the conventional black absorber technique (Kroy and Vonach 1969). The black absorber consisted of $Li_3FeF_6 + (NH_4)F_3(FeF_3)_3$ with 7.0 mg/cm^2 of ^{57}Fe. The elastic fraction, f^{exp}, measured by a RSMR experiment is given by:

$$f^{exp} = \frac{\eta_{2\theta}(0)}{\eta_0(0)} \tag{1}$$

where $\eta(0)$ is given by:

$$\eta(0) = 1 - \frac{Z(0)}{Z(\infty)} \tag{2}$$

$Z(0)$ denotes the counting rate in the detector when the source is at rest while $Z(\infty)$ gives the counting rate if the source is moved with a rather large velocity, destroying the Mössbauer resonance in the black absorber. The index of η characterizes the position of the black absorber between the source and the sample, (0), or between the sample and the detector, (2θ). A correction for collimator scattering was performed by a procedure similar to the one proposed by Champeney and Dean (1975). First the scattering fraction from an empty sample holder was determined experimentally, and then the correction for the absorption in the real sample was taken into account by multiplication with the appropriate absorption factor. The low temperature measurements were performed with the sample inserted into a copper rod which was connected to a copper container filled with dry ice or liquid nitrogen. The temperature of the sample could be continuously adjusted between 100 K and room temperature by means of an electric heating and temperature control system (Shchukin et al. 1976). The temperature measurement was done by a chromel-alumel thermocouple touching the sample holder. An independent temperature calibration was taken from RSMR experiments on acetone and destilled water. These solutions show a steep decrease of the inelastic fraction, f^{exp}, in a very narrow temperature interval near the melting point (at $T = 178$ K and $T = 273$ K, respectively). The observed small temperature interval with the steep decrease of f^{exp} proved that the temperature gradient over the sample holder was less than 3 K.

Results

In the following we give the experimentally obtained f^{exp} values for the different samples. The index at f^{exp} refers to the measured sample. The experi-

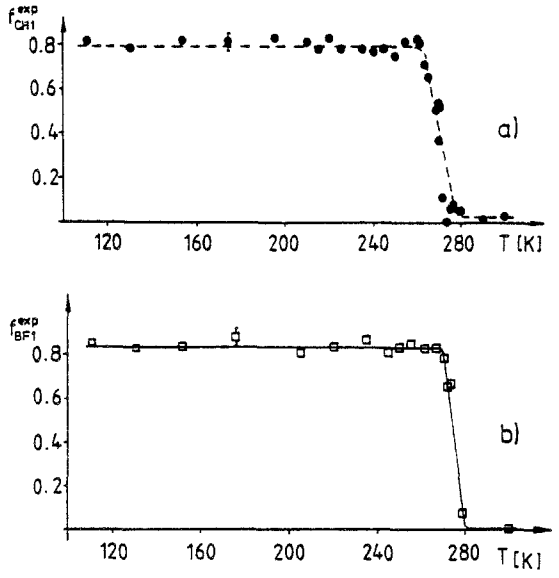

Fig. 1. The elastic fraction f^{exp} for a chromatophores in 0.05 M *Tris*-HCl (HCl (●)) and b 0.05 M *Tris*-HCl (BF1 (□)), (═══) least squares fit of f^{cal} according to Eq. (3) yielding the parameters given in Table 1. All data had the same error bars as indicated at 175 K. The error bars of the measurements at 300 are, however, a factor 2 smaller

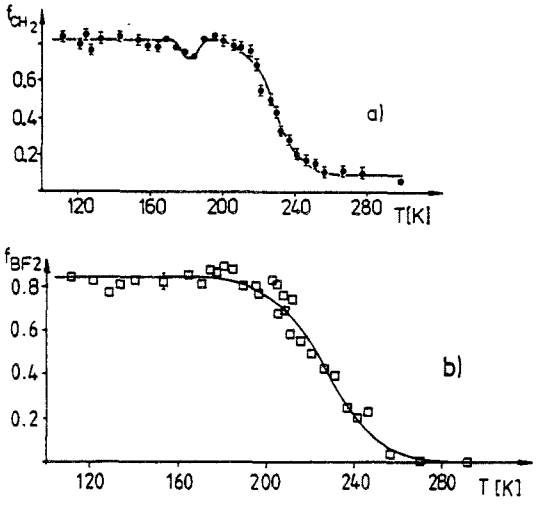

Fig. 2. The elastic fraction f for a chromatophores in 40% glycerol-60% water (CH2 (●)) and b 40% glycerol-60% water (BF2 (□)), (——) least squares fit of f^{cal} according to Eq. (3) yielding the parameters given in Table 1. In curve a the experimental data were measured with different statistics. Where no error bars are given the error corresponds to the diameter of the circles

mental results f^{exp}_{CH1} and f^{exp}_{BF1} from the samples CH1 and BF1 are given in Fig. 1 a, b. The results from the samples CH2 and BF2 are shown in Fig. 2 a, b while in Fig. 3 data for 100% glycerol are given. The qualitative behaviour of f^{exp}_{CH}, f^{exp}_{BF} and f^{exp}_{GLY} is rather similar.

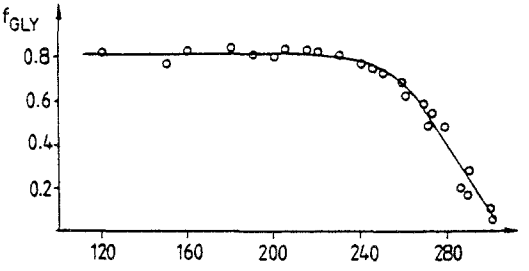

Fig. 3. The elastic fraction f for pure glycerol (GLY (○)), (——) least squares fit of f^{cal} according to Eq. (3) yielding the parameters given in Table 1

Starting from low temperatures f^{exp} is practically constant (with the exception of f^{exp}_{CH2}) within the experimental accuracy. Above a certain temperature, f^{exp} decreases rapidly to an unmeasurably low value. This overall behaviour suggests the description of the experimental data, f^{exp}, by an empirical function, f^{cal}, with a minimum set of variable parameters. One may use a function which is constant at high and low temperatures, thus representing average values of f in these temperature regions; (HT) characterizes the high temperature and (LT) the low temperature range. The decrease from the LT-region to the HT-region is assumed to be exponential:

$$f^{cal} = f(\mathrm{HT}) + [f(\mathrm{LT}) - f(\mathrm{HT})]/ [1 + \exp((T - T_0)/\Delta T_0)]\,. \qquad (3)$$

T_0 gives a characteristic temperature where f^{cal} decreases to $(f(\mathrm{LT}) + f(\mathrm{HL}))/2$ and ΔT_0 is a parameter for the width of the transition.

Eq. (3) evidently neglects a small increase of f in the temperature range from 170 K to lower temperatures. This increase is characteristic for all solids and reflects the decrease of the mean square displacements because of the freezing of acoustical and optical vibrations. The error bars of the data do not allow an unambiguous determination of the slope of f^{cal}. Moreover, the details of this temperature region are beyond the interest of the present discussion. We, therefore, feel that the function according to Eq. (3) is appropriate for our purpose.

A least-squares fit of the function f^{cal} to the experimental data then yields the lines in Fig. 1 to Fig. 3. The resulting fit parameters $f(\mathrm{HT})$, $f(\mathrm{LT})$, T_0 and ΔT_0 are given in Table 1. The chromatophores in glycerol-water solution indicate an unusual deviation from the simple fit at about 180 K. This deviation, which is slightly outside of the statistical error of the measurement can be empirically approximated by the addition of a symmetrical Lorentzian line with $\Delta T_l = 11$ K (FWHM) and $\Delta f = -0.09$ centered at $T_l = 181$ K.

Table 1. Results of the fit of f^{cal} according to Eq. (3) to the experimental data f^{exp} from CH1, BF1, CH2, BF2 and GLY.

Sample	f [LT]	f [HT]	T_0	ΔT_0
CH1	0.79 ± 0.01	0.02 ± 0.03	271 ± 0.5	1.8 ± 0.4
BF1	0.83 ± 0.01	$< 10^{-3}$	275 ± 0.5	1.3 ± 0.2
CH2	0.82 ± 0.01	0.09 ± 0.02	227 ± 1	7.4 ± 0.8
BF2	0.84 ± 0.01	$< 10^{-3}$	227 ± 2	12.0 ± 1.5
GLY	0.82 ± 0.06	0.05 ± 0.01	286 ± 3	15.0 ± 1.2

The indication of a similar behaviour was found in chromatophores of Rhodospirilium Rubrum (Krupyanskii et al. 1981).

Discussion

A comparison of the experimental data f_{CH}^{exp} and f_{BF}^{exp} in Figs. 1 and 2 shows directly that the mobility of the chromatophores roughly follows the mobility of the pure surrounding media. This fact is displayed in more detail in Table 1 which gives the parameters resulting from the least squares fit of Eq. (3) to the experimental data. The parameters T_0 are rather similar for chromatophores in solution and for the pure solvent (compare CH1 and BF1 or CH2 and BF2). Comparing the ΔT_0 values one has to take into account a small temperature gradient of about 3 K over the sample and, for CH2 and BF2, differences in the HT-region. Quantitative differences are revealed by the parameters f(HT) and f(LT). The value of f(LT) for CH1 and CH2 indicates that the mobility of the chromatophores in the low temperature range is slightly larger than the mobility of the solvent. At high temperatures, f(HT) approaches zero for both solvents while CH1 and CH2 yield $f(\mathrm{HT}) > 0$. Therefore, the average mobility of the chromatophores at room temperature is considerably smaller than the mobility of the solvent.

An unexpected decrease of f^{exp} near $T = 180$ K was indicated in CH2 but is absent in BF2, BF1 and CH1. This suggests that the mobility change at this point is not caused by the chromatophores alone. It may be taken as an indication for a phase transition in the chromatophore-solvent system. A similar behaviour of the chromatophores near 180 K was observed in specific heat measurements on chromatophores in glycerol-water (Knox et al. 1982), where a phase transition was also observed.

The data from the chromatophores in suspension reflects the average mobility of a system with at least three components: the chromatophores, the water bound to the chromatophores and the solvent. In order to analyse the different contributions one has to carry out a calculation similar to the one described by Krupyanskii et al. (1980b). It is based on the fact that the various components of the sample scatter incoherently. Therefore, one has to deal with the intensities scattered from the different atoms. We obtain the elastic fraction of the scattered Mössbauer quanta if we sum the elastically scattered intensity from all the components in the sample and divide that sum by the total scattered intensity. The scattered intensity is proportional to the number of atoms n_i of type i in each component and the scattering factors f_i^2 and F_i for elastic scattering and Compton scattering, respectively. One obtains:

$$f^{theo} = \frac{\sum_c \{u_c \sum_i n_i^{(c)} f_i^2 \exp(-2W_c)\}}{\sum_c \{u_c \sum_i n_i^{(c)} (f_i^2 + F_i)\}} \quad (4)$$

$n_i^{(c)}$ gives the number of the atoms i in one molecule (for one unit) of the component c while u_c gives the relative number of molecules (or units) of the component c hit by the beam of the γ-source ($\sum_c u_c = 1$). Eq. (4) assumes that the Debye-Waller factor $\exp(-2W_c)$ is the same for all atoms within one component. The values u_c and $n_i^{(c)}$ can be determined from the weight fractions and the composition of the different components and f_i^2 and F_i are taken from the literature (International Tables on X-ray Crystallography).

In our samples CH1 and CH2, we distinguish three components, c: the chromatophore membranes m, the bound water b and the free solvent s. The letters m, b and s refer to these components if used as indices instead of c. The amount of water bound to the chromatophores is unknown. We have taken it as one third (in weight) of the dry chromatophores. Then we can calculate u_m, u_b and u_s from the weight of the dry and wet samples. Eq. (4) can also easily be used for the calculation of f-values expected for the pure glycerol sample, GLY, and the samples BF1 and BF2. Here the $\sum_c$ vanishes since we treat these samples as a one component system. The chemical composition is taken into account by n_i. The index $\sum_c$ of Eq. (4) becomes s for the sample BF1 and BF2 and GLY for the sample GLY. In this way we identify the scattering of BF1 and BF2 with the scattering of the pure solvent in CH1 and CH2.

Using Eq. (4) we can now calculate values for f_{CH}^{cal} for several limiting cases of the chromato-

Table 2. Calculated values f_{CH}^{theo} according to Eq. (4) for the assumptions
(ia) $\exp(-2W_m) = 1$, $f_b^{exp} = f_{GLY}^{exp}$
(ib) $\exp(-2W_m) = 1$, $f_b^{exp} = f_{BF1}^{exp}$
(ii) $\exp(-2W_m) = \exp(-2W_s)$, $f_b^{exp} = f_{BF1}^{exp}$
(iii) $\exp(-2W_m) = 0$, $f_b^{exp} = f_{BF1}^{exp}$
compared to experimental results f_{CH}^{exp} for chromatophores in different solvents (iv)

Sample	T	Calculated f_{CH}^{cal}				Experimental f_{CH}^{exp}
	[K]	(ia)	(ib)	(ii)	(iii)	(iv)
CH1	140	0.854	0.853	0.828	0.664	0.79
CH2	140	0.873	0.872	0.832	0.600	0.83
CH1	300	0.190	0.196	$<10^{-3}$	$<10^{-3}$	0.02
CH2	300	0.273	0.281	0	0	0.09

phore mobility. In order to show the sensitivity of the method and the significance of our results we discuss three cases: i) $\exp(-2W_m) = 1$, ii) $\exp(-2W_m) = \exp(-2W_s)$ and iii) $\exp(-2W_m) = 0$. This corresponds to the values for f_{CH}^{exp} to be expected if the chromatophores are i) classically rigid, ii) behaving like free water and iii) are very mobile even at low temperatures. The calculation needs data for W_s and W_b. The values of W_s can be determined from f_{BF1}^{exp} together with Eq. (4). Values for f_b^{exp} yielding W_b are presently not available. There is, however, evidence that the bound water has dynamic properties different from free water (Singh et al. 1981). From NMR and EPR experiments with spin labels on proteins (Likhtenstein 1974) one may suggest that the dynamic behaviour of the bound water is very similar to 100% glycerol solution. We, therefore, include in our calculation the two possibilities: ia) W_b is obtained from $f_b = f_{GLY}^{exp}$ and ib) W_b is obtained from $f_b = f_{BF1}^{exp}$ which is identical with the assumption $W_b = W_s$. Calculated f_{CH}^{cal} values for the different cases are compared with experimental f_{CH}^{exp} values in Table 2. It shows that the chromatophores are far from being classically rigid (compare i) to iv)). The treatment of the bound water as glycerol or free water yields significant differences only at higher temperatures (compare ia) with ib)) which however, do not significantly influence the following discussion. As the most important result Table 2 shows that the chromatophores are slightly more mobile than frozen bulk water at low temperatures (compare ii) to iv)) and far less mobile than liquid water at room temperature (compare iii) to iv)).

We may now quantify the dynamic mobility of the chromatophores by taking the actual experimental results f_{CH}^{exp} and calculate the mean square displacement $\langle x_m^2 \rangle$ of the membranes. For that purpose one has to remember that

$$2W_c = (4\pi \sin\theta/\lambda)^2 \overline{\langle x_c^2 \rangle} \tag{5}$$

holds. Here, $\overline{\langle x_c^2 \rangle}$ is the mean square displacement averaged over all atoms of the component c and $\lambda = 0.86$ Å for ^{57}Fe, 2θ denotes the scattering angle. In order to get $\overline{\langle x_m^2 \rangle}$ one has to resolve Eq. (4) for the quantity $\exp(-2W_m)$. Using the compositions of our samples, equations for $\exp(-2W_m)$ are obtained which linearly depend on the experimentally obtained values f_{CH1}^{exp}, f_{CH2}^{exp}, f_{BF1}^{exp}, f_{BF2}^{exp} and f_{GLY}^{exp}. For the sample CH1 one obtains

$$\exp(-2W_m) = 5.266 f_{CH1}^{exp} - 0.4253 f_{GLY}^{exp} - 3.697 f_{BF1}^{exp} \tag{6a}$$

or

$$\exp(-2W_m) = 5.266 f_{CH1}^{exp} - 0.4253 f_{BF1}^{exp} - 3.697 f_{BF1}^{exp}. \tag{6b}$$

Eq. (6a) assumes that the bound water behaves like glycerol while Eq. (6b) treats the total amount of water as free water. For the sample CH2 we have obtained

$$\exp(-2W_m) = 3.66 f_{CH2}^{exp} - 0.4253 f_{GLY}^{exp} - 2.193 f_{BF2}^{exp} \tag{7a}$$

or

$$\exp(-2W_m) = 3.66 f_{CH2}^{exp} - 0.4253 f_{BF2}^{exp} - 2.193 f_{BF2}^{exp}. \tag{7b}$$

Again Eq. (7a) treats the bound water as equivalent to glycerol while Eq. (7b) makes no distinction between bulk solvent and bound solvent. Together with Eq. (5) it is now possible to calculate the mean square displacement $\overline{\langle x_m^2 \rangle}$, averaged over all atoms of the chromatophores obtained in the two samples. Mean square displacements, $\overline{\langle x_{BF}^2 \rangle}$ for the pure solvent can also be obtained from f_{BF}^{exp} together with Eqs. (4) and (5). Results are given in Table 3. The values for $\overline{\langle x_m^2 \rangle}$ in Table 3 can be compared to the corresponding data obtained from Mössbauer absorption experiments on chromatophores from *Rhodospirillum Rubrum*. Parak et al. (1980) found $\langle x^2 \rangle$ of ^{57}Fe in the chromatophores to be considerably smaller in frozen solution ($\langle x^2 \rangle = 0.03$ Å at 250 K and $\langle x^2 \rangle = 0.01$ Å at 180 K). In contrast to RSMR data, which averages over the whole system, Mössbauer absorption spectroscopy on ^{57}Fe labels the mobility of the system at the position of the iron. In the chromatophores the iron belongs to the protein part of the biological membrane, which amounts to about 40%. It is easy to imagine that the proteins are more rigid than the lipid bilayer. The comparison of

Table 3. Mobility in the solvent and the chromatophores. For CH1 and CH2 the values exp $(-2W_m)$ in brackets were obtained by using Eqs. (6b) and (7b) respectively instead of Eqs. (6a) and (7a). The values in brackets treat the whole solvent as free and unbound

Sample	T[K]	exp $(-2W_s)$	$\langle x_s^2\rangle$ [Å^2]
BF1	140	0.853	0.07 ± 0.03
BF2	140	0.867	0.06 ± 0.03
BF1	300	$< 10^{-3}$	> 3
BF2	300	0.002	> 2.7
		exp $(-2W_m)$	$\langle x_m^2\rangle$ [Å^2]
CH1	140	0.689 (0.686)	0.16 ± 0.04
CH2	140	0.805 (0.802)	0.09 ± 0.03
CH1	300	0.085 (0.116)	1.0 ± 0.4
CH2	300	0.298 (0.328)	0.52 ± 0.06

the RSMR data with the results of Mössbauer spectroscopy yields, therefore, no contradiction. Even within a protein molecule the mobility differs from part to part. For myoglobin we can also perform a comparison of Mössbauer absorption spectroscopy and RSMR data. While the ^{57}Fe atom in metmyoglobin crystals labelled a dynamic mean square displacement of $\langle x^2\rangle^\gamma = 0.06$ Å^2 at room temperature (Parak et al. 1981), RSMR yielded $\langle x^2\rangle^R = 0.74$ Å^2 for concentrated solution and $\langle x^2\rangle^R = 0.22$ Å^2 for met-Mb crystals (Krupyanskii et al. 1980b). A comparison of RSMR data obtained at 300 K for chromatophores and myoglobin crystals shows that chromatophores are clearly more flexible than a protein molecule. This difference is obviously caused by the lipid bilayers. The addition of glycerol drastically reduces the average flexibility at room temperature which is, however, still larger than the average mobility of a myoglobin molecule.

Finally one has to stress that at low as well as at high temperatures $\langle x_m^2\rangle$ is considerably smaller in CH2 than in CH1. This possibly shows that the water molecules do not interact with the chromatophores as strongly as the glycerol. The stabilisation of the chromatophores by the glycerol-water mixture is particularly evident from $\langle x_m^2\rangle$ at room temperature. In conclusion it must be emphasized that the glycerol-water solution strongly influences the dynamic properties of the chromatophores in the whole temperature range.

We now compare our experimental results with electron transfer data on chromatophores. In recent years many models have been developed for the correlation between the electron transfer process in chromatophores and their dynamic mobility. It should be noted in this context that the kinetic data of chromatophores of *Ectothiorhodospira Shaposhnikovii* were obtained from chromatophores in a glycerol-water mixture (Chamorovsky et al. 1976, 1980). Therefore, changes in the dynamical properties of chromatophores in glycerol-water compared to chromatophores in water are essential, and a comparison between the dynamic properties of chromatophores and the rate of electron transfer must be done for CH2.

Figure 4 gives the rate constant k_{A1A2} of the electron transfer from the primary to the secondary acceptor between 220 K and 260 K. The measurements were performed as described by Chamorovsky et al. (1976). It is obvious that the temperature dependence of k_{A1A2} correlates with the conformational mobility. The rate constant begins to increase at the temperature where the elastic fraction values start to decrease.

As described by Parak et al. (1981, 1982, 1984) the increasing mobility of bimolecules above 200 K can be understood as a fluctuation of the system between conformational substates as introduced by Frauenfelder et al. (1979). The activation energy for the transitions between conformational substates can be determined by models which describe the dynamic behaviour of a membrane fragment or a membrane protein fragment as an overdamped Brownian motion (Shaitan and Rubin 1980; Parak et al. 1982). According to Shaitan and Rubin (1980) one may calculate an activation energy from the temperature dependence of f^{exp} above 200 K. For CH2 it turns out to be 52.3 kJ/mol which is very close to the activation energy value for the electron transfer process, determined as 50.2 kJ/mol by Chamorovsky et al. (1976).

Following Beece et al. (1980), Knapp et al. (1982) and Shaitan and Rubin (1982) it is possible to show that the typical behaviour of the rate constant can be described not only by a radiationless transition (Jortner 1976) but also by a stochastic model for the electron transfer (Shaitan and Rubin

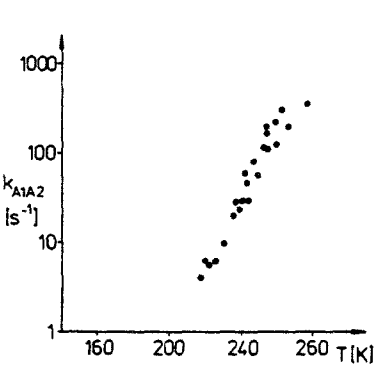

Fig. 4. The rate constant k_{A1A2} for the oxidation of the primary electron acceptor (A_1) by the secondary one (A_2) in *Ectothiorhodospira Shaposhnikovii*

1982). The picture of the Brownian motion of molecular fragments in a limited space suggests that the secondary quinone shares this quasi-diffusive motion. For some time it can come close to the primary quinone which makes the electron transfer process possible. The secondary quinone performs a random walk in a restricted space through a larger number of conformational substates which are unable to accept the electron. Only special conformational substates of the system allow a fast electron transfer in the picosecond range.

This situation can be compared to the situation in a Mb molecule, where the diffusion of a ligand through the semi-liquid part of the protein controls the ligand binding process. In our case all reaction rates between the dimer of bacteriochlorophyll (Dutton et al. 1979) and primary quinone are fast ($10^{10}\,s^{-1}$) and only the last step is slow: the electron transfer rate from the primary to the secondary quinone ($5 \times 10^{3}\,s^{-1}$). In this case the electron transfer rate is limited by the last step, and this last step is controlled by the quasi-diffusive motion of the secondary quinone in the semi-liquid part of the reaction center protein.

It should be mentioned that the oxidation rate of the high potential cytochrome *c* does not depend on temperature as shown by Chamorovsky et al. (1980). In spite of the slow oxidation rate of the cytochrome ($10^{6}\,s^{-1}$) the existence of an isotope effect at low temperature (Kihara and McGray 1973) shows that this process is not controlled by conformational fluctuations and must be considered as a normal radiationless process.

However, the amount of photooxidation strongly decreases with T in the temperature region around 180 K (Chamorovsky et al. 1980), where a phase transition is indicated by our experiment. It is also necessary to note that the oxidation rate for the low potential cytochrome in some cases changes its slope in the temperature region near the indicated phase transition. We have not enough experimental data to prove that the phase transition in CH2 and the changes in kinetic behaviour of the cytochrome oxidation are connected with each other. Additional experiments are necessary on this point, but the correlation observed seems to be not just accidental.

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