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Effects of dehydration and low temperatures on the oxidation of high-potential cytochrome *c* by photosynthetic reaction centers in *Ectothiorhodospira shaposhnikovii*

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Effects of temperature and dehydration on the efficiency of electron transfer from membrane-bound high-potential cytochromes c_h to the reaction-center bacteriochlorophyll (P-890) in *Ectothiorhodospira shaposhnikovii* have been studied. A kinetic analysis of the cytochrome oxidation suggests that there are at least two conformational states of the c_h-P-890 complex, of which only one allows photoinduced electron transfer from cytochrome to P-890$^+$. Lowering the temperature or dehydration leads to a change in the proportion of the populations in the two conformations. The observed 2-fold deceleration of cytochrome oxidation can be related only to the diminution of the amount of photoactive cytochromes per reaction center. The rate constant for the transfer of an electron from cytochrome c_h to bacteriochlorophyll is $2.8 \cdot 10^5$ s^{-1} and is independent of temperature and dehydration (as estimated within the accuracy of the experiments). The effects produced by low temperature and dehydration are completely reversible. The thermodynamic parameters of the transition of the cytochrome from the nontransfer to electron-transfer conformation were estimated. For room temperature (+20°C) in chromatophore preparations, $\Delta G = -5.4$ kJ · M^{-1}, $\Delta H = 60$ kJ · M^{-1}, $\Delta S = 0.22$ kJ · M^{-1} · K^{-1}. For Triton X-100 subchromatophore preparations, the absolute values of the above parameters are significantly lower: $\Delta G = -2.8$ kJ · M^{-1}, $\Delta H = 18$ kJ · M^{-1}, and $\Delta S = 0.075$ kJ · M^{-1} · K^{-1}. To a larger extent, the above parameters are diminished for chromatophore preparations in an 80% glycerol solution: $\Delta G = -1.7$ kJ · M^{-1}, $\Delta H = 6$ kJ · M^{-1}, $\Delta S = 0.025$ kJ · M^{-1} · K^{-1}. The data suggest the hydrophobic character of the forces that maintain the P-890-c_h complex in the electron-transfer conformation. The results obtained suggest that electron tunneling within the complex cannot occur until a specific conformational configuration of the complex is formed. The efficiency of cytochrome c_h oxidation is determined by the temperature, the degree of dehydration and the environmental conditions, whereas the transfer of an electron itself in the electron-transfer configuration is essentially independent of temperature and hydration.

Introduction

In purple bacteria, the electron donor for the photooxidized special pair of the reaction-center bacteriochlorophyll (P-890) is cytochrome *c* (for reviews see Refs. 1, 2). In a variety of species such as *Chromatium vinosum, Chromatium minutissimum, Ectothiorhodospira shaposhnikovii*, cytochromes *c* are membrane-bound proteins whose close association with the reaction-center protein is

maintained primarily through hydrophobic interactions [1,3,4]. This complex, characterized by a certain stoichiometry, consists of two photoactive hemes of the so-called 'high-potential' cytochrome c_h ($E_m \approx +300$ mV) and two hemes of a 'low-potential' cytochrome c_l ($E_m \approx 0$ mV) per reaction center. The two cytochromes are both capable of donating electrons to the reaction center under low-temperature conditions [1,5,6].

Experimental low-temperature studies of laser-induced cytochrome *c* oxidation stimulated the development of the physical electron-transport theory in biological systems. De Vault and Chance were the first to measure the temperature dependence of the rate of cytochrome c_l oxidation in whole cells of *C. vinosum* [7,8]. A biphasic temperature dependence of oxidation kinetics was found. It was characterized by a 1000-fold decrease factor as the temperature was lowered to 130 K. With further lowering of the temperature, even to that of liquid helium, the rate of the reaction remained unchanged. To explain these observations, a variety of electron-tunneling models have been proposed [9–12]. The concept that is most widely accepted to date is the so-called strong vibronic coupling theory [12].

In an attempt to obtain new data on the mechanisms of interactions between cytochromes *c* and reaction centers, we have measured the temperature-dependence of the rate of cytochrome c_h oxidation in *E. shaposhnikovii* chromatophores [13]. In contrast to what has been reported in Refs. 7 and 8, we observed the rate of the process to be practically independent of temperature in the range down to 115 K. At temperatures below 200 K, the extent of cytochrome c_h oxidation was seen to decrease noticeably. The results were obtained on preparations containing 80–90% glycerol in the suspension medium.

The reasons of such a difference in the temperature-dependence of cytochromes c_h and c_l oxidation is not yet clear. It is worth mentioning, however, that in the variety of species studied a 1000-fold decrease in the rate of cytochrome oxidation under low temperature has been observed only once – in case of *Chromatium*, D. In most other cases, the lowering of the reaction rate was much more modest [36]. We believe that structural factors are involved in the differences observed.

Recent studies of the primary events of bacterial photosynthesis show that the temperature-dependence pattern of some reactions which precede the oxidation of *c* cytochromes is very similar to that observed in our experiments [37,38]. The authors explain the results as reasonable behavior of activationless processes governed by the overlap of the electronic orbitals of the functional groups concerned. This seems adequate in an attempt to explain the kinetics of the photochemical action in a close association of porphyrin pigments of the reaction center [39]. However, if a redox process is taking place between the two macromolecular systems, for example between the reaction center and cytochrome c_h, such direct contact of their prosthetic groups (P-890 and heme) seems improbable.

As a quantitative explanation of the low-temperature results from our experiments, a kinetic model has been advanced [14], the essence of which is as follows.

(1) Relative to the reaction center bacteriochlorophyll P-890, each cytochrome c_h molecule (or active heme) can adopt at least two conformational states.

(2) Of the two states, only one allows a fast, temperature-independent transfer of an electron from c_h to the oxidized P-890.

(3) The equilibrium between the two states is dependent on temperature; at high temperatures the electron-transfer state is dominant.

(4) The rate with which the equilibrium is set up is significantly lower than the rate of an electron transfer: $c_h P^+\text{-}890 \rightarrow c_h^+ P\text{-}890$ in the electron-transfer state.

The proposed model is able to explain new experimental observations. It worth mentioning that Sarai and De Vault [15] have recently obtained qualitatively analogous results in experiments with *C. vinosum* and explained them in a way that does not differ much from what has been said above.

An original method has recently been applied in our laboratory to monitor photoinduced redox conversions of high-potential cytochromes in films of bacterial chromatophores with different humidity [16]. It appeared that with dehydration of preparations within a moderate humidity interval, from 0.65 to 0.35 P/P_s, the amount of photoactive cytochromes is reduced and the rate of their oxida-

tion slows somewhat [16].

In the present study we compare effects of low temperatures and dehydration on the transfer of electrons from the high-potential cytochrome c_h to P-890, and infer the role of conformational states of the system in this process. It is shown that for an electron to be transferred within the cytochrome c_h-reaction center complex the complex must take up a specific electron-transfer configuration. The rate for electron transfer itself in this configuration is independent of humidity and temperature within the ranges considered.

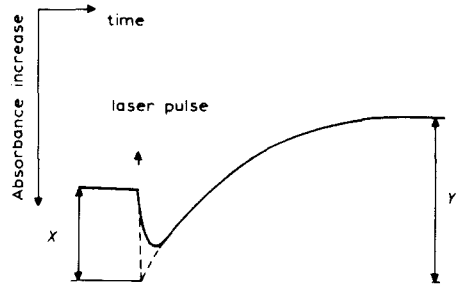
Materials and Methods

Chromatophore preparations were obtained by a standard method [17] from 2–3-day-grown *E. shaposhnikovii* cells. Subchromatophore particles enriched in reaction center pigment-protein complexes and cytochromes *c* were obtained by treatment of chromatophores with 0.1% Triton X-100 and purification by the chromatography on columns with hydroxyapatite. Properties of these complexes resemble those of the subchromatophore fraction A from *Chromatium* described by Thornber [18].

Two different reaction media were used to investigate the photo-induced oxidation of cytochrome *c* as a function of temperature. Medium I included 50 mM Tris-HCl (pH 8.0) and an electron donor system consisting of 50 μM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) and 2 mM sodium ascorbate to maintain the redox potential of the reaction medium within +150 to 200 mV. Medium II included 75–85% glycerol (by volume) in addition to the above reagents. In the medium without glycerol, on cooling the sample, the temperature around the freezing point was passed through very slowly, in about 10 min, in order to prevent the formation of polycrystalline structures and to have optically transparent samples at low temperatures [6].

Dry films of chromatophores prepared by the method described elsewhere [16] were used to investigate the effects of low humidity on the laser-induced oxidation of cytochrome *c*. For a quantitative kinetic study it was necessary to use a strictly controllable quantity of the preparation and to provide its homogeneity. Variable hydration levels of the chromatophore films were obtained by equilibrating the sample with the desired relative humidities provided by saturated solutions of different salts. The preparations were kept at given humidity for 48 h at +5°C before being transferred into sealed test cuvettes that were preincubated at the same humidity as the sample.

A single-beam laser spectrophotometer [19] was used to monitor the kinetics of photo-induced oxidation-reductions of cytochromes. Laser-induced absorption changes at around 424 nm are biphasic (Scheme I). The fast, unresolvable absorption increase (less than 0.2 μs) is due to the oxidation of P-890 (the *X* component). The relatively slow absorption decrease in a reflection of the concomitant oxidation of the high-potential cytochrome c_h and rereduction of bacteriochlorophyll [20–22]. The decomposition of this signal into the cytochrome and bacteriochlorophyll components has been described in Ref. 21. In this scheme, the contribution of P-890 is represented by the *X* length and that of cytochrome by the $(Y - X)$ length. The method is adequate where the donor-acceptor pairs of the cytochrome c_h reaction center complex are in the active state. At low temperatures and humidities this condition is not fulfilled.



Scheme I

As has been demonstrated earlier, the lowering of temperature and a moderate dehydration do not affect the amount of photoactive P-890 and its spectral characteristics in the 400–450 nm region to any sensible extent [16,23–25]. As for the photooxidazable cytochrome c_h, its amount was found to reduce in response to such exposures with no noticeable changes in its photo-induced differential spectrum in the Soret band [16,23]. This is accompanied by a diminution of the magnitude of

the Y component. Since the initial slope of the kinetics of Y component at any temperature and humidity and the whole kinetic pattern at normal conditions are close to the monoexponential fit, the theoretical approximation of the oxidation kinetics was performed by a linear regression method in semilogarithmic coordinates, as proposed in Ref. 21. The straight line obtained was extrapolated to the initial time (the onset of the reaction) to determine the amplitudes of the X and Y components. Since the extent of bacteriochlorophyll oxidation (the X component) was constant in our experiment, it was convenient to use the Y/X ratio, instead of Y, for quantitative estimations. The advantage of this is that one can compare the amplitude characteristics of different samples of the same series. Experiments on a single sample and a number of identical samples yielded quantitatively identical results (the amplitude measurements were typically accurate to within $\pm 10\%$). The value of the Y/X ratio for physiological conditions (room temperature and high humidity) was taken as the maximal; values of the Y/X ratios for different low temperatures and humidities were divided by this maximum value. In the following study the dimensionless parameter, $\Delta A_{424}/\Delta A_{max}$, is used.

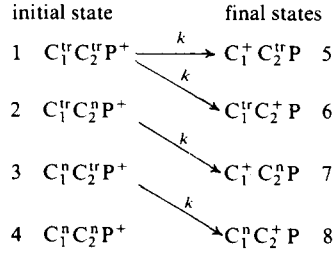
It is well known that the absorbance band at 424 nm in the spectrum of purple bacteria chromatophores is rather complex in character, with multiple components contributing to the absorbance changes at different kinetic domains. However, the complexity of absorbance changes at given wavelength does not matter too much in our experiments, because we studied only a narrow time range, from 0.1 to several microseconds, when all absorbance changes but those associated with cytochrome oxidation seem to be negligible.

Results and Discussion

Fig. 1 shows the amplitude characteristic and time-course of cytochrome c_h oxidation in *E. shaposhnikovii* chromatophores as a function of temperature and humidity. As is seen, on lowering the temperature in the 270–240 K range, or humidity in the 0.65 to 0.35 P/P_s interval, the amplitude of laser-induced cytochrome c_h oxidation is reduced and the reaction slows down. For a chromatophore suspension, the characteristic time of the reaction is 1.7 ± 0.2 μs at room temperature and 3.3 ± 0.2 μs at low temperature. For chromatophore films with a high humidity (0.75 P/P_2), the characteristic time is 1.8 ± 0.2 μs; in films with a low humidity (no greater than 0.35 P/P_2) it increases to 3.1 ± 0.2 μs. The changes are reversible: increasing the temperature or humidity resulted in the complete recovery of the signal. The greatest change in the rate of the reaction did not exceed 2-fold. Since at least two photoactive molecules of the high-potential cytochrome are associated with each reaction center [19,26], one can assume that the change in the reaction rate is due completely to a diminution of the number of the cytochromes in the system, rather than due to a change in the rate constant for electron transfer from the cytochrome to bacteriochlorophyll.

Since the behavior patterns of the amplitude and time-course of cytochrome oxidation in response to the lowering of temperature and humidity are similar, it seems reasonable to turn to the model used for the description of the temperature dependence in our previous work. Its basic features are recapitulated in the Introduction.

For a theoretical description of experimental observations we shall consider a simple kinetic diagram for electron transport in the c_h-P-890 system (Scheme II).



Scheme II

In the scheme, cytochrome c_h is represented by two molecules (or two hemes of a single molecule C_1 and C_2) which are potential electron donors for the photooxidized bacteriochlorophyll (P^+) of the reaction center [24,25]. Here C_i^{tr} and C_i^n stand for the ith heme of the cytochrome c_h in active ('transfer') and inactive ('non-transfer') configurations, respectively. In the kinetic sense, the C_1 and

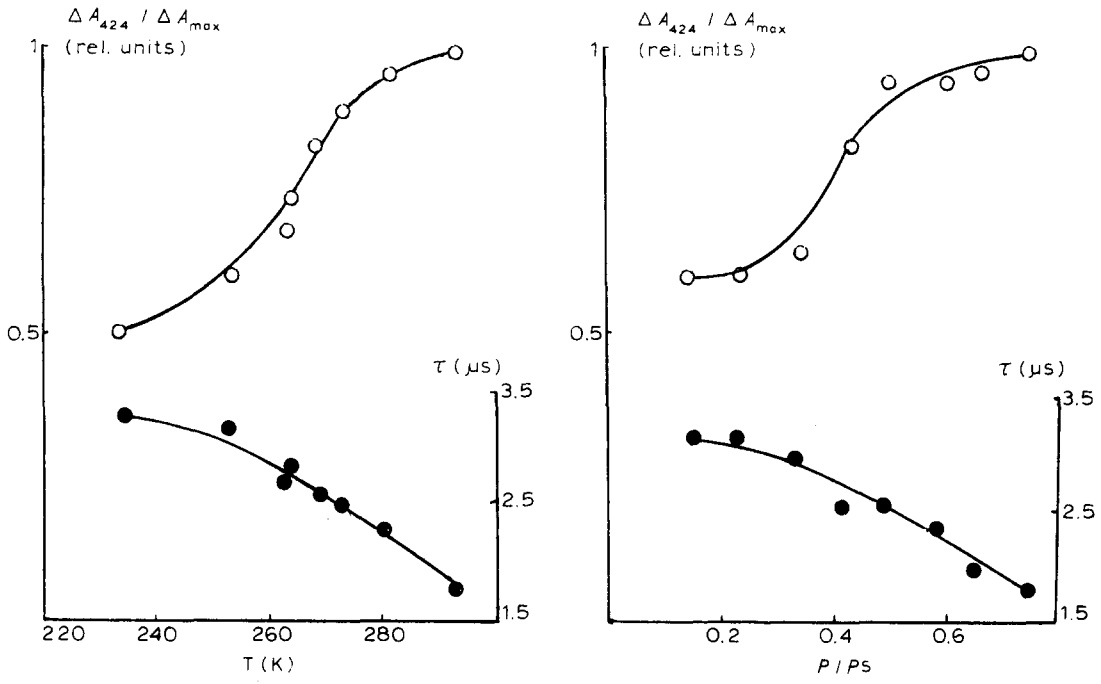


Fig. 1. The amplitude (upper curves, O——O) and time-course (lower curves, ●——●) of the oxidation of high-potential cytochrome c_h in *E. shaposhnikovii* chromatophores as a function of temperature and relative humidity. The amplitude of absorption changes is proportional to the amount of reaction centers containing one or two hemes of c_h. A single laser flash (694 nm, 30 ns, 10 mJ) was used as excitation source. Reaction medium I (see Materials and Methods).

C_2 molecules are identical and interact with the reaction center bacteriochlorophyll independently of each other *; k is the rate constant for electron transfer from a molecule of cytochrome to the bacteriochlorophyll. Rather simple calculations omitted here show that the kinetics of cytochrome oxidation according to Scheme II can be described as:

$$\frac{\Delta A(t)}{\Delta A_{max}} = P_1(0)(1-e^{-2kt}) + (P_2(0)+P_3(0))(1-e^{-kt}) \quad (1)$$

where $P_i(0)$ is the probability for the cytochrome being in the ith state at the onset of the reaction; $\Delta A(t)$ is the amplitude of the absorption changes associated with the oxidation of cytochrome for the time between zero and t; ΔA_{max} is the normalized factor, the maximum amplitude of the absorption changes when all the cytochromes are in the electron-transfer conformation.

Since the conformational transitions of C_1 and C_2 are independent, the probability, $P_i(0)$, of the complex's being in the ith state can be expressed in terms of the probability, P, of the cytochrome's being in the electron-transfer configuration. Using the formula describing the probability for the simultaneous occurrence of two independent events, Eqn. 1 can be rewritten as

$$\frac{\Delta A(t)}{\Delta A_{max}} = 1-[1-P(1-e^{-kt})]^2. \quad (2)$$

Using Eqn. 2 we can derive an exact relation between the two kinetic parameters, the amplitude $\eta = \Delta A(\infty)/\Delta A_{max} = 1-(1-P)^2$ and characteristic time, τ, or cytochrome photooxidation that satisfies the condition $\Delta A(\tau)/\Delta A_{max} = 1-e^{-1}$. Namely,

$$k\tau = \ln\frac{1-\sqrt{1-\eta}}{\sqrt{1-(1-e^{-1})\eta}-\sqrt{1-\eta}} \quad (3)$$

* The simplest model is considered. According to the structural data obtained on *Rhodopseudomonas viridis* reaction centers using X-ray diffraction technique [39] the cytochromes c in the reaction center may not be equivalent. However, the organization of cytochrome compartment in *E. shaposhnikovii* may differ from one in *Rps. viridis* [40].

The minimum value of $\tau = 1/2k$ was observed in the experiment when all the cytochromes were in the electron-transfer configuration ($\eta = 1$). For a room-temperature chromatophore suspension this is 1.7 ± 0.2 μs. To the accuracy of experiment, this time is nearly the same as the characteristic time of cytochrome oxidation in chromatophore films with a high humidity content ($P/P_2 = 0.75$), which is 1.8 ± 0.2 μs. Using this value one finds $k = 2.8 \cdot 10^5\ s^{-1}$. A theoretical curve derived from Eqn. 3 is presented in Fig. 2 along with the corresponding experimental points obtained from the data of Fig. 1. The curve gives a good description of temperature and humidity-dependent changes in the amplitude and time-course of cytochrome oxidation. A good consistency between the theoretical and experimental results suggests that dehydration and low temperature cause a similar kinetic effect. The effect is in changing the populations of the electron-transfer (tr) and nontransfer (n) configurations, the latter dominating at low temperatures and humidities. The rate constant for the transfer of an electron from cytochrome c_h to the P-890 bacteriochlorophyll obviously is independent of the environmental conditions, within the accuracy of experiment. Under physiological conditions when the two c_h hemes compete for donation of an electron to the bacteriochlorophyll molecule, the rate constant is $k = 1/2\tau$, instead of $1/\tau$.

Using the data on the temperature-dependent oxidation of the high-potential cytochrome within the above kinetic description, it is possible to determine the change in the free energy of a cytochrome molecule transition from the nontransfer to electron-transfer configuration: $\Delta G = -RT \ln K_p$ at any temperature T where $K_p = p/(1-p)$ is the equilibrium constant for the transition at a given temperature. Plotting its temperature dependence in the Van 't Hoff coordinates will yield the enthalpy of the transition. To approximate the experimental data (Fig. 4, curve 1) we used $\Delta H(T)$ decomposition, restricting the description to the linear term $\Delta H = a + bT$. By this approach a smooth curve can be obtained in the Van 't Hoff coordinates over the study temperature region. The nonlinearity, which is the greatest in the low temperature interval, may arise from changes in the microenvironment of the cytochrome c_h-reaction center complex in the heterogeneous membrane system. Our estimates of the room-temperature ($T = 293$ K) change in the free energy and Van 't Hoff's enthalpy associated with the non-transfer to electron-transfer conformational transition are: $\Delta G = -5.4\ kJ \cdot M^{-1}$, $\Delta H = 60\ kJ \cdot M^{-1}$. Using these values and the relation $\Delta G = \Delta H - T\Delta S$ one can find the entropy change at this temperature to be $\Delta S = 0.22\ kJ \cdot M^{-1} \cdot K^{-1}$.

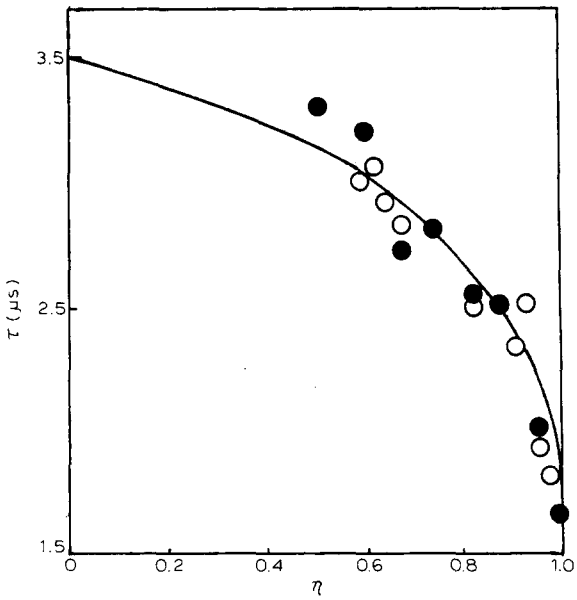


Fig. 2. Theoretical dependence of the characteristic times of cytochrome c_h oxidation (τ) as a function of the normalized amplitude of the signal (η). The dependence was calculated from Eqn. 3. Experimental points are plotted from the data of Fig. 1. Open circles, experimental points from the humidity dependence of the reaction, and closed circles, from its temperature dependence.

The data on the humidity-dependent oxidation kinetics of the high-potential cytochrome give additional information on the mechanism of the process. A gravimetric and NMR-spin-echo monitoring of the water content and state in bacterial photosynthetic membranes shows that the desorption of weakly bound mobile water with a proton spin-spin relaxation time, T_2, of several milliseconds takes place as the relative humidity content of the preparation became less than 0.5 P/P_2 [27]. Similar observations were made by us in preliminary experiments on *E. shaposhnikovii* chromatophore preparations. Within the same humidity range, we observed a change in the effectivity of electron transfer from cytochrome *c* to the

reaction center bacteriochlorophyll (Fig. 1).

The shift in the equilibrium between the electron-transfer and non-transfer populations may be caused by structural and dynamic rearrangements in the lipid surrounding of the cytochrome c_h-reaction center complex.

Dehydration and temperature decrease obviously leads to solidification of lipid matrix. In this case, cytochrome c_h, and likewise other peripheral membrane proteins [28,29], may be forced out of the hydrophobic region of the bilayer. It is worth noting that with a moderate dehydration of chromatophores of purple bacteria, from 0.6 to 0.3 P/P_s, the membrane viscosity increases significantly, as can be seen by a change in the mobility of hydrophobic probes [30], the consequence of which may be the disappearance of the direct "end-to-end" contact between the integral protein of the reaction center and cytochrome c_h that is necessary for electron transfer.

On the other hand, the stabilization of such a configuration may be maintained by hydrophobic interactions between the protein groups themselves. Then, the structuring of the surrounding water that will take place, in accordance with the classical mechanism of hydrophobic binding, will change the entropy of the system to a lower value, the lowering being compensated only partly by the lowering of the system enthalpy through the formation of new hydrogen bonds in the aqueous environment [31]. Such rearrangements can obviously be observed only at a fairly high degree of hydration.

Our experimental estimation of the enthalpy and entropy changes during cytochrome c_h-reaction center transition from the non-transfer to the electron-transfer configuration (see above) are in a good agreement with the concept of hydrophobic stabilization of the functionally active state of this complex.

The hydrophobic interactions will be weakened by addition of a detergent or a partial replacement of water for a less polar solvent. We examined the effect of temperature on the kinetics of cytochrome c_h oxidation in chromatophores from *E. shaposhnikovii* in the presence of 80% glycerol and in subchromatophore pigment-protein complexes containing 0.1% Triton X-100. The results are presented in Fig. 3. As is seen, changes in the

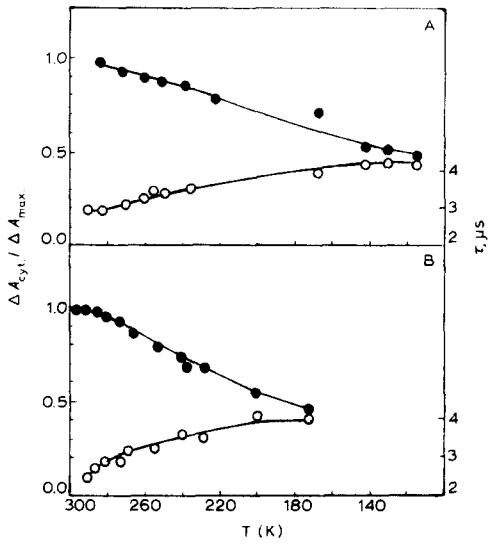


Fig. 3. The effect of temperature on the amplitude (●———●) and time-course (○———○) of the oxidation of high-potential cytochrome c_h. (a) *E. shaposhnikovii* chromatophores, medium II; (b) *E. shaposhnikovii* subchromatophore preparation, Medium I. Other conditions as in Fig. 1.

kinetics occur over a wider range of temperatures than in the case of an aqueous chromatophore suspension. At room temperature, the oxidation of the cytochrome c_h in these preparations occurs with a slightly higher characteristic time than in an aqueous chromatophore suspension; at low temperatures, however, the rate of c_h oxidation is the same for all types of preparation. Rather than a change in the elementary rate constant of the electron transfer from the cytochrome to P-890, it seems reasonable to suggest within the context of the model discussed that a change in the balance of the nontransfer and electron-transfer populations in response to a variation in the environmental conditions is responsible for the observed effect.

The experimental dependence agree well with the predictions of the theoretical model if we assume that the population in the nontransfer state is 0.33 for chromatophores in an 80% glycerol solution at room temperature and 0.2 for a subchromatophore preparation. These estimates were used to determine the K_p value for the Van 't Hoff presentation of the data (Fig. 4). From the obtained curves it is possible to determine the thermodynamic parameters for the transition of

the cytochrome at 293 K from the non-transfer to electron-transfer configuration. For a chromatophore suspension in 80% glycerol we found $\Delta G = -1.7$ kJ · M^{-1}, $\Delta H = 6$ kJ · M^{-1} and $\Delta S = 0.025$ kJ · M^{-1} · K^{-1}. For Triton X-100 subchromatophore particles, $\Delta G = -2.8$ kJ · M^{-1}, $\Delta H = 18$ kJ · M^{-1}, $\Delta S = 0.075$ kJ · M^{-1} · K^{-1}. As expected for the latter, the weakening of the hydrophobic interactions that stabilize the complex in the electron-transfer configuration is accompanied by a diminution of absolute values of the changes in the parameters associated with the transition, the smaller difference in the free energies between the two configurations being the result of a smaller entropic contribution. The drop of the steady-state level of photooxidized cytochrome *c* observed at low temperatures and humidities [6,16] suggests that the rate of its oxidation in the non-transfer state cannot exceed the rate of its reduction under these conditions ($\tau = 3$–100 s). This gives rise to the question of what underlying physical phenomena are responsible for such a large change in the electron-transfer rates in the two configurations.

As is known, quantum-mechanical vibronically coupled electron-tunneling models predict that the rate of the process depends on the distance between the redox centers, the height of the energetic barrier and the spectrum of the continuum of coupled vibrations [9–12]. Any noticeable change in the spectrum after a moderate dehydration of the preparation is most improbable. On the other hand, by varying some electron-transfer parameters, for instance, the distance between the redox centers, one can estimate that a change in the electron-transfer rate by 6–8 orders, from microseconds to tens of seconds, corresponds to a change of about 2 nm in the distance between the nearest electron carriers. The occurrence of such a large-scale change seems little probable because of its complete reversibility under obviously non-destructive exposures such as moderate degree of dehydration; however, we cannot exclude this possibility entirely at present.

Strictly speaking, there is some inadequacy in representing electron transfer between two macromolecules as tunneling between two local centers, since such a description does not differentiate the electron transfers between macromolecules and those within a single macromolecule. There is,

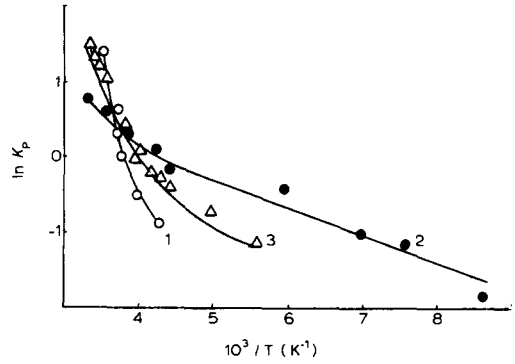


Fig. 4. The Van 't Hoff plot of the temperature dependence of the equilibrium constant for a conformational transition of the cytochrome c_h-reaction center complex from the nontransfer to electron-transfer configuration. (1) *E. shaposhnikovii* chromatophores, medium I; (2) *E. shaposhnikovii* chromatophores, medium II; (3) *E. shaposhnikovii* subchromatophore preparation, medium I. See text for the details.

however, evidence, obtained from model systems, that the rate of cytochrome *c* oxidation by myoglobin depends upon the nature of the two macromolecules, the contact being formed via certain interacting amino acid residues [32]. In this connection the idea of electron tunneling along 'electron paths' formed by structural elements of macromolecules seems rather realistic. The mechanisms of long-distance electron tunneling (over several nanometers) have been discussed elsewhere (Ref. 33 and references therein). It appears by an analysis made in Refs. 33 and 34 that the joining of individual 'electron-transfer paths' between the cytochrome and the reaction center to form a direct contact is a critical step for electron transfer. If, during the transition into the nontransfer configuration, the contacting groups are separated by only 0.3–0.5 nm (this is the amplitude of vibrations of segments of α-helices of proteins in a condensed medium [35]), the overlapping of their wave functions disappears almost entirely, with the result that the rate of the electron transfer within the complex slows down by six orders of magnitude. This deceleration is due to a simultaneous increase in the tunneling distance and the height of the barrier at the site of contact, whereas the parameters of the electron transfer within a macromolecule remain unchanged.

On the basis of the data reported here it is concluded that the kinetics of cytochrome *c* oxida-

tion by the reaction center consist of two successive steps: a thermally activated formation of a conformation favorable for electron transfer, and an electron-transfer reaction, which is an activationless process.

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