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Temperature dependence of cytochrome photooxidation and conformational dynamics of *Chromatium* reaction center complexes

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Abstract

A temperature dependence of multiheme cytochrome *c* oxidation induced by a laser pulse was studied in photosynthetic reaction center preparations from *Chromatium minutissimum*. Absorbance changes and kinetic characteristics of the reaction were measured under redox conditions where one or all of the hemes of the cytochrome subunit are chemically reduced ($E_h = +300$ mV or $E_h = -20$ to -60 mV respectively). In the first case photooxidation is inhibited at temperatures lower than 190–200 K with the rate constant of the photooxidation reaction being practically independent on temperature over the range of 300 to 190 K ($k = 2.2 \times 10^5 s^{-1}$). Under reductive conditions ($E_h = -20$ to -60 mV) lowering the temperature to 190–200 K causes the reaction to slow from $k = 8.3 \times 10^5 s^{-1}$ to $2.1 \times 10^4 s^{-1}$. Under further cooling down to the liquid nitrogen temperature, the reaction rate changes negligibly. The absorption amplitude decreases by 30–40% on lowering the temperature. A new physical mechanism of the observed critical effects of temperature on the rate and absorption amplitude of the multiheme cytochrome *c* oxidation reaction is proposed. The mechanism suggests a close interrelation between conformational mobility of the protein and elementary electron tunneling act. The effect of "freezing" conformational motion is described in terms of a local diffusion along a random rough potential.

Introduction

Cytochromes *c* are immediate secondary electron donors for the photooxidized reaction center bacteriochlorophyll dimer in purple bacteria (see reviews by Bartsch 1978, Dutton and Prince 1978).

In a variety of bacteria such as *Chromatium vinosum*, *C. minutissimum*, *Rhodopseudomonas viridis*, *Rps. gelatinosa* etc. the photosynthetic reaction center is closely asociated with a multiheme subunit containing two high-potential (C_h) and two low-potential (C_l) hemes *c* (Case et al. 1970, Dutton 1971, Dutton and Prince 1978, Seibert and De Vault 1970).

In a number of bacteria (*Rhodobacter sphaeroides*, *Rb. capsulatus*, *Rhodospirillum rubrum*) a soluble cytochrome c_2, which resembles the single-heme cytochrome of the mitochondrial respiration chain, acts as a secondary electron donor (Bartsch 1978, Dutton and Prince 1978).

Chance and Nishimura were first to observe the low temperature oxidation of cytochromes *c* in *Chromatium vinosum* (Chance and Nishimura 1960).

De Vault and Chance, in their subsequent fundamental work (De Vault and Chance 1966) measured the temperature dependence of this reaction and observed that the characteristic time of the reaction increased from 1 μs to 2.5 ms as the temperature was lowered from 300 K to 120 K and that with further lowering of the temperature the rate of the reaction did not change any more.

Later, the pattern of this temperature dependence was confirmed using subcellular preparations of *C. vinosum* (Dutton et al. 1971).

It seems apparent, that the temperature depen-

dence observed by De Vault and Chance is related to the low-potential cytochrome, for it was observed only under anaerobic conditions and because the chemical preoxidation of the low-potential cytochromes of *Chromatium* virtually completely inhibited the photoinduced cytochrome reactions at 80 K (Dutton et al. 1971, Dutton and Prince 1978).

Kihara and Chance have undertaken an immense study of the low-temperature cytochrome oxidation of different species of photosynthesizing bacteria (Kihara and Chance 1969).

The ability to oxidize at low temperature was found to be correlated with neither taxonomic group of the bacteria nor the capacity of utilizing sulphur or other substrates. Neither was it a property of obligate or facultative anaerobes.

In organisms in which cytochrome c_2 acts as an electron donor, the low-temperature electron transfer appeared to be completely inhibited.

Only low-potential cytochromes c_1, with a rare exception, were found to undergo photo-induced oxidation at 77 K, the reaction being irreversible.

Different species exhibited different extents of slowing the reaction at low temperature. For instance in *Rps. gelatinosa*, the characteristic time of cyt. c_1 oxidation (τ is 0.7 μs at 300 K and 4.6 μs at 80 K). In *Rps. sp.* NW $\tau_{300} = 0.55\,\mu s$ and $\tau_{80} = 4.2\,\mu s$.

The data on temperature dependence of the photo-induced oxidation of the high-potential cytochrome in *Chromatium* are not so complete. It has been found in earlier investigations (Vredenberg and Duysens 1964, Dutton 1971, Seibert 1971) that the reaction is blocked at low temperature and that over the positive temperature range (310–270 K) the characteristic time of the reaction increases from 2 μs to 6 μs, which corresponds to an activation energy of 2.6 $\pm$ 0.6 kcal/mol.

In a later work, reported as a short communication, Sarai and De Vault have observed in *C. vinosum* that the characteristic time of cytochrome *c* oxidation remained unchanged ($\tau_{1/2} = 2\,\mu s$), as the temperature was lowered from 300 K to 260 K and that temperature lowering further to 200 K caused $\tau_{1/2}$ to increase to 9 μs with a simultaneous drop of the quantum efficiency of the reaction from 1 to almost zero (Sarai and De Vault 1983). The observations appeared similar to those seen in another very resembling species, *Ectothiorhodospira shaposhnikovii* (Chamorovsky et al. 1980, 1986).

The three-dimensional structure of the cytochrome subunits of the *Rps. viridis* reaction center was investigated by a highly-resolved X-ray diffraction analysis (Deisenhofer et al. 1984, 1985). It appears that the hemes in the cytochrome subunits form a linear array with the special pair dimer standing 21 Å apart from the nearest heme and 60–70 Å from the most distant heme.

In subsequent investigations (Shopes et al. 1987, Weyer et al. 1987, Dracheva et al. 1988, Nitscke and Rutherford 1989) the nearest heme was identified as a high-potential heme (c_h), the following hemes being located as a sequence c_l–c_h–c_l (or c_h–c_l–c_l, which seems less probable).

The low-temperature cytochrome *c* oxidation data for *Rps. viridis* cytochromes are sometimes not unambiguous; neither are they with *Chromatium*.

In early work by Chance and coworkers using intact cells and subcellular extracts, the characteristic time of the photooxidation of cytochrome *c* was seen to slow from 0.6–0.8 μs to 250–350 μs, as the temperature was lowered from 300 K to 77 K (Kihara and Chance 1969, Chance 1974). The insensitivity of the reaction to moderate aeration and its partial reversibility at low temperature suggest the involvement of high-potential heme.

It was also found in (Shopes et al. 1987) that the high-potential cytochrome *c* in *Rps. viridis* reaction center preparations is capable of low-temperature oxidation, with the characteristic time increasing from 270 ns at 300 K to 2.8 ms at 125 K. There are however data (Nitscke and Rutherford 1989) that it is the high-potential hemes of *Rps. viridis*, that are not photooxidized at low temperatures (4 K). The inhibition of photooxidation of the high-potential hemes at as low a temperature as 80 K was also observed in (Kaminskaya et al. 1989).

It seems important to make a kinetic and amplitude analysis of the effect of temperature on the photo-induced oxidation of the high- and low-potential hemes of the multiheme cytochrome subunits of the reaction center using a single purified preparation poised under strictly fixed redox conditions with a selective chemical reduction of the high-potential or low-potential hemes.

In the present work we measured the temperature dependence of the cytochrome oxidation reaction, both for the high- and low-potential

cytochromes, using reaction center preparations from *Chromatium minutissimum* under conditions where only 1 high-potential heme undergoes oxidation and the rest of the cytochromes are oxidized chemically, and also under conditions where all the cytochromes are chemically reduced.

The oxidation kinetics of the high-potential heme were found to be independent of temperature over the temperature range studied and the absorbance changes corresponding to cytochrome oxidation were observed to decrease sharply, as the temperature was lowered to 200 K. When the low-potential cytochrome undergoes oxidation, the rate of the reaction slows down with lowering the temperature to ~190–200 K and remains practically unchanged at lower temperatures. The absorbance changes due to cytochrome oxidation fall in this case to about 70%.

Materials and methods

The bacterium *Chromatium minutissimum* was grown and chromatophores isolated by the method described elsewhere (Chamorovsky et al. 1977).

Chromatophores were suspended in a Na-phosphate buffer (10 mM) (A^{1cm}_{595nm} = 12) and 10^{-2} M sodium dithionite was added.

Cooled acetone (60%) was added to suspend chromatophores. Sedimentation was done by centrifugation (2000 g, 5 min, 4°C). The sediment was kept in the cold for removal of the acetone remnants. The dry sediment was solubilized in a solution containing 0.2% Triton X-100, and 0.5% sodium cholate in 10 mM Na-phosphate buffer in the presence of 10^{-2} M dithionite (48–64 h, 4–10°C). The preparation was sedimented by centrifugation (2000 g, 30 min, 4°C). The supernatant was diluted to 1% Triton X-100 concentration and chromatographed on a hydroxyapatite column. The pure reaction center preparation was eluated by a 0.1% Triton X-100 solution in a Na-phosphate buffer. The stoichiometry was $A_{775}:A_{800}:A_{882}$ = 1.1:2.4:1. Electrophoresis in polyacrylamide gel gave a molecular mass of 120 ± 10 kD for the reaction center protein. The properties of the obtained reaction centers are described in greater detail in (Sabo et al. 1989).

Spectral measurements were made on a single-beam differential spectrophotometer with laser excitation (Chamorovsky et al. 1989).

A local-made cryostat, similar to that described in (Chamorovsky et al. 1986), was used for low-temperature measurements at poised redox potentials.

Glucose oxidase ("Sigma") was used to remove dissolved oxygen from the water-glycerol suspension of reaction centers when poising the system at low redox poentials.

Results and discussion

In previous work using *C. vinosum*, *C. minutissimum*, *Rps. viridis* and similar bacteria, it was assumed that the high- and low-potential cytochromes *c* are pairwise equivalent (Case et al. 1970, Chamorovsky et al. 1977, 1986).

Redox titration of reaction center preparations containing active cytochromes showed that all the hemes have different redox potentials (Shopes et al. 1987, Dracheva et al. 1988, Nitscke and Rutherford 1989).

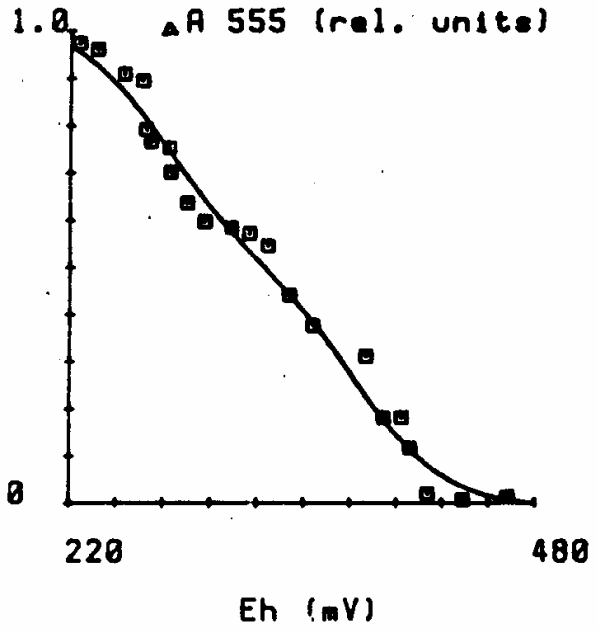

Fig. 1. Absorption changes at 555 nm as a function of the redox potential of the medium for reaction centers of *C. minutissimum*. The reference wavelength, 540 nm. The incubation medium contained 50 mM Na-phosphate buffer, pH 7.0, 67% glycerol (v/v), 0.1% Triton X-100, 300 μM diaminoduren, 100 μM benzoquinone. Titration was done by adding sodium ascorbate (reductive titration) or potassium ferricyanide (oxidative titration). The theoretical plot was derived using Nerst equation for two one-electron transitions for E_m = +380 mV and E_m = +270 mV, whose relative contributions are equal.

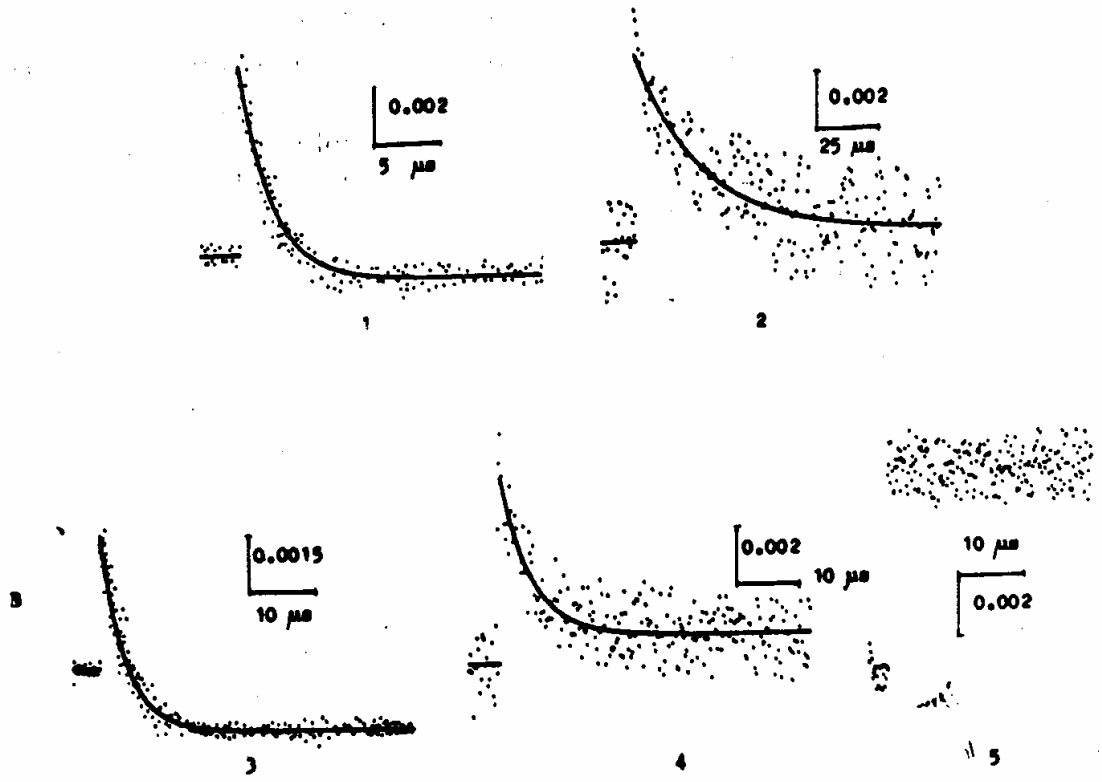

Fig. 2. Kinetics of absorption changes at 423 nm in *Chromatium* reaction center preparations for different temperatures and redox potentials of the medium: A, $E_h = -40$ mV (1—300 K; 2—198 K); B, $E_h = +300$ mV (3—300 K; 4—250 K; 5—168 K). The medium contained 50 mM Na-phosphate buffer, pH 8.0 and 70% glycerol (v/v). 1, 2—additions: glucose + glucose-oxidase and catalase; 10 μM phenazine methosulphate, sodium dithonite. 3, 4, 5—additions: N,N,N′,N′-tetramethylphenilene diamine 50 μM; ferricyanide.

In our dark redox titration experiments we observed the increase in absorbance at 550–555 nm, related to α-band of cytochrome, as the redox potential was lowered in the range from +480 to +200 mW, which reflects the reduction of two high-potential hemes with mid-point potentials of $E_{m_1} = +380$ mV and $E_{m_2} = +270$ mV (Fig. 1). With further lowering the redox potential, the absorbance further increased, which is obviously associated with the reduction of the low-potential hemes. At redox potentials below −100 mV the absorbance did not exhibit any further increase.

There is, therefore, good reason to believe that at redox potentials around +300 mV one high-potential heme is predominantly reduced; at redox potentials in the range −50 to −100 mV all the hemes of the multiheme cytochrome subunit are chemically reduced.

It is under these conditions that we measured the temperature dependences of the amplitude and kinetics of the photo-induced oxidation of cytochromes.

Presented in Fig. 2 are the corresponding absorption changes at 423 nm. It is to be noted that the low-temperature photo-induced oxidation under reducing conditions is an irreversible reaction. Moreover, as a result of the blockage of electron transfer through the acceptor pathway at low temperature, the repeated excitation may cause the formation of the triplet state of the reaction center. For this reason, the low-temperature kinetic curves were measured in a single-accumulation mode, whereas signal averaging was used in room-temperature measurements.

The absorbance increase at 423 nm reflects the photooxidation of the special pair bacteriochlorophyll molecules and the absorbance decrease is due to cytochrome oxidation. For all cases, the Marquart method (Himmelblau 1973) was used to approximate the cytochrome oxidation kinetics.

Presented in Fig. 3A is an Arrhenius plot of the logarithm of the rate constant (reciprocal of the characteristic time τ) versus temperature (curve 1 for the high-potential heme, $E_h = +300$ mV, and curve 2 for the low-potential heme, $E_h = -20$ to −60 mV).

Curve 2 is biphasic in character, similar to that described by De Vault and Chance (De Vault and Chance 1966). The rate constant logarithm decreases from 13.5 ($\tau = 1.2$ μs) at room temperature

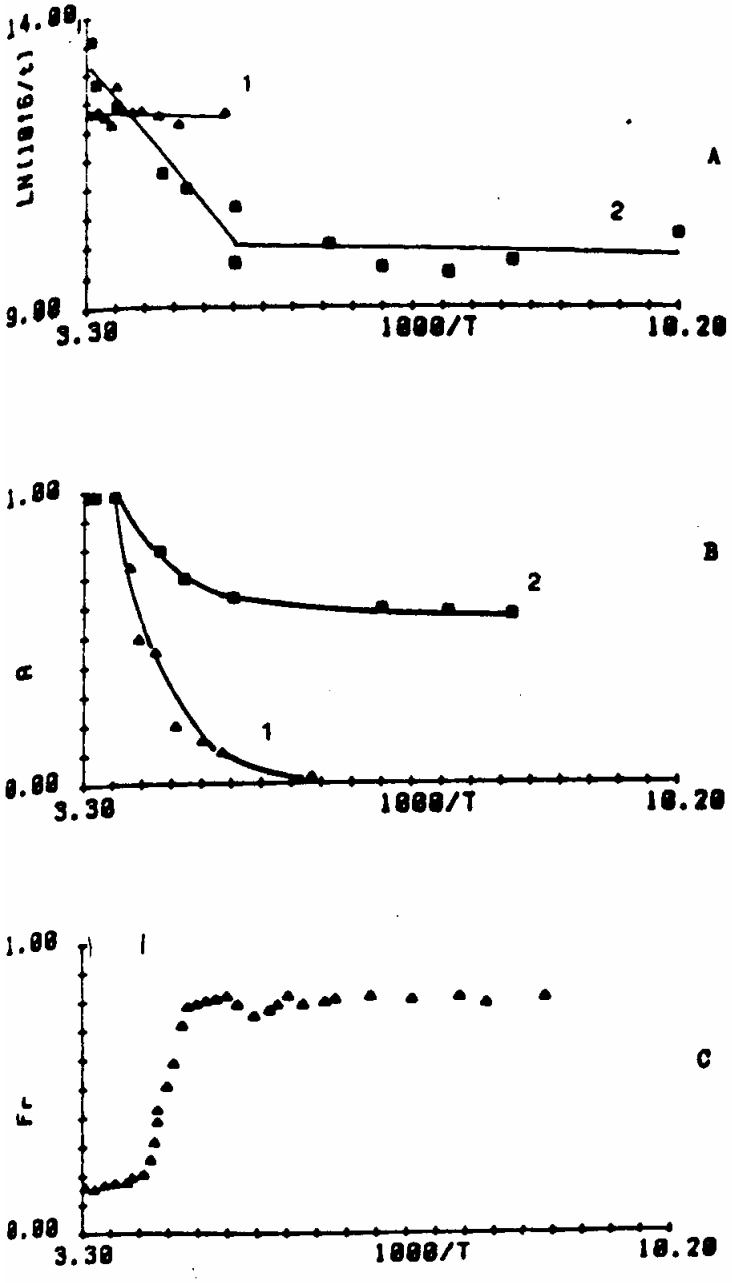

Fig. 3. Temperature dependence of the rate of cytochrome *c* oxidation (A) and of absorption changes corresponding to the total oxidation (seen as absorbance flattening) (B) for *Chromatium* reaction center preparations. (1) $E_h = +300$ mV (oxidation of high-potential heme); (2) $E_h = -20$ to -60 mV (oxidation of low-potential heme). Other conditions as in Fig. 2. (C) Temperature dependence of elastic Rayleigh scattering of Mössbauer radiation (f_R) in *E. shaposhnikovii* membrane preparations (Krupyanskii et al. 1985).

to about 10 (τ = 45–50 μs) at 200 K; with further lowering the temperature to 80 K, it decreases negligibly. The activation energy for the two parts of the temperature dependence is 15.0 ± 10 kJ/mol and 0.3 ± 0.3 kJ/mol, respectively. The tolerances are variances.

The rate constant for the high-potential cytochrome photooxidation (curve 1) shows a negligible dependence on temperature over the temperature range 300–200 K (E_a = 0.55 ± 0.25 kJ/mol). At lower temperatures no oxidation of the high-potential cytochrome was observed within the experimental error.

As seen from Fig. 3B, the lowering of temperature to 200 K results in a pronounced decrease in the absorbance changes corresponding to high-potential cytochrome *c* oxidation at $E_h = +300$ mV (curve 1).

Under reducing conditions ($E_h = -20$ to -60 mV), the absorbance decrease due to cytochrome oxidation is less pronounced (curve 2).

It is worth noting that the observed temperature-dependent changes in the rate constant (Fig. 3A, curve 2) and the photo-induced absorption changes (Fig. 3B, curve 1) both occur over nearly the same temperature range.

We have earlier suggested (Chamorovsky et al. 1986) that the temperature-induced inhibition of high-potential cytochrome oxidation may be a consequence of the slowing of conformational mobility of protein fragments of the reaction center complex. It is within this temperature range that a sharp change in the parameters of Rayleigh scattering of Mössbauer radiation was observed in chromatophores of *Ectothiorhodospira shaposhnikovii* (Fig. 3C). The scattering intensity is known to be associated with changes in the dynamic behavior of the system (Krupyanskii et al. 1985) and the rise in elastic scattering at low temperature is a manifestation of damping of the conformational mobility of a chromatophore suspension.

To evaluate the contribution of chromatophores to the elastic scattering of Mössbauer radiation it was necessary to exclude the effects brought about by the water–glycerol solvent. It is well known that the Mössbauer spectrum of a solvent is drastically broadened above the melting point so that its contribution to f_R at room temperature is practically negligible. On the other hand, for chromatophores the spectra show almost no line broadening, the line shape becoming non-Lorentzian. The latter makes it possible to determine the temperature-induced changes in the dynamic parameters of the protein–lipid matrix. The physical mechanism of these effects has been discussed in detail in many papers relating to Mössbauer spectroscopy of proteins (see for example Basovets, Uporov et al. 1989).

A good deal of data are available to date showing the influence of the conformational dynamics of macromolecules on their functional activity (Case and Karplus 1979, Gavish and Werber 1979, Welch et al. 1982). However, the observed effects of temperature on the kinetic and amplitude characteristics associated with cytochrome *c* oxidation cannot be interpreted within the framework of commonly accepted theoretical models which are concerned mainly with the effect of temperature on the electron transfer rate only. The concepts used earlier for interpreting the effects of temperature and humidity on the organization and functioning of the electron-transfer pathways were of qualitative character (Chamorovsky et al. 1986). A thorough analysis of the behavior of the absorption amplitude has led us to a new theoretical formulation in an attempt to describe the mechanisms of the reaction in question. Much consideration is given to conformational degrees of freedom and to the dynamics and phase transitions which occur in systems with stochastic potentials. Our approach does not contradict the generally accepted electron tunneling concept for protein media (Kuznetsov et al. 1978, Jortner 1980, Marcus and Sutin 1985, Kuhn 1986). There is only an aspect we want to draw attention to.

The specific biphasic temperature dependence of cytochrome *c* oxidation in *Chromatium* detected by De Vault and Chance 20 years ago gave rise to a number of ideas in theoretical and molecular physics regarding elementary processes in biological systems.

The first step was the refusal of the semiconduction hypothesis. Instead, electron tunneling was invoked (De Vault and Chance 1966). This also gave rise to a hypothesis which explained the observed effects by changes in the effective donor–acceptor distance induced by thermal fluctuations (Hales 1976). On the other hand, it is perfectly obvious that electron transfer in protein, in its physical essence, differs little from oxidation-reduction reactions in solution (Dogonadze and Kuznetsov 1973, Kestner et al. 1974). Hence, the temperature dependnce of the rate of cyt. *c* oxidation was explained by a rearrangement of the nuclear subsystem following a change in the electronic state of the donor–acceptor couple (Jortner 1980).

With regard to this approach, there are several aspects which present a problem. Firstly, for the oxidative reaction determined only by electron-exchange interactions within the heme-pigment system, those models fail to explain large differences in the $k(T)$ dependence for different bacteria (Bixon and Jortner 1986). Secondly, it was not possible to establish electron vibronic coupling parameters consistently in a chain of successive oxidation-reductions in the reaction center protein. Thirdly, the inflexion of the $k(T)$ dependence at low temperatures is much sharper than predicted by vibronic coupling theory. These suggest a more complicated mechanism of the reaction in question (Bixon and Jortner 1986).

Definitely, apart from the vibronically-coupled electron-exchange interactions, there are other mechanisms of the electron-transfer reactions in proteins. It is very important to understand the role of the protein structure in efficient and unidirectional electron transfer. Preliminary quantum-chemical calculations (Kuhn 1986) indicate that there is a strong dependence of reaction rates on the orientation of the donor and acceptor groups. Several lines of evidence show the involvement of π-bridges of aromatic amino-acid residues in long-distance electron transfer (Bixon and Jortner 1986, Michel-Beyerle et al. 1988). In other words, there are good reasons to believe that specific electron pathways exist in the protein structure. We believe that thermal fluctuations of the structure or conformational motions in the protein may exert influence on the efficiency of operation of the electron-transfer pathway (Shaitan and Rubin 1982, Krupyanskii et al. 1985, Rubin et al. 1987). The fact that the changes in the characteristics of cytochrome *c* photooxidation occur over the same temperature range as the restriction on freedom of protein conformational mobility obviously confirms the suggestion. On the basis of Mössbauer spectroscopy data we have earlier discussed the concept of conformational mobility as a local diffusion in the rough potential field (Shaitan and Rubin 1986) with coupling of electron transfer and conformational fluctuations. The latter may be described by a system of coupled Fokker-Planck equations containing additional terms representing chemical reactions (Shaitan and Rubin 1982). According to this concept for motion along one degree of freedom, and also as the general theory of vibronically-coupled electron transfer predicts

(Rips and Jortner 1987, Zusman 1988), the rate of the reaction depends on the conformation relaxation time, τ_c. However, the complexity of the above experimental data is that the chemical oxidation of the low-potential hemes C_l (that do not participate directly in the $C_h \xrightarrow{e} P^+$ reaction) not only changes drastically the temperature dependence of the rate of this reaction, making it temperature-independent, but also largely changes the amount of donor–acceptor couples $C_h P^+$ that can undergo this reaction in a frozen state (see Fig. 3).

There are two interpretations (thermodynamic and kinetic) of the sharp decrease in the amplitude and yield of the cytochrome *c* oxidation reaction. If we assume that thermodynanmic equilibrium is established during the kinetic measurements at all temperatures used in the study, the decrease of the amplitude may be ascribed to the shift of redox equilibrium in the system $C_h P^+ \rightleftharpoons C_h^+ P$ *when* C_l is pre-oxidized chemically. This seems a realistic situation, bearing in mind that the positive charge that appears on the C_l heme lowers the energetic level of the electron localized on C_h (Dutton and Prince 1978, Rubin et al. 1987, Kaminskaya et al. 1989).

The alternative interpretation involves the strong effect of conformational fluctuations on an electron transfer rate. We apply a well-developed method using the stochastic character of the reaction conformational coordinates (Shaitan and Rubin, 1982). The latter arises as a consequence of the existing hierarchy of times of vibrational relaxation ($\sim 10^{-12}$ s) and conformational motions ($\gtrsim 10^{-10}$ s). By definition, conformational motions are relative displacements of atom groups having amplitudes noticeably larger than those of valent vibrations (~ 0.1 Å).

Let us consider the simplest situation where some group "I" changes in state (for instance, it donates an electron) which is accompanied by its random walk along the conformation energy surface $U_I(x)$. The dynamics of this process may be described by the Fokker–Planck equation:

$$\frac{\partial P_I(x, t)}{\partial t} = \frac{\partial}{\partial x} D_I(x) \left[\frac{\partial P_I}{\partial x} + \beta P_I \frac{\partial U_I}{\partial x}\right] - W_I(x) P_I, \qquad \beta = 1/k_B T. \quad (1)$$

$P_I(x, t)$ is the probability density for group I being in a given chemical state and in conformation x at time t. $U_I(x)$ and $U_{I^+}(x)$ (Fig. 4) are conformation energy surfaces for groups I and I^+, which, generally speaking, have complicated shapes; $D_I(x)$ is a diffusion coefficient for diffusion of I along the conformational substates; $W_I(x)$ is the dependence of the oxidation rate of I on conformation. The sharp conformation dependence of the reaction rate is presumably caused by steric factors, i.e., by the change in the mutual orientation of the reactants and the distance between them, on the one hand, and by the dependence of the enthalpy of the reaction on conformation, on the other hand. The latter changes in proportion to the difference $[U_I(x) - U_{I^+}(x)]$.

This scheme can easily be generalized for a series of successive processes (Shaitan and Rubin 1982). We shall show below that both the amplitude and kinetic behavior observed can be interpreted within the framework of the scheme.

Diffusion along the conformation energy surface and the sharp parametric dependence of the rate of

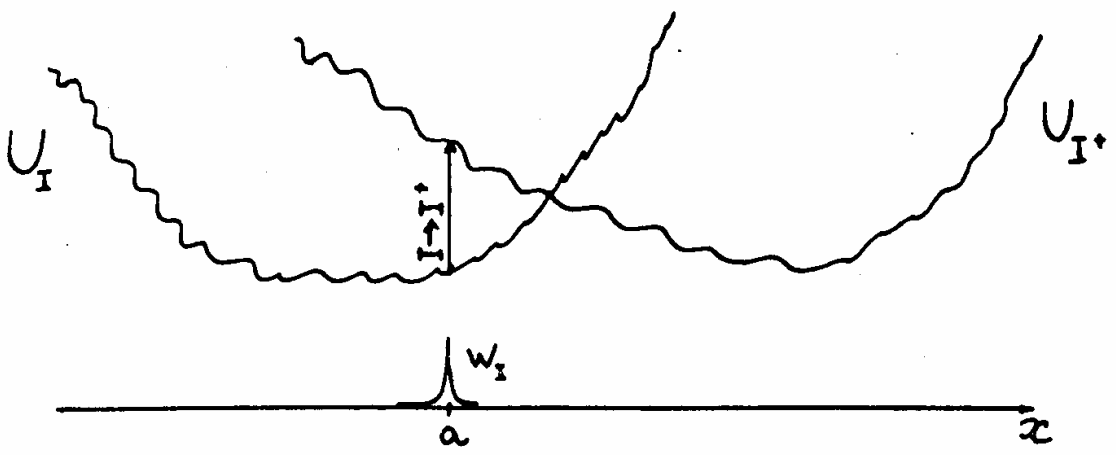

Fig. 4. Conformation energy surfaces for groups I and I^+. $W(x) \sim W_a(x - a)$. *The arrow shows the* I *to* I^+ *transition.*

a reaction on conformational coordinate may cause unusual effects.

Let us consider solution to Eqn. (1) with initial condition $P(x, 0) = \delta(x - x_1)$. Denote it $P(x, t|x_1, 0)$. The quantity

$$G(x_1, t) = \int_{-\infty}^{+\infty} P(x, t|x_1, 0)dx \tag{2}$$

is the probability that particles located initially at point x_1 will not enter the reaction at time (t). (For convenience of presentation, here and in what follows the number of particles is the number of groups that did not undergo the reaction. The particle disappears after the reaction has occurred.) The mean time of the reaction is then expressed as

$$\langle t, x_1 \rangle = -\int_0^{\infty} t \frac{\partial G}{\partial t} dt. \tag{3}$$

If at $t = 0$, the distribution of the particles along the coordinate is $P_0(x_1)$, the mean time of the process will be

$$\langle t \rangle = \int_{-\infty}^{+\infty} \langle t, x_1 \rangle P_0(x_1)dx_1. \tag{4}$$

Let us determine conditions under which the amplitude of the reaction decreases, a situation where some amount of particles do not reach the "active zone" at which the reaction can occur ($x = a$), i.e., they "stick" somewhere in the route. This is a situation for which the mean time of the reaction, (3) or (4), tends to infinity. With the diffusion coefficient $D \neq 0$ and a smooth profile of the conformation potential $U_0(x)$, such a behavior of the system is impossible. However, the situation changes drastically in case of a random rough potential (Fig. 5) in which the particle may be localized. An idea that the conformation potential has such a profile has come earlier in interpreting the Mössbauer spectra of proteins (Frauenfelder et al. 1979, Shaitan and Rubin 1980, Bauminger et al. 1983, Knapp et al. 1983).

We shall explain the situation in a simple example. Assume that at point $x = a$ the rate of disappearance of particles, as a result of relaxation, is very high. Let $\langle t, x_1 \rangle$ denote the mean first passage time to point a. This time is expressed as (Gardiner 1985):

$$\langle t, x_1 \rangle = \int_{x_1}^{a} dy \frac{e^{\beta U(y)}}{D(y)} \int_{-\infty}^{y} e^{-\beta U(z)} dz. \tag{5}$$

For certainty, we take $x_1 < a$. Let the conformational potential be a sum of the smooth $U_0(x)$ and a random function $u(x)$ of a "ridge"-like profile

$$U(x) = U_0(x) + u(x). \tag{6}$$

The random function $u(x)$ at each point has the distribution $\rho_x(u)$. It can be shown (Zwanzig 1988) that for this situation Eqn. (5) is identical to the formula using the re-normalized potential and diffusion coefficient

$$\langle t, x_1 \rangle = \int_{x_1}^{a} dy \frac{e^{\beta U^*(y)}}{D^*(y)} \int_{-\infty}^{y} e^{-\beta U^*(z)} dz \tag{5a}$$

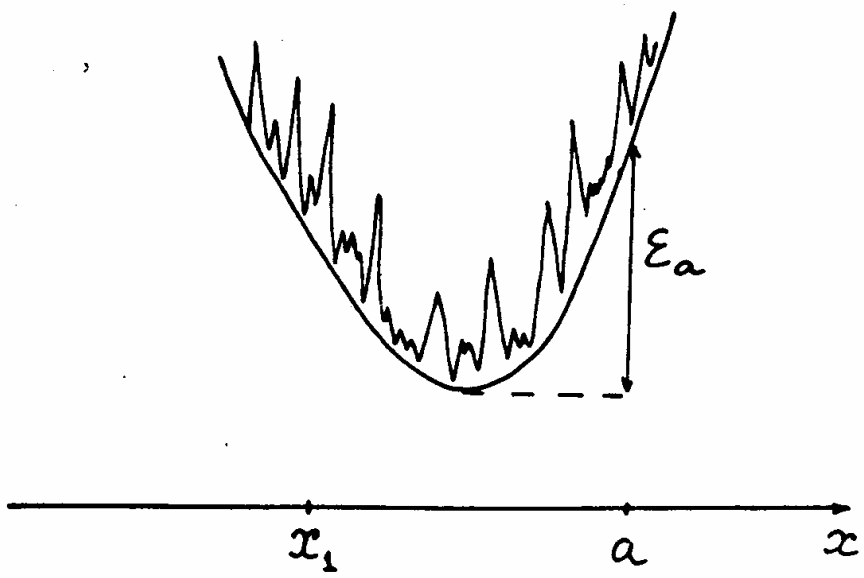

Fig. 5. Stochastic "ridge"-like potential profile. $U(x) = U_0(x) + u(x)$. a is the drain coordinate; x_1 is the initial position of the particle.

where

$$U^*(x) = U_0(x) - k_B T\psi^-(x) \quad (7)$$

$$D^*(x) = D\,e^{-[\psi^+(x)+\psi^-(x)]} \quad (8)$$

$$e\psi^{\pm}(x) = \langle e^{\pm\beta U(x)} \rangle \quad (9)$$

$\langle \ldots \rangle$ denotes the averaging of the random function at point x. $D(x)$ in Eqn. (8) is an initial diffusion coefficient for diffusion along a smoothly varying potential and is taken to be independent of T. The potential barrier height distribution function may be presented in a general form as

$$\rho_x(u) = [\varepsilon(x)]^{-\alpha} \frac{u^{\alpha-1}}{\Gamma(\alpha)} e^{-u/\varepsilon(x)} \quad (10)$$

$\varepsilon(x)$ characterizes the decay steepness of the potential barrier height distribution. Using Eqns (8)–(10) one obtains an expression for the diffusion coefficient.

$$D^*(x) = \quad (11)$$

$$\begin{cases} D(1 - T_0^2(x)/T^2)^x; & T > T_0(x) \\ 0; & T \leqslant T_0(x); \quad T_0(x) = \varepsilon(x)/k_B. \end{cases}$$

At around the critical temperature (T_0 is the inflexion point on the temperature dependence at Fig. 3A), the diffusion coefficient D^* is $D^* \sim (T - T_0)^x$. This means that for a one-dimensional motion —(for instance, rotation of some group in the field of a random potential) at a temperature below the critical temperature $T < T_0(x)$ which, generally speaking, depends on the position of the particle — the particle becomes localized. The situation is termed as "freezing" of the degree of freedom, or conventionally, as "phase transition". The conventionality of the latter means that this transition occurs without signficant change in the heat capacity of the system.

Thus for a situation depicted in Fig. 5 particles located to the left of point x_1, for which D^* is zero, cannot reach the drain region $x = a$ and do not particpate in the reaction.

This model may provide a key to the understanding of both the amplitude and kinetic effects simultaneously associated with low temperature cytochrome *c* oxidation. It can be shown (Shaitan and Rubin 1982) that for a system being localized at point x_1 at the onset the characteristic time of the reaction is the sum of the time of the chemical reaction itself, W^{-1}, and the mean diffusion time of the reaction zone:

$$\tau = \frac{1}{k(x_1)} = \langle t, x_1 \rangle + \frac{1}{W}. \quad (12)$$

Equation (12) should be slightly corrected for a situation when the configuration favourable for the occurrence of the reaction zone has a lower energy than that of x_1 but is on the same slope of the $U^*(x)$ potential profile and simultaneously $W \ll 1/\langle t, x_1 \rangle$. However, this has no effect on the final result and will be omitted from consideration.

We shall now turn to a simplified situation when the parameter α in distribution (10) is constant and the mean height of the potential barriers between x_1 and a (Fig. 5) does not increase. According to (5) the temperature dependence of $\langle t, x_1 \rangle$ in this case takes the form:

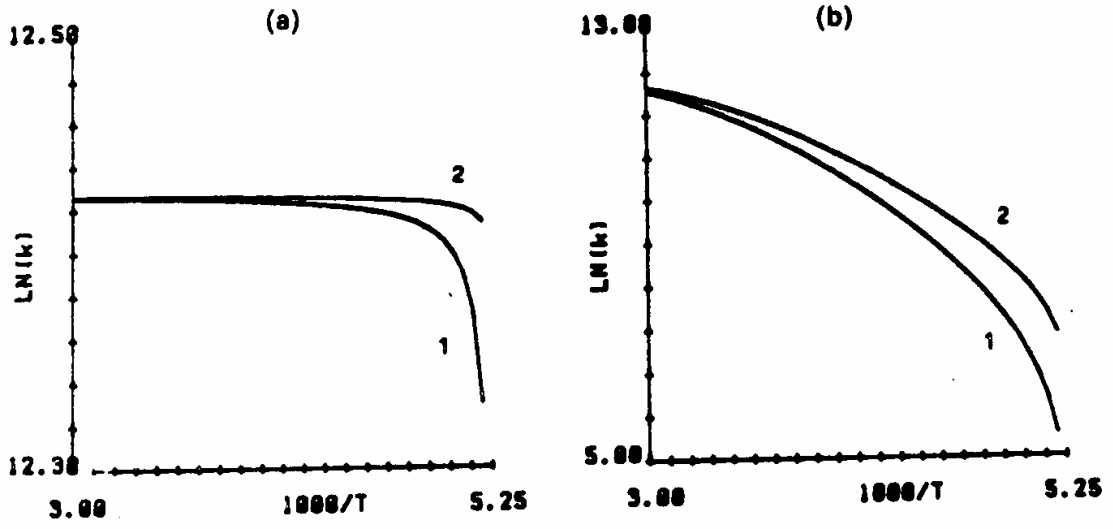

Fig. 6. The temperature dependence of the rate of cytochrome *c* oxidation derived from Eqns. (12) and (13). (A) $\varepsilon_a = 0$; (B) $\varepsilon_a = 15$ kJ/mol; (1) $\alpha = 1$; (2) $\alpha = 0.5$.

$$\langle t, x_1 \rangle \simeq \tau_0 (1 - T_0^2(x_1)/T^2)^{-\alpha} e^{\varepsilon_a / k_B T} \qquad (13)$$

where τ_0 is the characteristic conformation relaxation time at room temperature and $\varepsilon_a = U^*(a)$. τ_0 takes a value of 10^{-8}–10^{-10} s as estimated by different methods (Frauenfelder et al. 1979, Shaitan and Rubin 1980, Bauminger et al. 1983, Knapp et al. 1983, Shaitan and Uporov 1986, Krupyanskii et al. 1987, Rubin et al. 1987). Figure 6 shows the temperature dependence $k(T)$ calculated from Eqn. (12) for $T_0 = 190$ K, $\tau_0 = 10^{-8}$ s, $W = 10^6$ s^{-1} or 2.5×10^5 s^{-1} for $\varepsilon_a = 0$ and $\varepsilon_a = 15$ kJ/mol.

In all the cases, there is a very sharp threshold at which the reaction ceases. The reaction may require activation energy or not, depending on the position of the zone favorable for the reaction on the potential curve. For $\varepsilon_a = 0$, the rate of the reaction is constant at $T > T_0$ because the condition $\tau_0 \ll 1/W$ is fulfilled. It is worth noting that a rather weak temperature dependence of the conformational relaxation time has recently been observed for individual modes in human serum albumin by Mössbauer Fourier spectroscopy (Basovets et al. 1988, Basovets et al. 1989, Uporov et al. 1989).

The above model provides a consistent interpretation of the effect of temperature on the amplitude and kinetic pattern of reactions for instance, the oxidation reaction of the cytochrome complex $C_h C_l$. Let us turn to a situation where the fast oxidation of the high-potential heme is a conformation-controlled reaction. This is quite possible as a result of motion of one aromatic amino acid residue I (for instance tyrosine (Deisenhofer et al. 1985, Knapp and Fischer 1987)). A bridge is formed by π–π-orbital overlapping and the efficiency of the reaction being largely dependent on its orientation (Kuhn 1986). Assume that the position of this bridge is most favourable for

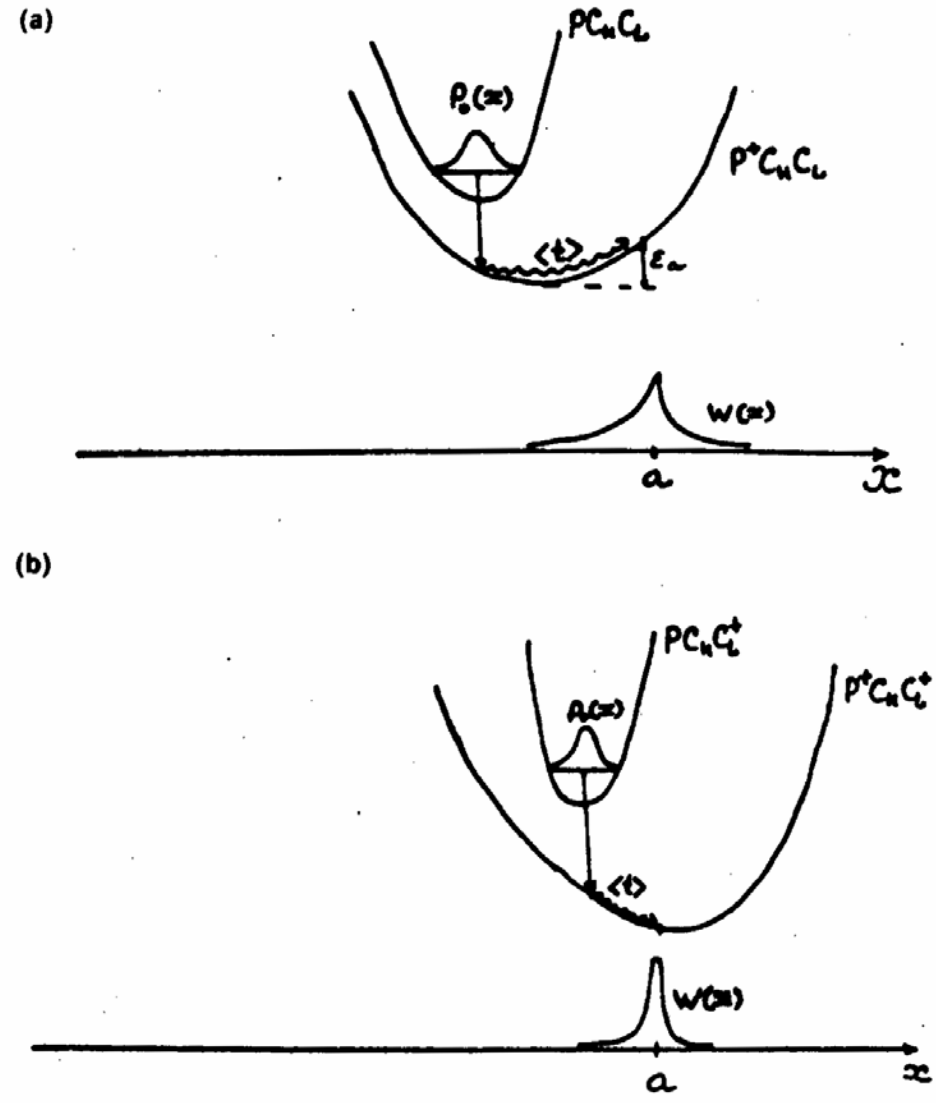

Fig. 7. Change in the conformation energy surface of group I following the photooxidation of the reaction center pigment for different charge states of the hemes. a is the reaction zone in which fast photooxidation of C_h occurs.

the electron-transfer reaction when its conformation coordinate is $x = a$ (Fig. 7). Another aspect which must be taken into account is the change in the equilibrium position of group I caused by a change in the charged state of pigment P and hemes. One possible situation is illustrated by Fig. 7.

A change in the charged state of the pigment shifts an equilibrium of group I. For I to enter the reactive zone "a" (Fig. 7A), an activation energy of ε_a is required. Oxidation of the low-potential hemes C_l shifts the equilibrium of group I ultimately in such a way that the reaction zone appears in the minimum of the lower potential curve for the $P^+C_hC_l^+$ state (Fig., 7B).

In the first case (Fig. 7A), for the system to enter the reaction zone, a conformational strain or upward motion along the potential curve are necessary. It determines the temperature dependence of the reaction rate for which the activation energy is required. A much slower but temperature independent oxidation at $T < T_0$ (Fig. 3A, curve 2) corresponds to the particle being beyond the "active zone" where the reaction between C_h and P^+ proceeds due to the "tail" of $W(x)$ distribution. An independent route may be also possible to fill the hole on P^+ by an electron from C_l. In the second case (Fig. 7B), the reactive configuration can be attained through temperature independent relaxation to the potential minimum only. This is a situation in which the rate of the reaction is temperature independent when $T > T_0$ (Fig. 3A, curve 1). At $T < T_0$, particle is localized or the conformational mode becomes "frozen" and no reaction can occur. Here in contrary to the case on Fig. 7A the reaction beyond the active zone does not proceed alone due to the strong Coulomb interaction between the electron on C_h and C_l^+.

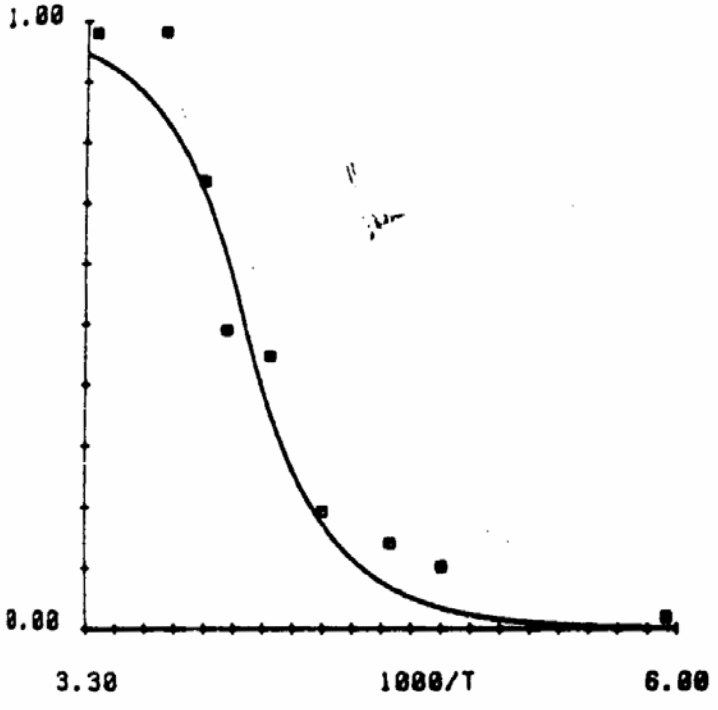

Fig. 8. The temperature dependence of the amplitude signal derived from Eqns. (14)–(17) for a linear potential profile. Dots —experimental data (see Fig. 3B, curve 1).

Let us consider this amplitude drop effect within the simplest model. The flash-induced oxidation of the pigment projects on the lower potential curve a distribution function $P_0(x)$ for group I (Fig. 7B). For a given temperature T, the reaction "zone" may be reached only by particles located to the right of the critical coordinate x_1 (Fig. 5), for which $T_0(x_1) = T$. It is easy to see that the amplitude of the reaction is

$$A(T) = \int_{x_1(T)}^{\infty} P_0(x)\,dx. \tag{14}$$

It is then assumed that the mean potential barrier height reduces linearly toward the potential minimum

$$T_0(x) \simeq (a - x)/\eta. \tag{15}$$

Then

$$x_1(T) \simeq \eta(T^* - T). \tag{16}$$

The T^* value is determined from the condition $A(T^*) = 1/2$. For the simplest case when the potential changes linearly $U(x) = \xi|x|$, one obtains

$$A(T) = \begin{cases} 1 - \frac{1}{2}B^{[(T^*/T)-1]}; & T \geqslant T^* \\ \frac{1}{2}B^{[(1-(T^*/T)]}; & T < T^* \end{cases} \tag{17}$$

where $B = e^{\eta\xi/k_B}$. The dependence $A(T)$ for $T^* = 245$ K and $B = 2000$ is shown in Fig. 8. The obtained results correspond to a quite reasonable physical situation in which, for instance, the conformational energy $U_0(x)$ increases by 10 kcal/mol in response to the Δx shift (for instance, 0.5 Å) and mean barrier heights increase by approximately 1.5 kcal/mol, when group I is shifted by the same amount. It is not our goal to get a fit to the experimental $A(T)$ and $k(T)$ dependences because much uncertainty exists with regard to the nature of the conformational mode that controls the reaction. The main point of the model is to describe the

mechanism of coupling between the chemical reaction and diffusion in a random-potential field. This provides a single consistent physical picture within which the effect of temperature on both the kinetic and amplitude features can be well accommodated. The most complicated aspect of this model is the nature of the randomly-behaved potential, or the random "ridge"-like potential pattern with a wide distribution of the heights of the potential barriers. It should be emphasized that atomic vibrations with amplitudes higher than those of valent vibrations (~ 0.1 Å) cannot occur without the re-adjustment of the environment. The configurations, and the corresponding energies, occurring in such a complex system as proteins hardly are strictly deterministic, which forms a basis for the developed phenomenological model.

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