Biochimica et Biophysica Acta, 589 (1980) 151—155

BBA Report

BBA 41322

THE OXIDATION RATE OF HIGH-POTENTIAL *c*-TYPE CYTOCHROME IN THE PHOTOCHEMICAL REACTION CENTRE IS TEMPERATURE-INDEPENDENT

S.K. CHAMOROVSKY, A.A. KONONENKO, S.M. REMENNIKOV and A.B. RUBIN

Biology Department, Moscow State University, Moscow 117234 (U.S.S.R.)

(Received October 1st, 1979)

Key words: Bacterial photosynthesis; Cytochrome c; Oxidation kinetics; Temperature dependence

Summary

The temperature dependence of laser-induced (694.3 nm, 30 ns, 10 mJ·cm^{-2}) high-potential cytochrome *c* (E_m = +290 mV) oxidation kinetics was studied in *Ectothiorhodospira shaposhnikovii* chromatophores. It was shown that the rate constant of this reaction is independent of temperature in the range of 300 K to 120 K.

Chance and Nishimura [1] found that photoinduced oxidation of cytochrome *c* in whole cells of *Chromatium* strain D can occur at 80 K. In later works [2—4], low-temperature redox reactions of *c*-type cytochromes have also been observed in cells of some other photosynthesizing bacteria.

Chance and de Vault [5,6] were first to measure the temperature dependence of the rate of laser-induced oxidation of cytochrome *c* in *Chromatium* D. The rate constant for the reaction decreases exponentially with an activation energy of 3.3 kcal/mol on lowering the temperature from 300 K to 93 K, but then the rate remains unchanged down to liquid helium temperatures. The half-time for the reaction is 2 μs at 300 K and 2 ms below 123 K. A similar complex temperature-dependence has subsequently been observed in some other species of photosynthesizing bacteria [7,8].

These data have been made the basis for the quantum-mechanical theories of the kinetics of electron transfer in the photochemical reaction centre [5,9—11].

It is well established that in cells of sulphur purple bacteria there are two cytochromes *c*, high-potential and low-potential, which are both oxidized by a single reaction centre [12,13]. For this reason it has been suggested in

early studies that the complex biphasic temperature dependence indicates involvement of two different reaction mechanisms in the photo-induced oxidation of both low- and high-potential cytochromes or only the low-potential cytochrome [5,14]. The latter view is generally accepted now in theoretical papers [9—11]. It is supported experimentally by a preference for the low-potential haems to be oxidized on a laser flash at room temperature, when the high-potential haems are initially also maintained in reduced form [12,13]. However, it remains to be proved whether the preference for the low-potential cytochrome *c* oxidation by the reaction centre holds over a wide temperature range as well. Also unclear is the possible contribution to the reaction of the high-potential haems.

The selective photooxidation of high-potential cytochrome with bacteriochlorophyll (*P*-890) in the reaction centre is easy to observe after 'switching off' low-potential cytochrome *c* by chemical oxidation. This reaction has already been studied under a steady-state activation with a spectroscopic technique of a low time resolution (approx. 0.1 s). It was shown in experiments with *Ectothiorhodospira shaposhnikovii* [3] and *Rhodopseudomonas gelatinosa* [4] that under appropriate redox conditions some high-potential cytochrome *c* undergoes a reversible photooxidation even at 80 K. But the temperature dependence of the rate constant for cytochrome oxidation has not been measured.

This problem was the main object of the present work. It was done on bacterial chromatophores under oxidizing conditions (poised at E_h = +180 + 250 mV) where high-potential cytochrome was the only constitutive electron donor to the reaction centre. The results reported here show that the rate constant of the laser-induced oxidation of high-potential cytochrome is independent of temperature at least over the range 300 to 120 K.

Chromatophores were isolated after ultrasonic disruption of 2—3-day-old cells of *E. shaposhnikovii.* Kinetic measurements of photoinduced absorbance changes at controlled redox potential of the reaction medium were done on a pulse laser spectrophotometer as described elsewhere [15]. For low temperature experiments, chromatophores in 0.05M Tris-HCl buffer (pH 7.6) were mixed with glycerol (up to 80—90% v/v).

We found that in such samples cytochrome oxidation on single laser pulse (694.3 nm; 30 ns, 10 $mJ \cdot cm^{-2}$) occurs over a wide temperature range. Fig. 1 shows kinetic traces of the laser-induced oxidation of high-potential cytochrome at 298 and 235 K. Absorbance changes were measured at 424 nm. The signal at this wavelength has a biphasic character. The fast absorbance increase is caused by *P*-890 oxidation, which has a spectral component *P*-440 in this spectral region [13]. Due to insufficient resolving time of our spectrophotometer, we were not able to resolve *P*-440 kinetics. The time course of the kinetics (approx. 0.1 μs) is the characteristic resolving time of the spectrophotometer. The slow absorbance decrease is a reflection of cytochrome *c* oxidation [13]. Between 300 and 200 K the extent of the cytochrome oxidation is independent of temperature (Fig. 2a) and the reaction is reversible. At much lower temperatures, however, the amount of cytochrome photooxidation diminishes (Fig. 2a), following

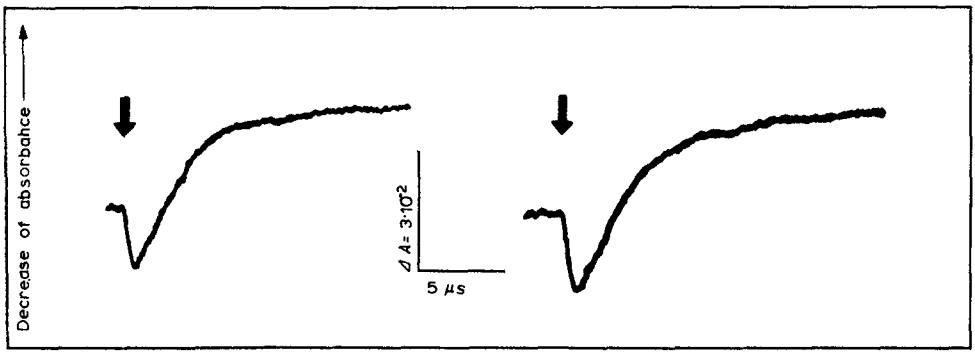

Fig. 1. Kinetic traces of laser-induced (694.3 nm, 30 ns, 10mJ•cm^{-2}) absorbance changes at 424 nm in *E. shaposhnikovii* chromatophores. Chromatophores were poised at redox potentials from +250 to +180 V and suspended at 80—90% glycerol before freezing. Arrows indicate the time of a laser flash. A, at 298 K; B, at 235 K.

the logarithm of amplitude vs. $1/T$ dependence, formally corresponding to an activation energy, E_a, of approx. 1.3 kcal/mol (Fig. 2b).

The half-time of the laser-induced cytochrome oxidation, measured at room temperatures, was 2.5 ± 0.3 μs (Fig. 1). This estimate is in agreement with data on whole cells and chromatophores of *E. shaposhnikovii* in water-buffer suspension without glycerol [13,15], and thus one may conclude that considerable changes of viscosity in the reaction medium (80—90% of glycerol in this case) do not influence the kinetics of the process studied.

We also found that the rate of laser-induced cytochrome oxidation remains practically constant under temperature lowering, at least down to 120 K where the amplitude of the signal is still high enough to allow accurate kinetic determinations (Fig. 3). This observation is quite opposite to that seen for the low potential cytochrome in cells of *Chromatium* D [5] and *E. shaposhnikovii* [7], which showed a marked slowing down of the reaction with decreasing temperature from 300 to 123 K. It is worth

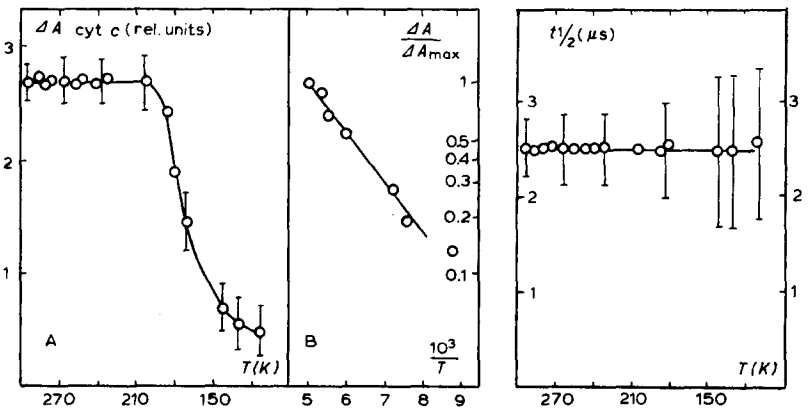

Fig. 2.A. Temperature dependence of laser-induced oxidation of high-potential cytochrome in *E. shaposhnikovii* chromatophores. A new dark-adapted sample was taken for each temperature point. Experimental conditions as in legend to Fig. 1. B. The same curve as a log amplitude vs. $1/T$ plot.

Fig. 3. Temperature dependence of half-time of laser-induced oxidation of high-potential cytochrome in *E. shaposhnikovii* chromatophores. Experimental conditions as in legend to Fig. 1.

noting in this respect that both high- and low-potential cytochromes are similar in situ in their spectral characteristics. The difference between them lies in their midpoint redox potentials (near +290 mV for high-potential, but 0 + +10 mV for low-potential cytochromes [15,16]. In the case of *E. shaposhnikovii* the bias in their electronic donor-acceptor levels with respect to *P*-890 ($E_{m7,0}$ = + 390 mV [15] is 0.4 eV and 0.1 eV low- and high-potential cytochromes, respectively. This may possibly be the reason for a marked difference in the temperature dependence of the oxidation rates of the two cytochromes.

Recent results of picosecond fluorescence [17] and absorption [18] studies suggest little if any temperature sensitivity of the primary events of bacterial photosynthesis, which are delivery of the electronic excitation to the reaction centre and the separation of charges in this pigment-protein complex. These steps preceed the oxidation of *c*-type cytochromes. Thus interactions involved in the initial electron-transfer steps may be 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 act in the closely associated porphyrin pigments of the reaction centre [18]. However, if a redox process is taking place between the two macromolecular systems, for example between the reaction centre and the cytochrome *c*, direct contact of their prosthetic groups (*P*-890 and haem) seems improbable. We suggest that the appropriate electron pathway can be formed by specific conformations of the reaction centre and the high-potential cytochrome *c* proteins. Breaking of conformational dynamics of macromolecular components below some threshold temperature (approximately 200 K) may be the reason for the observed inhibition of the high-potential cytochrome *c* oxidation. Such a pattern of temperature dependence for mobility of membrane components was recently obtained in experiments with spin- and ^{57}Fe-labelled bacterial chromatophores [19].

References

1 Chance, B. and Nishimura, M. (1960) Proc. Natl. Acad. Sci. U.S.A. 46, 19—24
2 Kihara, T. and Chance, B. (1969) Biochim. Biophys. Acta 189, 116—124
3 Grigorov, L.N., Kononenko, A.A. and Rubin, A.B. (1969) Proc. Acad. Sci. U.S.S.R., Ser. Biol. 3, 448—451
4 Dutton, P.L. (1971) Biochim. Biophys, Acta 226, 63—80
5 De Vault, D. and Chance, B. (1966) Biophys. J. 6, 825—867
6 De Vault, D. Parkes, J.H. and Chance, B. (1967) Nature 215, 642—644
7 Remennikov, S.M. (1974) Thesis, 5th Symp. Methods Res. Photosynthetic Electron Transport (Akulova, E.A., ed.), pp. 172—183, Puschino
8 Thornber, J.P., Dutton, P.L., Fajer, J., Forman, A., Holten, D., Olson, J.M., Parson, W.W., Prince, R.G., Tiede, D.M. and Windsor, M.W. (1977) Proc. 4th Int. Congr. Photosynthesis (Hall, D.O., Coombs, J. and Goodwin, T.W., eds.)
9 Grigorov, L.N. and Chernavskii, D.S. (1972) Biophys. (U.S.S.R.) 17, 195—202
10 Hopfield, J.J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3640—3644
11 Jortner, J. (1976) J. Chem. Phys. 64, 4860—4867
12 Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232—245
13 Kononenko, A.A., Remennikov, S.M., Rubin, A.B., Rubin, L.B., Venedictov, P.S. and Lukashev, E.P. (1973/74) J. Photochem. 2, 371—376
14 Chance, B. (1974) Ann. N.Y. Acad. Sci. 227, 613—626
15 Remennikov, S.M., Chamorovsky, S.K., Kononenko, A.A., Venedictov, P.S. and Rubin, A.B. (1975) Studia Biophys. 51, 1—13

16 Remennikov, S.M., Chamorovsky, S.K., Kononenko, A.A. and Rubin, A.B. (1976) Studia Biophys. 60, 15—33
17 Kononenko, A.A., Korvatovsky, B.N., Paschenko, V.Z., Rubin, A.B., Rubin, L.B. and Tusov, V.B. (1979) Rep. Acad. Sci. U.S.S.R. 242, 1421—1424
18 Peters, K., Avorius, P. and Rentzepis, P.M. (1978) Biophys. J. 23, 207—217
19 Berg, A.I., Knox, P.P., Kononenko, A.A., Frolov, E.N., Chrymova, I.N., Rubin, A.B., Likhtenshtein, G.I., Goldansky, V.I., Parak, F., Bucl, M. and Mossbauer, R. (1979) Mol. Biol. (U.S.S.R.) 13, 81—89