BBA 43177

Rhodopseudomonas spheroides cytochrome c_2

The occurrance of soluble c_2-type cytochromes in photosynthetic bacteria has been well established. This report concerns the isolation, purification and some properties of the c_2-type cytochrome from *Rhodopseudomonas spheroides*.

The methods established by KAMEN and co-workers[1,2] were employed with some modifications. Lyophilized powders of 48 h cells grown photosynthetically in culture medium described by COHEN-BAZIRE, SISTROM AND STANIER[3] were extracted overnight with one of the following buffer systems (1:10, w/v): 0.1 M Tris–HCl, pH 8.0; 0.1 M sodium citrate, pH 5.4; 0.1 M sodium phosphate, pH 7.5, or 0.1 M sodium acetate, pH 5.2. Final yields of cytochrome c_2 were comparable irrespective of the extracting buffer. The extract was centrifuged at 18000 $\times$ *g* for 20 min, then at 100000 $\times$ *g* for 90 min and the supernatant fraction containing cytochrome c_2 was dialyzed against several changes of 0.01 M Tris–HCl buffer, pH 7.5, at 4°. The dialysate

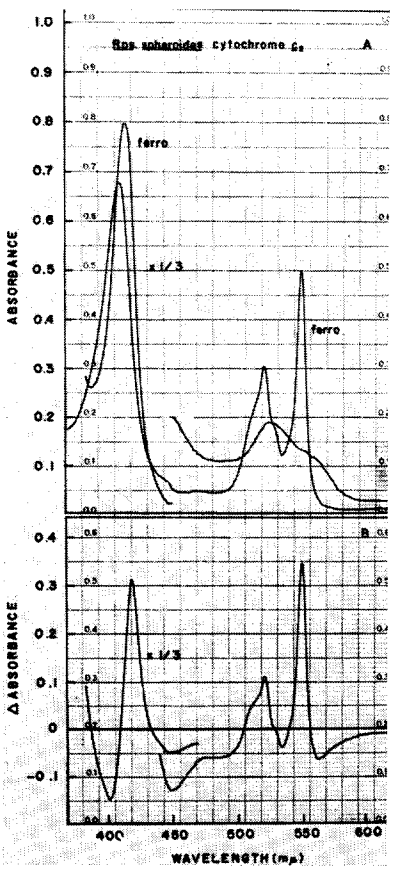

Fig. 1. Absolute spectra (A) and difference spectra (B) of ferri- and ferro-cytochrome c_2. Spectra were determined in 0.05 M potassium phosphate buffer, pH 7.0, with a Cary Model 14 spectrophotometer. The concentration of cytochrome c_2 for the visible region was 20.3 μmoles and 6.78 μmoles for the Soret region.

was charged onto a 4 cm by 15 cm column of DEAE-cellulose (Selectal, Type 20, Brown Paper Company, Berlin, N.H.) equilibrated to pH 7.5 with 0.01 M Tris–HCl. Cytochrome c_2 was adsorbed at the top of the column and was eluted with 0.15 M Tris–HCl, pH 7.5. The eluate was pooled and concentrated on DEAE. After elution with 0.2 M Tris–HCl, pH 7.5, the concentrated cytochrome c_2 fraction was brought to 80 % ammonium sulfate saturation to remove cytochromoid C. Cytochrome c_2 was precipitated by raising the concentration of ammonium sulfate to 100 %. The cytochrome c_2 pellet was solubilized in a minimal volume of water and the ammonium sulfate treatment repeated until the supernatant fraction was free of cytochromoid C. This procedure resulted in a cytochrome c_2 sample of high purity and crystallization was simple to accomplish. The purity index of a 2 times recrystallized sample of cytochrome c_2 was $A_{550\,m\mu}/A_{275\,m\mu} = 0.90$.

The absorption spectra presented in Fig. 1 are typical of c_2-type cytochromes[4] with absorption maxima for the reduced form at 550 mμ, 522 mμ and 416 mμ. The oxidized form is characterized by a broad absorption band at 525 mμ and a Soret maximum at 411 mμ.

In Table I, data are presented for the molar extinction coefficients at pH 7.0. The values were calculated in terms of heme content by forming the alkaline pyridine haemochromogen. One heme per mole was assumed and the $\varepsilon_M \times 10^{-3}$ l/mole·cm = 29 for horse heart cytochrome *c* at 550 mμ (ref. 5) was used in the calculations. The molecular weight was estimated by gel filtration on Sephadex G-100 according to the method of ANDREWS[6] and found to be 12400. *Rps. spheroides* cytochrome c_2 is a high potential cytochrome and the mid-point oxidation–reduction potential of +0.342 V was calculated by titration against buffered ferri–ferrocyanide[7].

The results presented here indicate that the c_2-type cytochrome extracted from light-grown *Rps. spheroides* has physical and chemical properties similar to those of

TABLE I

MOLAR EXTINCTION COEFFICIENTS OF ABSORPTION MAXIMA OF *Rps. spheroides* CYTOCHROME c_2 (pH 7.0)

	Wavelength (*mμ*)		$\varepsilon_M \times 10^{-3}$ (*l/mole·cm per heme*)
State of cytochrome			
ferri-cytochrome	560	shoulder	5.70
	525		9.37
	411		106.0
ferro-cytochrome	550		24.6
	522		15.1
	416		117.0
Difference spectrum			
ferro- —ferri-cytochrome	564	trough	−3.0
	550		18.2
	534.8	trough	−1.7
	522		5.9
	448	trough	−6.9
	420		45.3
	402	trough	−28.0

the cytochrome c_2 isolated from the related photoheterotroph, *Rhodospirillum rubrum*[2].

This research was supported by Grant GB 3908 from the National Science Foundation.

Department of Biology, Boston College, Chestnut Hill, Mass. (U.S.A.) JOSEPH A. ORLANDO

1 M. D. KAMEN, R. G. BARTSCH, T. HORIO AND H. DE KLERK, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. VI, Academic Press, New York, 1963, p. 391.
2 T. HORIO AND M. D. KAMEN, *Biochim. Biophys. Acta*, 48 (1961) 266.
3 G. COHEN-BAZIRE, W. R. SISTROM AND R. Y. STANIER, *J. Cellular Physiol.*, 49 (1957) 25.
4 R. G. BARTSCH, in H. GEST AND A. SAN PIETRO, *Bacterial Photosynthesis*, Antioch Press, Yellow Springs, Ohio, 1963, p. 475.
5 D. DRABKIN, *J. Biol. Chem.*, 146 (1942) 605.
6 D. ANDREWS, *Biochem. J.*, 91 (1964) 222.
7 S. F. VELICK AND P. STRITTMATTER, *J. Biol. Chem.*, 221 (1956) 265.

Received June 23rd, 1967

Biochim. Biophys. Acta, 143 (1967) 634–636

BBA 43174

Electron paramagnetic resonance studies on the cytochrome oxidase from yeast

The availability of preparations of purified mammalian cytochrome oxidase (cytochrome $c:O_2$ oxidoreductase, EC 1.9.3.1) has stimulated an extensive chemical and physical investigation of the properties of this complicated and extremely important terminal oxidase (a collection of articles which documents much of this work is to be found in ref. 1). In addition to the two heme components, the enzyme also contains stoichiometric amounts of copper. EPR techniques have proved invaluable in documenting the presence, nature and functional role of the component[2–4], although it is well established that EPR only accounts for about one-half of the chemically detectable copper present in the protein[5].

Recently a highly purified preparation of cytochrome oxidase has been obtained from *Saccharomyces cerevisiae*[6] and it is the purpose of this communication to report on the results that we have obtained by EPR spectroscopy of this preparation. As reported previously[6], the preparations of cytochrome oxidase contain an average of 5.4, 6.3, 4.4 and 5.8 mμmoles of cytochromes $a + a_3$, copper, non-heme iron, and labile sulfide, respectively, per mg of protein and catalyze the oxidation of approx. 40 μmoles of ferrocytochrome *c* per min per mg of protein. More recently, however, preparations of enzyme have been prepared by passage of the final preparations through a sucrose gradient with specific activities as high as 80 to 90. Fig. 1A is the EPR spectrum of the oxidase as prepared in the most highly purified form. It resembles markedly that exhibited by beef-heart cytochrome oxidase and is not at all typical of conventional