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ISOLATION AND CHARACTERIZATION OF CYTOCHROME c_1 FROM PHOTOSYNTHETIC BACTERIUM *RHODOPSEUDOMONAS SPHAEROIDES* R-26

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SUMMARY-- Cytochrome c_1 of photosynthetic bacterium *R. sphaeroides* R-26 has been purified from isolated cytochrome b-c_1 complex to a single polypeptide, using a procedure involving Triton X-100 and urea solubilization, calcium phosphate column chromatography and ammonium sulfate fractionation. The purified protein contains 30 nmoles heme per mg protein and has an apparent molecular weight of 30,000, as determined by sodium dodecylsulfate polyacrylamide gel electrophoresis. Bacterial cytochrome c_1 is soluble in aqueous solution in the absence of detergent and has spectral characteristics similar to mammalian cytochrome c_1. The amino acid compositions of these two proteins, however, are not comparable.

Although the involvement of the c-type cytochromes in the photosynthetic electron transfer is well established, the participation of cytochrome c_1 in the cyclic photosynthetic electron transport system of *R. sphaeroides* was demonstrated only recently (1). The redox potential heterogeneity of cytochrome c_2 in the cytochrome b-c_2 complex was observed in 1975 (2). Recent developments in isolation and characterization of the cytochrome b-c_1 complex from photosynthetic bacteria have confirmed the existence of cytochrome c_1 and its essential role in photosynthetic electron transfer (3-5). A molecular weight of 30 thousand daltons for cytochrome c_1 was established by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) from lysed chromatophores or purified cytochrome b-c_1 complex from *R. sphaeroides* strain GA or R-26. The redox potential of cytochrome c_1 was estimated to be 290 mV (1, 4) in the membrane and 230 mV (3) in the isolated cytochrome b-c_1 complex. This is comparable to the redox potential of mammalian cytochrome c_1, which is 228 mV (6). Little is known about the molecular properties of the photosynthetic

Abbreviations: SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis

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bacterial cytochrome $\underline{c}_1$, as isolation of the active cytochrome was not attempted until recently. Taking advantage of the availability of the highly purified bacteria cytochrome $\underline{b}$-$\underline{c}_1$ complex in our laboratory, we have developed a simple procedure for isolation this cytochrome $\underline{c}_1$. In this communication, we report the isolation procedure and some properties of this purified cytochrome $\underline{c}_1$.

MATERIALS AND METHODS

The cell culture of R. sphaeroides R-26, was a gift from Drs. Okamura and Feher, Dept. of Physics, UCSD. The growth conditions were exactly as reported (7). The preparation of chromatophores and the cytochrome $\underline{b}$-$\underline{c}_1$ complex were carried out according to method developed recently (3). sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (8), determination of cytochrome content (6) and protein (9) were performed by the reported methods. Spectral measurements were done in a Cary spectrophotometer, model 219.

RESULTS AND DISCUSSION

Purification of Cytochrome $\underline{c}_1$ from Purified Cytochrome $\underline{b}$-$\underline{c}_1$ Complex-- The cytochrome $\underline{b}$-$\underline{c}_1$ complex, isolated from R. sphaeroides R-26, was dialyzed against 50 mM Tris-acetate buffer, pH 7.8, overnight, with one change of buffer, to remove phosphate and glycerol. The dialyzed cytochrome $\underline{b}$-$\underline{c}_1$ complex (3.4 ml) was precipitated with 50% ammonium sulfate saturation. The precipitates were collected by centrifugation and redissolved in 1.7 ml 50 mM Tris-acetate buffer, pH 7.8, containing 1.5% Triton X-100 and 2 M urea. The protein concentration was approximately 5 mg/ml. The solution was incubated in ice for 20 min and frozen at - 20 ^{o}C for one hour. The frozen cytochrome $\underline{b}$-$\underline{c}_1$ complex was thawed and applied to a column (0.8 x 4.0 cm) packed with a calcium phosphate:cellulose (1:1) mixture and equilibrated with 50 mM Tris-acetate buffer, pH 7.8, containing 1.5% Triton X-100 and 2 M urea. The column was then washed with 8 ml of 25 mM phosphate buffer, pH 7.4, containing 0.25% sodium cholate. Under these conditions, cytochrome $\underline{b}$ and other unwanted proteins were not absorbed by the calcium phosphate column and appeared in the effluent. The washing step serves the purpose of separating cytochrome $\underline{b}$ from cytochrome $\underline{c}_1$, and replaces the Triton X-100 with sodium cholate. After washing, crude cytochrome $\underline{c}_1$ was eluted out by 0.2 M K-phosphate buffer, pH 7.4, containing 1% sodium cholate. The cytochrome $\underline{c}_1$ fractions were combined (2.1 ml) and brought to 20% ammonium

sulfate saturation by adding 0.107 gm ammonium sulfate per ml solution. The mixture was stirred at 0^{o} for 20 min before being centrifuged at 40,000 x g for 20 min to remove the resulting precipitates. The supernatant solution obtained was brought to 30% ammonium sulfate saturation by adding 0.143 ml saturated ammonium sulfate solution per ml and the precipitate formed was again removed by centrifugation. The supernatant solution, which contains cytochrome c_1, was then brought to 45% ammonium sulfate saturation by adding 0.27 ml saturated ammonium sulfate solution, and the cytochrome c_1 was recovered in the precipitates after centrifugation. The collected cytochrome c_1 was dissolved in 0.12 ml of 50 mM K-phosphate buffer, pH 7.4, and stored at - 70 ^{o}C until use. A summary of purification data is given in Table I. About 27% of the cytochrome c_1 present in the cytochrome b-c_1 complex was recovered in the final purified preparation.

Properties of Purified Cytochrome c_1-- Purified cytochrome c_1 is soulble in aqueous solution in the absence of detergent. The preparation contains 30 nmoles cytochrome c_1 per mg protein, and shows only one protein band in the SDS-polyacrylamide gel electrophoresis. The electrophoretic mobility of the isolated bacterial cytochrome c_1 is the same as that of the mammalian cytochrome c_1, which has a molecular weight of 30,000, determined by SDS-PAGE and 27,874 by amino acid sequence. Fig. 1 shows the SDS-PAGE pattern of cytochrome c_1. Purified cytochrome b-c_1 complex is also included for comparison. In contrast

Table I. Summary of Purification Data

Treatment	Volume	Protein	Cytochrome c_1	
			Concentration	Recovery
	ml	mg/ml	μM	%
Cytochrome b-c_1 Complex	3.4	2.7	20	100
Triton X-100 & Urea Treatment	1.7	5.0	35	88
Calcium Phosphate Column Eluate	2.1	1.4	18	55
$AmSO_4$ Fractionation, 30-45% Sat'd	0.12	5.1	154	27

to mammalian cytochrome $\underline{c}_1$, which until recently has been isolated in a two-polypeptide form, the bacterial cytochrome $\underline{c}_1$ contains only one polypeptide. This suggests that the active cytochrome $\underline{c}_1$ is indeed one polypeptide, although the single polypeptide mammalian cytochrome $\underline{c}_1$ preparation is seldom obtained.

Figure 2 shows the spectral properties of purified cytochrome $\underline{c}_1$. The oxidized form shows a Soret absorption at 409 nm with a shoulder at 360 nm. Similar to mammalian cytochrome $\underline{c}_1$, the spectral characteristics at the α- and β- regions are less defined. Upon reduction by ascorbate, the α-absorpiton at 552.5 nm and β-absroption at 522 nm with a shoulder at 530 nm, were observed. The Soret absorption maximum of the reduced protein was at 417 nm.

Cytochrome $\underline{c}_1$ as prepared is fully ascorbate reducible, and addition of sodium dithionite does no further reduction. This suggests that the isolated protein is in the fully active state and is thus suitable for study of the electron transfer reaction between the electron donating or accepting components

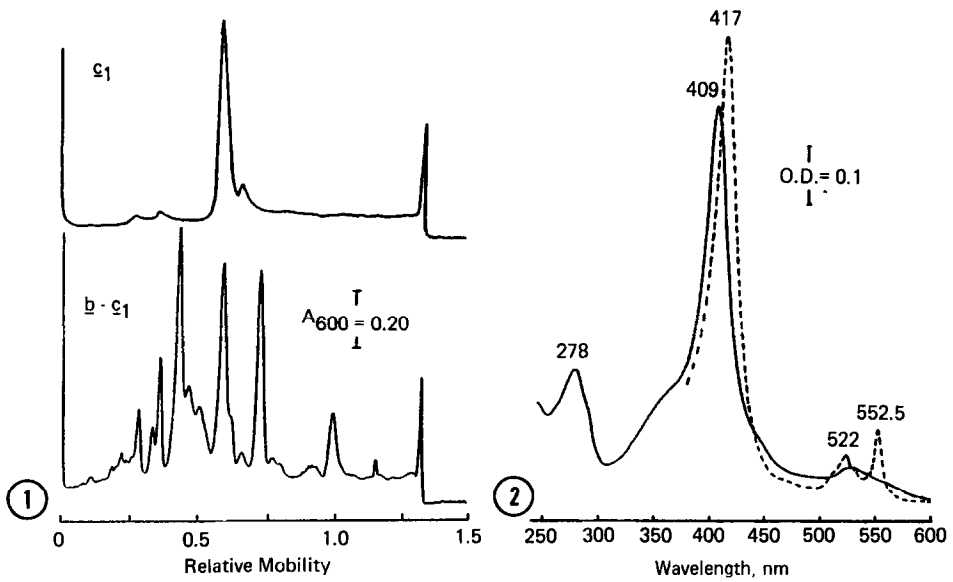

Figure 1. Densitometric tracing of SDS-polyacrylamide gel electrophoresis of purified cytochrome $\underline{c}_1$ and its starting material, the cytochrome $\underline{b}$-$\underline{c}_1$ complex. The mobility of horse cytochrome $\underline{c}$ was used as reference.

Figure 2. Absorption spectra of purified cytochrome $\underline{c}_1$ of R. sphaeroides R-26. The purified cytochrome $\underline{c}_1$ was diluted to 0.2 mg/ml in 50 mM phosphate buffer, pH 7.4, containing 0.2% sodium cholate. Spectra were measured in a Cary spectrophotometer, model 219, at 23°. The solid (——) and broken (- - -) lines represent oxidized and sodium ascorbate reduced forms, respectively.

and cytochrome $\underline{c}_1$. Although the molecular weight determined by SDS-PAGE is 30,000, cytochrome $\underline{c}_1$ as prepared is in aggregated form with an apparent molecular weight of over 125,000 appearing at the void volume of HPLC using an I-125 column.

Despite the spectral and molecular weight similarity between mammalian and bacterial cytochrome $\underline{c}_1$, the amino acid composition of bacterial protein bears no resemblance to mammalian counterpart. A comparison of amino acids composition of mammalian and bacterial cytochrome $\underline{c}_1$ is given in Table II. The amino acids composition of mammalian cytochrjome $\underline{c}_1$ is calculated from the sequence data (10). Since the molecular weight of bacterial cytochrome $\underline{c}_1$ is not yet precisely determined, the amino acid composition data are expressed in μmole amino acids per gram protein. The obvious difference in amino acid composition between these two cytochromes suggests that the primary structure

Table II. Comparison of Amino Acids Composition between Mammalian and Photosynthetic Bacterial Cytochrome $\underline{c}_1$

Amino Acids	μmol/gm protein	
	Beef Mitochondria	R. sphaeroides
Aspartic Acid	697.1	761.3
Threonine	256.8	463.7
Serine	587.0	459.3
Glutamic Acid	770.5	982.0
Proline	807.2	874.7
Glycine	623.7	905.7
Alanine	660.4	1028.0
Half-Cystine	183.4	n.d
Valine	550.3	269.7
Methionine	366.9	271.0
Isoleucine	146.8	249.3
Leucine	917.2	706.0
Tyrosine	550.3	238.0
Phenylalanine	293.5	440.0
Lysine	440.3	433.7
Histidine	330.2	192.0
Arginine	550.3	374.3
Tryptophan	110.1	60.3

may be quite different. On the other hand, the similarity in spectral properties indicates that both cytochromes have similar heme environment. A more detailed structural study is needed for this particular component of cyclic electron transfer system before the electron transfer mechanism can be elucidated.

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