Biochimica et Biophysica Acta, 976 (1989) 70–76
Elsevier

BBABIO 43037

The *Rhodospirillum rubrum* cytochrome bc_1 complex: peptide composition, prosthetic group content and quinone binding

Aidas Kriauciunas [1], Linda Yu [2], Chang-An Yu [2], R. Max Wynn [3] and David B. Knaff [1]

[1] *Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX,* [2] *Department of Biochemistry, Agricultural Experiment Station, Oklahoma State University, Stillwater, OK and* [3] *Division of Molecular Plant Biology, University of California at Berkeley, Berkeley, CA (U.S.A.)*

(Received 26 January 1989)

Key words: Cytochrome bc_1 complex; Electron transport chain; Quinone binding; Purple bacteria; (*R. rubrum*)

A cytochrome bc_1 complex, essentially free of bacteriochlorophyll, has been purified from the photosynthetic purple non-sulfur bacterium *Rhodospirillum rubrum*. The complex catalyzes electron flow from quinol to cytochrome *c* (turnover number = 75 s^{-1}) that is inhibited by low concentrations of antimycin A and myxothiazol. The complex contains only three peptide subunits: cytochrome *b* (M_r = 35000); cytochrome c_1 (M_r = 31000) and the Rieske iron-sulfur protein (M_r = 22400). E_m values (pH 7.4) were measured for cytochrome c_1 (+320 mV) and the two hemes of cytochrome *b* (−33 and −90 mV). Electron flow from quinol to cytochrome *c* is inhibited when the complex is pre-illuminated in the presence of a ubiquinone photoaffinity analog (azido-Q). During illumination, the azido-Q becomes covalently attached to the cytochrome *b* peptide and, to a lesser extent, to cytochrome c_1.

Introduction

The cytochrome bc_1 complexes of mammalian and bacterial electron-transport chains and the related cytochrome b_6f complex of oxygenic photosynthetic organisms play a central role in energy transduction associated with respiratory and photosynthetic electron transfer [1–3]. The cytochrome bc_1 complexes of photosynthetic purple non-sulfur bacteria have proven particularly useful for studying mechanisms of electron flow, for the following reasons. (1) Kinetic studies of electron flow through the complexes of *Rhodobacter sphaeroides, Rhodobacter capsulatus* and *Rhodospirillum rubrum* induced by single turnover flashes have provided a detailed picture of the electron-transfer steps [4,5]. (2) The peptide composition of these complexes, which contain 3 or 4 subunits, is much simpler than those of the corresponding mitochondrial complexes [1,3,6] and (3) The amino acid sequences, deduced from nucleotide sequences of the corresponding genes, are known for all three electron-carrier subunits of the *Rb. capsulatus* complex [7,8]. The cytochrome bc_1 complex isolated from *R. rubrum* [9] has several additional advantages, including its simple three peptide subunit composition, its stability and the fact that it possesses a high-affinity binding site for its electron-accepting substrate, cytochrome c_2 [10]. We present below an improved purification protocol for the *R. rubrum* cytochrome bc_1 complex and data on the oxidation-reduction and quinone-binding properties of the complex.

Materials and Methods

Wild-type (strain S1) *R. rubrum* cells were grown photosynthetically and chromatophores prepared as described previously [9]. The *R. rubrum* cytochrome bc_1 complex was solubilized by suspending chromatophores which had been washed once in 20 mM Tris-acetate buffer (pH 8.0) containing 5 mM EDTA to a final bacteriochlorophyll (BChl) concentration of 200 μM in 35 mM Mops buffer (pH 7.4) containing 1 mM $MgSO_4$ and 25 mM dodecylmaltoside. After stirring for 1 h at 4°C, the suspension was centrifuged at 200000 × *g* for 90 min. The cytochrome bc_1 complex was then purified according to a modification of the procedure of Ljung-

Abbreviations: $Q_{10}C_{10}BrH_2$, 2,3-dimethoxy-5-methyl-6-(10-bromodecyl)-1,4-benzoquinol; azido-Q, 3-azido-2-methoxy-6-(3,7-dimethyloctyl)-1,4-benzoquinone; SDS, sodium dodecyl sulfate; Mops, 4-morpholinepropanesulfonic acid; E_m, oxidation–reduction midpoint potential; BChl, bacteriochlorophyll.

Correspondence: D.B. Knaff, Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409, U.S.A.

dahl et al. [6]. Sodium chloride was added to the 200 000 $\times g$ supernatant to a final concentration of 100 mM, which was then applied to a DEAE-Biogel A column equilibrated with 35 mM Mops (pH 7.4), 1 mM $MgSO_4$ and 0.1 mg dodecylmaltoside per ml (buffer A) containing 100 mM NaCl. The column was washed with 20 column volumes of buffer A containing 200 mM NaCl and then eluted with buffer A containing 350 mM NaCl. Fractions containing the cytochrome bc_1 complex were pooled, diluted 1 : 1 with buffer A and applied to a DEAE-Sepharose 6B column equilibrated with buffer A containing 100 mM NaCl. The column was washed with 10 column volumes of buffer A containing 300 mM NaCl and then eluted with buffer A containing 450 mM NaCl. Fractions containing the purified complex were pooled, glycerol was added to a final concentration of 5% (v/v) and the sample stored at $-20\,^{\circ}C$ until required.

The antibody against the *Rb. capsulatus* Rieske iron-sulfur protein was a gift from Dr. Nadia Gabellini. The antibody against *Rb. sphaeroides* cytochrome c_1 was prepared as described previously [11].

Antimycin A and equine cytochrome *c* (Type VI) were obtained from Sigma Chemical Co. Dodecylmaltoside was obtained from Calbiochem-Behring Corp. Myxothiazol was purchased from Boehringer Mannheim. Protein molecular-weight standards and material for the preparation of polyacrylamide gels for electrophoresis were obtained from Bio-Rad Laboratories. 2,3-Dimethoxy-5-methyl-6-(10-bromodecyl)-1,4-benzoquinol ($Q_0C_{10}BrH_2$) was synthesized as described previously [12].

Absorbance spectra were measured using Aminco DW-2a, SLM-Aminco DW-20 and Perkin Elmer Lambda 5 spectophotometers. Oxidation-reduction titrations were performed electrochemically as described previously [13]. BChl *a* concentrations were determined after extraction into 7 : 2 (v/v) acetone/methanol [14]. Protein concentration was determined according to a modification [15] of the procedure of Lowry et al. [16]. Heme analyses were performed according to the method of Takaichi and Morita [17], none-heme iron was determined as described by Massey [18] and acid-labile sulfide determined as described by Brumby et al. [19]. Ubiquinone content was determined using a modification of the method of Takamiya and Dutton [20] in which detergent was removed by precipitating the complex with 72% (v/v) trichloroacetic acid prior to ubiquinone extraction.

The quinol : cytochrome *c* oxidoreductase activity of the complex was assayed essentially as described previously [9], using 25 μM $Q_0C_{10}BrH_2$ as the electron donor and 50 μM equine ferricytochrome *c* as the electron acceptor. Polyacrylamide gel electrophoresis was conducted in the presence of sodium dodecyl sulfate (SDS) according to the method of Laemmli [21] using a 1.5 mm thick slab gel with a 4% cross-linked stacking gel and a 10–15% gradient resolving gel. Prior to electrophoresis, samples were solubilized with 200 mM dithiothreitol, 2% SDS in 20 mM Tris-HCl buffer (pH 8.3) at $50\,^{\circ}C$ for 10 min. The gels were stained for protein with Coomassie Brillant Blue. Immunoblots were performed according to a modification of the method of Towbin et al. [22], using 2% non-fat milk in Tris-buffered saline as a blocking agent and horse radish peroxidase-linked, goat anti-rabbit IgG as the second antibody.

Photoaffinity labeling of the cytochrome bc_1 complex by UV-photolyzed 3-[^{3}H]azido-2-methyl-5-methoxy-6-(3,7-dimethyloctyl)-1,4-benzoquinone (Azido-Q) was carried out essentially as described previously [23]. For studies of azido-Q incorporation and inactivation during photolysis, the purified complex was precipitated with 50% saturated ammonium sulfate to remove glycerol and detergent and then resuspended in 50 mM potassium phosphate buffer (pH 7.0). Azido-Q was added in a 100-fold molar excess (compared to cytochrome c_1) and allowed to incubate for 10 min at $0\,^{\circ}C$ prior to photolysis. Samples were removed at various times after the onset of illumination and assayed for quinol : cytochrome *c* oxidoreductase activity or for radioactivity uptake. Radioactivity was measured after paper chromatography on Whatman No. 3 filter paper in 2 : 1 chloroform/methanol to separate the protein from unincorporated azido-Q. The remainder of the sample was extracted with chloroform/methanol and then with chloroform. The solvent was removed by passing a stream of N_2 over the sample, and the residue was then lyophilized and subjected to SDS-polyacrylamide gel electrophoresis according to the method of Weber and Osborn [24] except that *N'*,*N*-diallytartardiamide replaced bisacrylamide as the cross-linker. The gel was stained with Coomassie Brillant Blue to locate the protein subunits of the cytochrome bc_1 complex and, after de-staining, was cut into slices which were dissolved in 1 ml of 2% periodic acid prior to mixing with 5 ml of Insta-Gel scintillation cocktail and counting for radioactivity using a Packard liquid scintillation counter (Model 1900CA). Gel slices sizes were designed so that the gel was cut halfway between the limits of Coomassie Blue-staining regions associated with the resolved Rieske iron-sulfur and cytochrome c_1 peptides to insure that each slice contained radioactivity associated with azido-Q binding to no more than one peptide.

Results

Fig. 1 shows the absolute and reduced minus oxidized difference spectra of the purified *R. rubrum* cytochrome bc_1 complex. The absorbance spectrum is similar to that of the complex prepared from *R. rubrum* in one of our laboratories, using a different method [9]. Although neither BChl nor carotenoid makes significant contribu-

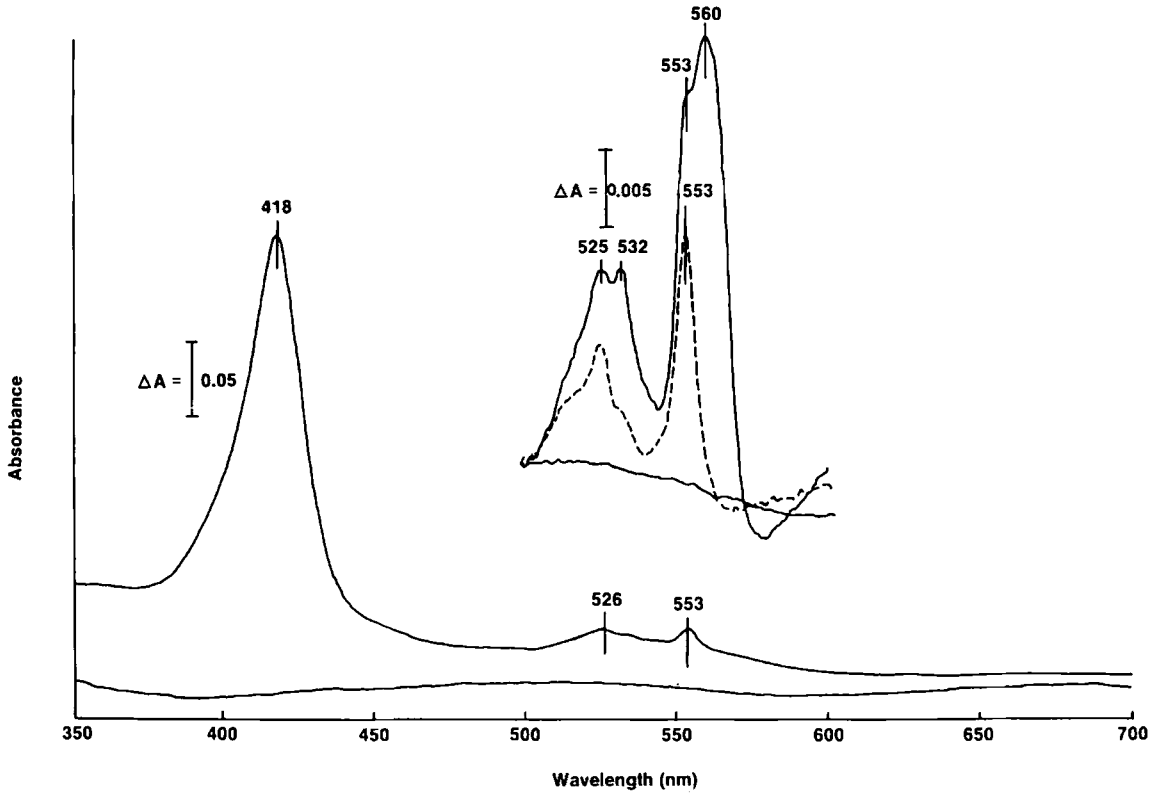

Fig. 1. Absorbance spectra of the *R. rubrum* cytochrome bc_1 complex. The solid line extending from 350 to 700 nm represents the absolute spectrum of the complex as isolated. (The instrumental baseline is included for comparison.) The two insets, shown at 10-fold higher sensitivity, show the ascorbate-reduced minus ferricyanide-oxidized (- - - - -) and dithionite-reduced minus ferricyanide-oxidized (———) difference spectra. The spectra were obtained in a 1 cm optical path length cuvette. The complex was dissolved in at 35 mM Mops buffer (pH 7.4) containing 0.1 mg/ml dodecylmaltoside.

tions to the absorbance spectrum, a small amount of BChl was detected in some preparations by direct chemical analysis (Table I). In other preparations, no BChl could be detected within the sensitivity limits of the assay. The BChl : cytochrome c_1 heme ratio never exceeded 0.03. The ascorbate-reduced minus ferricyanide-oxidized difference spectrum shows an α-band maximum at 553 nm and a β-band maximum at 525 nm due to reduced cytochrome c_1 [1,6,9,11]. The dithionite-reduced minus ferricyanide-oxidized difference spectrum, in addition to containing features attributable to cytochrome c_1, shows α-band and β-band features at 560

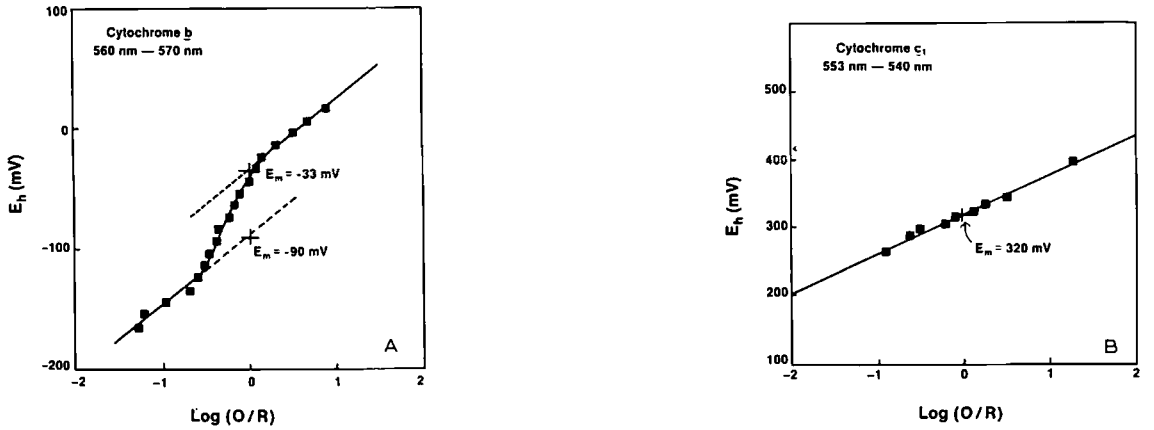

Fig. 2. Oxidation-reduction titrations of the *R. rubrum* cytochrome bc_1 complex. The sample contained 30 μM complex in the buffer used for obtaining Fig. 1. The following redox mediators were present: 10 μM potassium ferricyanide; 50 μM 1,4-benzoquinone; 50 μM 2,5-dihydroxy-1,4-benzoquinone; 50 μM diaminodurene; 50 μM 1,2-naphthoquinone; 10 μM phenazine methosulfate; 50 μM duroquinone; 50 μM 2-hydroxy-1,4-naphthoquinone and 20 μM anthraquinone-2-sulfonate. Optical pathlength 0.3 mm; spectral resolution, 1 nm; $T = 15\,^{\circ}$C. (A) Cytochrome *b*. Absorbance changes at 560–570 nm were monitored to measure % reduction and the log of the ratio of oxidized-to-reduced cytochrome plotted vs. E_h. (B) Cytochrome c_1. As in (A), except that ΔA at 553–540 nm was utilized. The solid lines are best fits to the Nernst equation.

TABLE I

Prosthetic group content of the R. rubrum cytochrome bc_1 complex.

Component	Content (nmol/mg protein)
Cytochrome c_1 (heme *c*)	6.1
Cytochrome *b* (protoheme)	12.1
Acid-labile sulfide	12.4
Non-heme iron	14.0
Ubiquinone	6.8
Bacteriochlorophyll	0.18

and 532 nm, respectively, due to reduced cytochrome *b* [1,6,9]. Table I shows that, on a heme basis, the *R. rubrum* complex contains two cytochrome *b* per cytochrome c_1, as is the case for other cytochrome bc_1 complexes [1–3,6,9]. Table I also shows that the *R. rubrum* complex exhibits a non-heme iron/inorganic sulfide/heme *c* ratio near 2:2:1, indicative of the presence of equimolar amounts of cytochrome c_1 and the [2Fe-2S]-containing Rieske iron-sulfur protein [1–3,5,9]. We have previously established the presence of a Rieske iron-sulfur protein in the *R. rubrum* complex using electron paramagnetic resonance (EPR) spectroscopy [9]. The complex also contains approximately one ubiquinone per cytochrome c_1, in agreement with our previous observations on a *R. rubrum* complex prepared using a different purification protocol [9]. The *R. rubrum* cytochrome bc_1 complex catalyzed electron flow from $Q_0C_{10}BrH_2$ to equine cytochrome *c* with a turnover number of 75 s^{-1}. Electron flow was inhibited by both antimycin A (87% inhibition at 0.8 μM) and myxothiazol (96% inhibition at 1 μM).

Although the data of Table I established that the *R. rubrum* complex contains cytochrome *b* and cytochrome c_1 in a 2:1 ratio, the difference spectra were not well enough resolved to allow a determination of whether two different forms of cytochrome *b* were present, as is the case for other cytochrome bc_1 complexes [1–3,5,9]. However, oxidation-reduction titrations of cytochrome *b*, monitored at 560–570 nm (Fig. 2A), did reveal two different cytochrome *b* components with E_m values (at pH 7.4) of −33 mV ($n = 0.98$) and −90 mV ($n = 1.02$), respectively. Titrations monitored at 553–540 nm (Fig. 2B), to maximize contributions from cytochrome c_1 and minimize those from the *b* cytochromes, revealed a single component with $E_m = +320$ mV ($n = 0.98$). A series of titrations indicated that these E_m values were reproducible to within ± 4 mV. All titrations were fully reversible and the E_m and *n* values were independent of redox mediator concentration over a 5-fold range.

The peptide composition of the purified *R. rubrum* complex was determined by electrophoresis in the presence of SDS followed by staining for protein with Coomassie Brillant Blue (Fig. 3A). Three peptide components were detected, with apparent molecular masses of 35 kDa, 31 kDa and 22.4 kDa, respectively. These values differ slightly from those observed for a *R. rubrum* complex prepared using a different purification protocol [9]. No components with molecular masses in the 8–12 kDa range were detected, in contrast to several reports of such a component being present in the *Rb. sphaeroides* and *Rb. capsulatus* complexes [1,6,25–27]. Results similar to those of Fig. 3 were obtained if the gels were stained with silver instead of Coomassie Brilliant Blue (data not shown). Western blots using antibodies raised against either *Rb. sphaeroides* cytochrome c_1 or the *Rb. capsulatus* Rieske iron-sulfur protein (Fig. 3B) have identified the 31 kDa peptide as cytochrome c_1 and the 22.4 kDa peptide as the Rieske iron-sulfur protein, respectively. We had previously assigned the 31 kDa component to the cytochrome c_1 peptide on the basis of heme-staining [9]. Therefore, the 35 kDa molecular mass peptide, the only remaining component, must contain the two cytochrome *b* hemes. This apparent molecular mass value for the *R. rubrum* cytochrome *b* is lower than that observed for cytochrome *b* in cytochrome bc_1 complexes isolated from other photosynthetic bacteria [1,3,6,15,16].

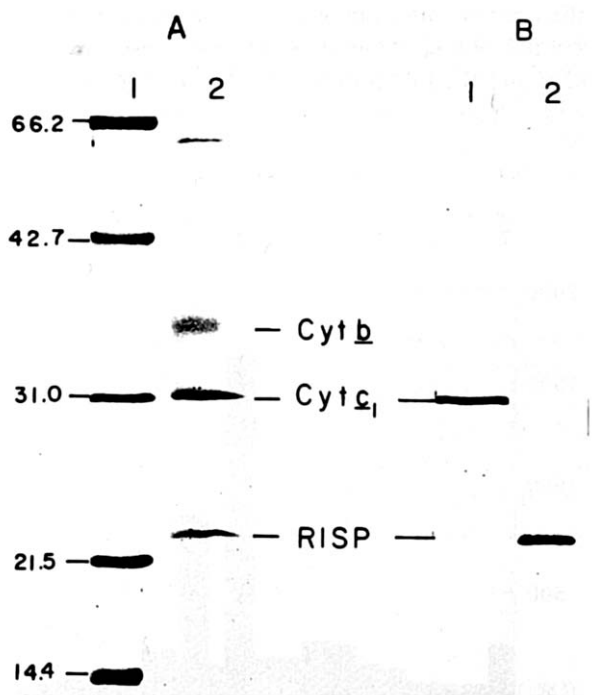

Fig. 3. Peptide composition of the *R. rubrum* cytochrome (Cyt) bc_1 complex. Electrophoresis was performed as described in Materials and Methods with 130 pmol of complex loaded per lane. Molecular weight standards (lane 1A) used were: bovine serum albumin (M_r = 66200); ovalbumin (M_r = 42700); carbonic anhydrase (M_r = 31000); soybean trypsin inhibitor (M_r = 21500) and lysozyme (M_r = 14400). (A) Staining with Coomassie Brilliant Blue for protein. (B) Samples were transferred to nitrocellulose paper as described in Materials and Methods and treated with antibody raised against either *Rb. sphaeroides* cytochrome c_1 (lane 1) or the *Rb. capsulatus* Rieske iron-sulfur protein (RISP) (lane 2).

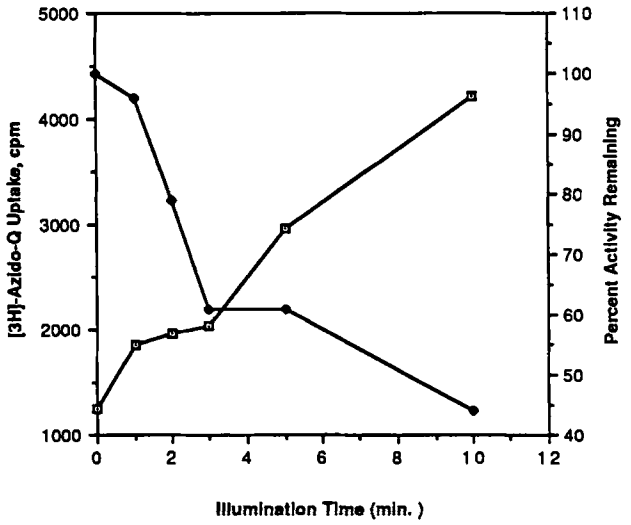

Fig. 4. Effect of illumination time on the binding of azido-Q to the *R. rubrum* cytochrome bc_1 complex and on the ubiquinol : cytochrome *c* oxidoreductase activity of the complex. [^{3}H]azido-Q binding (□) and activity were (◆) were measured as described in Materials and Methods.

The most widely accepted scheme for electron flow through the cytochrome bc_1 complex is the so-called 'Q-cycle' model [1,2,4,5]. This model features quinone binding at two sites, one where quinol is oxidized in two sequential one-electron steps by the Rieske iron-sulfur protein and the low-potential cytochrome *b* heme and a second, where quinone is reduced by the high potential cytochrome *b* heme. To explore the location of quinone binding by the *R. rubrum* complex, photoaffinity labeling with a ubiquinone analog was carried out. Fig. 4 shows the time-course for inhibition of the quinol : cytochrome *c* oxidoreductase activity of the *R. rubrum* cytochrome bc_1 complex and the simultaneous incorporation of ^{3}H-labeled azido-Q during UV-photolysis. In control experiments, incubation of the *R. rubrum* complex with azido-Q in the dark or UV illumination of the complex in the absence of azido-Q produced no inhibition of quinol : cytochrome *c* oxidoreductase activity (data not shown). Fig. 5 shows that azido-Q incorporation into the *R. rubrum* cytochrome bc_1 complex was confined to two fractions that were shown to correspond to the cytochrome *b* peptide (slice 11) and the cytochrome c_1 peptide (slice 13). The cytochrome *b* peptide was labeled to approximately twice the extent of the cytochrome c_1 peptide. Very little azido-Q was incorporated into the Rieske iron-sulfur protein (slice 14) and no incorporation was detected into any low molecular weight, putative quinone-binding peptides [28] that might have escaped detection by staining for protein.

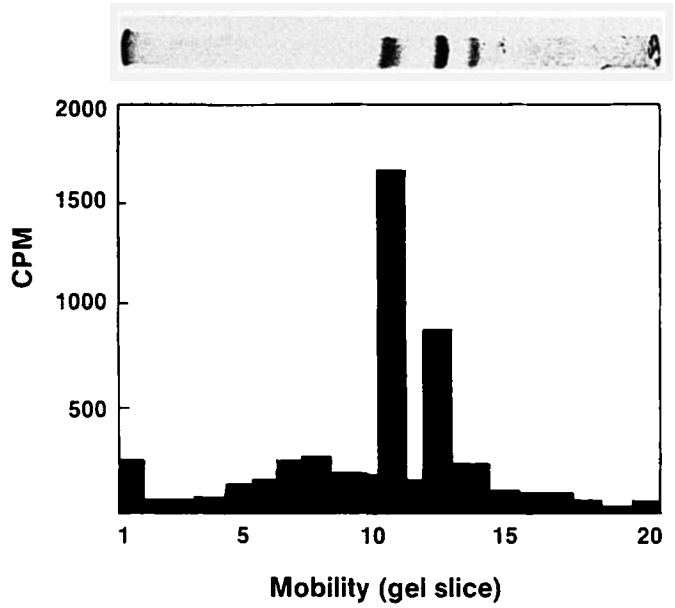

Fig. 5. Distribution of azido-Q radioactivity after photoaffinity labeling of the *R. rubrum* cytochrome bc_1 complex. Approx. 40 μg of protein were loaded onto the gel and azido-Q binding measured as described in Materials and Methods. The gel was first stained for protein (top) and subsequently used for radioactivity determination (bottom). The cytochrome *b* peptide was located entirely in slice 11, the cytochrome c_1 peptide in slice 13 and the Rieske iron-sulfur protein in slice 14.

Discussion

A method has been developed for purifying a cytochrome bc_1 complex from *R. rubrum*.. The complex is vitually free of BChl, is highly stable, shows a high turnover number and exhibits activity that is sensitive to inhibition by two highly specific inhibitors of cytochrome bc_1 complexes. The isolated *R. rubrum* complex has an electron transfer prosthetic group content similar to that observed for other cytochrome bc_1 complexes [1–3,5]. The two different E_m forms of cytochrome *b* typical of other cytochrome bc_1 complexes [1,2,4,5] are also present in the *R. rubrum* complex. The E_m values obtained for the cytochrome components in the isolated *R. rubrum* complex at pH 7.4 differ somewhat from those obtained in redox titrations of the complex in situ, using *R. rubrum* chromatophore membranes at pH 7.0 [29]. However, the values obtained in this study for cytochromes c_1 and the lower potential *b* cytochrome are comparable (i.e., they differ by 30 mV or less) to those observed with other isolated complexes (Refs. 1 and 27; and Crofts, A.R. and Robertson, D.E., personal communications). The E_m value obtained in this study for the higher potential cytochrome *b* is approximately 70 mV more negative than those reported for complexes from other photosynthetic bacteria.

The most striking property of the *R. rubrum* cytochrome bc_1 complex is the simplicity of its peptide composition. The only peptides detected were those that carry the electron-transfer prosthetic groups: the protoheme-containing cytochrome *b* peptide (M_r = 35 000); the cytochrome c_1 peptide (M_r = 31 000) and the Rieske

iron-sulfur protein ((M_r = 22400). The minimal molecular mass of the complex, assuming one copy of each peptide, is thus 88.4 kDa and one would thus predict a heme *c* content of 11.3 nmol per mg protein instead of the 6.1 nmol per mg protein we detected. It is possible that the subunit molecular masses determined by electrophoresis are inaccurate, as is often the case for membrane-bound proteins, or that the method used for protein determination in this work is somewhat inaccurate because the standard (bovine serum albumin) and the complex subunits react differently. It is also possible that there are inaccuracies in the heme determination due to the contribution of other pigments to the measured absorbance. The molecular masses of cytochrome c_1 and of the Rieske iron-sulfur protein in the *R. rubrum* cytochrome bc_1 complex are similar to those reported for cytochrome bc_1 complexes isolated from other photosynthetic bacteria. However, the molecular mass of the *R. rubrum* cytochrome *b* peptide is significantly lower than the 42–48 kDa values observed for the *Rb. sphaeroides* and *Rb. capsulatus b* cytochromes [1,3,6,25]. Attempts are currently underway in one of our laboratories to determine the true molecular mass of this *R. rubrum* protein from the nucleotide sequence of its gene (Harman, J.G., Knaff, D.B., Shanker, S. and Kim, Y.-A., unpublished results).

No evidence for the presence of a low molecular mass (i.e., 8–12 kDa) peptide in the complex was found, although the presence of such peptides has been reported for cytochrome bc_1 complexes isolated from other photosynthetic bacteria [1,6,25–28]. Since genetic studies have located only genes for the three prosthetic group-containing peptides in these bacteria [7,8], it is possible that the fourth peptide observed in some photosynthetic bacterial cytochrome bc_1 complex preparations may be either a fortuitous contaminant or a proteolytic degradation product of one of the three subunits (Ref. 3, and Robertson, D.E. and Daldal, F., personal communication). It is also possible that the *R. rubrum* complex contains a fourth peptide in situ which is lost during purification. However the fact that the turnover number for the *R. rubrum* complex compares favorably with those observed for complexes isolated from *Rb. capsulatus* and *Rb. sphaeroides,* which have been reported to have four subunits [6], and the observation that the *R. rubrum* complex retains sensitivity to two highly specific inhibitors [1,2,4,5] of electron flow through the cytochrome bc_1 complex, antimycin A and myxothiazol, make this possibility seem unlikely.

The photoaffinity labeling experiments with the ubiquinone analog azido-Q identify a major site for quinone binding on the cytochrome *b* peptide, consistent with the Q-cycle model for electron transport which postulates semiquinone oxidation and quinone reduction by the low- and high-potential protohemes of the cytochrome *b* peptide, respectively [1,2,4,5]. Although the Q-cycle model also postulates quinol oxidation by the Rieske iron-sulfur protein, no azido-Q binding to the *R. rubrum* Rieske protein could be observed at levels significantly above those of the background. Somewhat surprisingly, since there is no evidence for direct interaction between ubiquinone and cytochrome c_1 [1,2,4,5], significant azido-Q binding to the cytochrome c_1 peptide was observed. We attribute this to some non-specific binding of azido-Q to the complex. In contrast to results obtained with azido-Q photoaffinity labeling of the *Rb. sphaeroides* cytochrome bc_1 complex [28], which showed extensive labeling of a 12 kDa peptide, no low-molecular-mass peptides were labeled in the *R. rubrum* complex. This finding eliminates the possibility that a low-molecular-weight, quinone-binding protein is present in the isolated *R. rubrum* complex but escaped detection because it stained poorly with either Coomassie Brilliant Blue or with silver.

The results reported above represent the first detailed characterization of a cytochrome bc_1 complex, isolated from a photosynthetic bacterium, that unambiguously contains only three peptide subunits (see Ref. 3 for a recent discussion of the subunit composition these complexes). However, a three-subunit complex has been isolated from the non-photosynthetic bacterium *Paracoccus denitrificans* [30,31]. The *R. rubrum* complex differs from the *P. denitrificans* complex in that the former contains a cytochrome c_1 peptide similar in size to that found in mitochondria [1] while the *P. denitrificans* cytochrome c_1 is considerably larger, with M_r = 62000 [30,32].

Acknowledgements

This research was supported in part by grants from the National Science Foundation (DMB-8806609 to D.B.K.) and National Institutes of Health (GM 30721 to C.-A.Y.). The authors would like to thank Dr. Nadia Gabellini for the generous gift of the Rieske iron-sulfur protein antibody and Drs. Nadia Gabellini, Fevzi Daldal, Antony Crofts, James Harman and Dan Robertson for helpful discussions.

References

1 Hauska, G., Hurt, E., Gabellini, N. and Lockau, W. (1983) Biochim. Biophys. Acta 726, 97–133.
2 Rich, P.R. (1984) Biochim. Biophys. Acta 768, 53–79.
3 Gabellini, N. (1988) J. Bioenerg. Biomembr. 20, 59–83.
4 Crofts, A.R., Meinhardt, S.W., Jones, K. and Snozzi, M. (1983) Biochim. Biophys. Acta 723, 202–218.
5 Dutton, P.L. (1986) in Encyclopedia of Plant Physiology, Vol. 19 (Staehelin, A. and Arntzen, c.J., eds.), pp. 197–237, Springer-Verlag, Berlin.
6 Ljungdahl, P.O., Pennoyer, J.D., Robertson, D.E. and Trumpower, B.L. (1987) Biochim. Biophys. Acta 891, 227–241.
7 Gabellini, N. and Sebald, W. (1986) Eur. J. Biochem. 154, 569–579.

8 Davidson, E. and Daldal, F. (1987) J. Mol. Biol. 195, 13–24.
9 Wynn, R.M., Gaul, D.F., Choi, W.-K., Shaw, R.W. and Knaff, D.B. (1986) Photosynth. Res. 9, 181–195.
10 Bosshard, H.R., Wynn, R.M. and Knaff, D.B. (1987) Biochem. 26, 7688–7693.
11 Haley, P.E., Yu, L., Dong, J.-H., Keyser, G.C., Sanborn, M.R. and Yu, C.-A. (1986) J. Biol. Chem. 261, 14593–14599.
12 Yu, C.-A. and Yu, L. (1982) Biochemistry 21, 4046–4101.
13 Smith, M., SMith, W.H. and Knaff, D.B. (1981) Biochim. Biophys. Acta 635, 405–411.
14 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 312–323.
15 Peterson, C. (1983) Methods Enzymol. 91, 95–119.
16 Lowry, O., Rosebrough, A., Farr, A. and Randall, R. (1951) J. Biol. Chem. 193, 265.
17 Takaichi, S. and Morita, S. (1981) J. Biochem. 89, 1513–1519.
18 Massey, V. (1957) J. Biol. Chem. 229, 763–775.
19 Brumby, P., Miller, R. and Massey, V. (1965) J. Biol. Chem. 240, 2222–2228.
20 Takamiya, K. and Dutton, P.L. (1979) Biochim. Biophys. Acta 546, 1–16.
21 Laemmli, K. (1977) Nature 227, 688–685.
22 Towbin, H., Staehelin, T., Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
23 Yu, L., Yang, F. and Yu, C.-A. (1985) J. Biol. Chem. 260, 963–973.
24 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
25 Yu, L., Mei, Q.-C. and Yu, C.-A. (1984) J. Biol. Chem. 259, 5752–5760.
26 Wilson, E., Farley, T.M. and Takemoto, J.Y. (1985) J. Biol. Chem. 260, 10288–10292.
27 Andrews, K. (1988) Ph.D. Thesis, University of Illinois.
28 Yu, L. and Yu, C.-A. (1987) Biochemistry 26, 3658–3664.
29 Venturoli, G., Fenoll, C. and Zannoni, D. (1987) Biochim. Biophys. Acta 892, 172–184.
30 Yang, X. and Trumpower, B.L. (1986) J. Biol. Chem. 261, 12282–12289.
31 Yang, X. and Trumpower, B.L. (1988) J. Biol. Chem. 263, 11962–11970.
32 Kurowski, B. and Ludwig, B. (1987) J. Biol. Chem. 262, 13805–13811.