Isolation of cytochrome $b$ from the cytochrome $bc_1$ complex of
Rhodopseudomonas sphaeroides GA

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Cytochrome $b$ has been isolated from the cytochrome $bc_1$ complex of Rhodopseudomonas sphaeroides GA. It represents the largest of the 3 polypeptides of this complex (40, 34, 25 kDa). Spectral heterogeneity is lost, but redox heterogeneity is retained to some extent, and the pH-dependence of the midpoint potential is preserved during isolation.

Rps. sphaeroides  Bacterial cytochrome $bc_1$ complex  Cytochrome $b$
Ubiquinol--cytochrome $c$ oxidoreductase

1. INTRODUCTION

A cytochrome complex which has ubiquinol--cytochrome $c$ oxidoreductase activity can be isolated from chromatophores of Rhodopseudomonas sphaeroides [1,2]. It contains the Rieske FeS center and ubiquinone, and 2 hemes $b$/cytochrome $c_1$ [1]. The 2 cytochromes $b$ in the complex correspond [3] to cytochrome $b$-561 and $b$-566 in the parent membrane with respect to redox potentials [4,5], absorption spectra [5,6] and spectral effects of the inhibitory antibiotics, antimycin A and myxothiazol [7].

The complex consists of 3 major polypeptides with app. $M_\text{r}$ 40000, 34000 and 25000 [1]. Here we show that the 40 kDa polypeptide corresponds to cytochrome $b$. It can be isolated from the complex and some of its properties are reported.

2. MATERIALS AND METHODS

The cytochrome $bc_1$ complex from Rps. sphaeroides was prepared as in [1] with the omission of Triton X-100 [3]. Cytochrome $b$ was prepared from the complex by chromatography on hydroxyapatite in the presence of Triton X-100, modified (i.e., omitting urea) from a procedure also successfully employed for the isolation of cytochrome $b$ from the mitochondrial complex [8]. The cytochrome complex from Rps. sphaeroides, which was suspended in 50 mM glycyglycine (pH 7.4), 0.25% cholate, 30 mM octylglucoside and ~30% (w/v) sucrose from the density gradient centrifugation, was loaded onto a short OH-
apatite column, equilibrated with 5 mM phosphate (pH 7.4) and 0.1% Triton X-100. Cytochrome $b$ was then eluted from the complex with 10 mM phosphate/0.1% Triton X-100. The residue of the complex, containing cytochrome $c_1$ and residual cytochrome $b$ was eluted subsequently with 50 mM phosphate/0.1% Triton X-100.

Protein [9] and pyridine hemochrome [10] were determined by standard procedures. Other methods are described in the legends.

3. RESULTS AND DISCUSSION

The SDS--PAGE pattern of the cytochrome $bc_1$ complex isolated from Rps. sphaeroides GA is shown (fig.1) in 3 different amounts (track 4--6), together with the reaction center (track 3) and cytochrome $c_2$ (track 2), both from the same organism [1]. The preparation presented here shows the dominant polypeptides of 40 and 34 kD [1], but contains relatively little of the 25-kD polypeptide. The small polypeptide below 10 kD
Fig. 1. Heme-carrying polypeptides in the preparation of the cytochrome bc₁ complex from *Rps. sphaeroides*. SDS-PAGE was carried out after Laemmli [11], on a gradient gel of 12–18% polyacrylamide. The same gel was first stained for heme [12] (right); then, after destaining, with Coomassie blue (left). Track: (1) protein standards with 92, 66, 45, 31, 21 and 14 kDa; (2) 0.2 nmol cytochrome c₂ from *Rps. sphaeroides*; (3) 0.2 nmol reaction center complex from *Rps. sphaeroides* GA [1]; (4–6) cytochrome bc₁ complex from *Rps. sphaeroides* GA, 0.3, 0.15 and 0.05 nmol cytochrome c₁.

Fig. 2 shows that the isolated cytochrome b corresponds to the 40-kD polypeptide, which loses the heme upon SDS-PAGE (fig.1). Spectra of this cytochrome b preparation are shown in fig.3. In contrast to the cytochrome b in the complex [3], the low-temperature spectrum does not reveal a split α-peak. Up to 80% of cytochrome b from the complex could be isolated, as determined by the pyridine hemochrome [10]. This suggests that all of the cytochrome b is represented by the 40-kD polypeptide. There was a tendency for loss of heme from the cytochrome b after purification.

The midpoint potential of the cytochrome b isolated from the complex was pH-dependent (fig.4). The slopes of the titrations at both pH-values in fig.4 are < 1, indicating redox heterogeneity. It is not possible, however, to resolve the titrations in fig.4 into two components accurately, as done for the cytochrome in the complex [1,3]. Heterogeneity and pH-dependence of the midpoint potential has been also reported for
Fig. 2. SDS-PAGE of cytochrome b isolated from the cytochrome bc₁ complex from Rps. sphaeroides. SDS-PAGE was carried out after Laemmli [11] on 14% polyacrylamide: (1) standard proteins as in fig. 1; (2) cytochrome bc₁ complex, 75 pmol cytochrome c₁; (3) 60 pmol isolated cytochrome b.

cytochrome b isolated from the mitochondrial cytochrome bc₁ complex [8,17], and for cytochrome b₆ isolated from the cytochrome b₆f complexes from chloroplasts and a cyanobacterium (in preparation). Therefore, cytochrome b of these complexes has universal properties (reviewed in [18]).

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Fig. 3. Spectra of the isolated cytochrome b. The spectra were recorded with an Aminco DW2 UV/Vis spectrophotometer as in [1,3]. (A) Redox difference spectrum, dithionite minus ferricyanide, at RT: (B) absolute spectrum of the cytochrome reduced with dithionite, at RT; in both cases cytochrome b was 290 nM as determined by pyridine hemochrome [10]. (C) Redox difference spectrum, dithionite minus ferricyanide, over liquid N₂, 120 nM cytochrome b, 0.2 mm cuvette.

Fig. 4. Redox titration of the isolated cytochrome b at two pH values. Redox titrations were done as in [1]. The pH was adjusted to either 6.0 or 7.4 in the presence of 20 mM Tris–HCl, 20 mM MES–NaOH and 5 mM phosphate. Cytochrome b was 350 nM.

REFERENCES


