# 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. $M_r$ 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 omittance 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 glycylglycine (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

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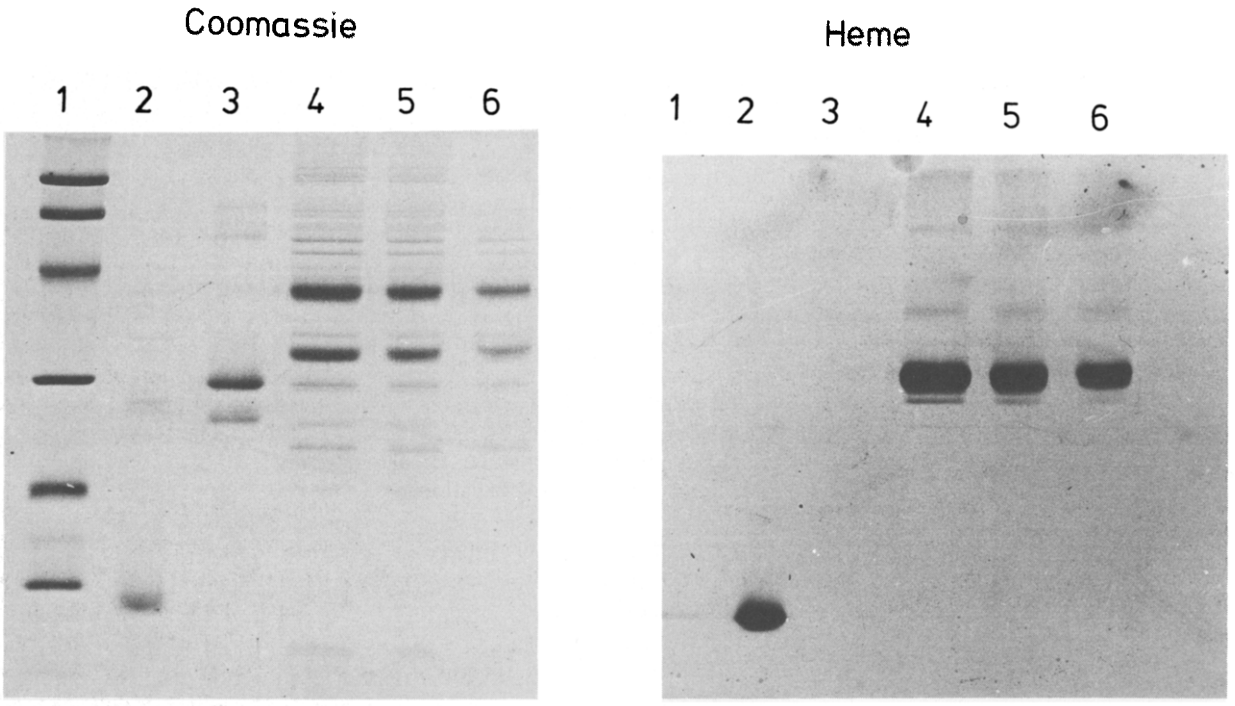


Fig.1. Heme-carrying polypeptides in the preparation of the cytochrome $bc_1$ complex from *Rps. sphaeroides*. SDS–PAGE was carried out after Laemmli [11], on a gradient gel of 12–18% polyacrylamide. The same gel was first stained for heme [12] (right); then, after destaining, with Coomassie blue (left). Track: (1) protein standards with 92, 66, 45, 31, 21 and 14 kDa; (2) 0.2 nmol cytochrome $c_2$ from *Rps. sphaeroides*; (3) 0.2 nmol reaction center complex from *Rps. sphaeroides* GA [1]; (4–6) cytochrome $bc_1$ complex from *Rps. sphaeroides* GA, 0.3, 0.15 and 0.05 nmol cytochrome $c_1$.

reported in [1] is seen. In addition it is still contaminated by reaction center (the larger two subunits of the reaction center [13] are not resolved on the gel presented), and by some other polypeptides. The right part of fig.1 shows the identical gel, but stained for heme [12]. Cytochrome $c_2$ (track 2) and the 34-kD polypeptide of the complex, corresponding to cytochrome $c_1$ [14], are heavily stained. In addition some fainter heme-positive bands are seen in the pattern of the complex, which do not, however, correspond to major polypeptide bands seen in the left part of the figure. As concluded in [1], the pattern in fig.1 can be interpreted either by assuming that in accordance with the mitochondrial complex but in contrast to cytochrome $b_6f$ complexes [15,16], cytochrome *b* loses its heme during SDS–PAGE, or that the 34-kD band represents polypeptides of cytochrome *b* and $c_1$. To clarify this point cytochrome *b* was isolated from the complex as in section 2.

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 $\alpha$-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

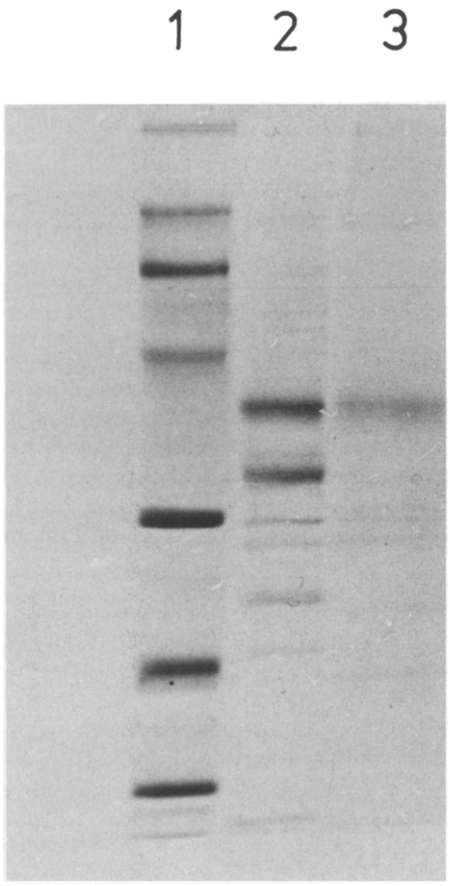


Fig.2. SDS–PAGE of cytochrome *b* isolated from the cytochrome $bc_1$ complex from *Rps. sphaeroides*. SDS–PAGE was carried out after Laemmli [11] on 14% polyacrylamide: (1) standard proteins as in fig.1; (2) cytochrome $bc_1$ complex, 75 pmol cytochrome $c_1$; (3) 60 pmol isolated cytochrome *b*.

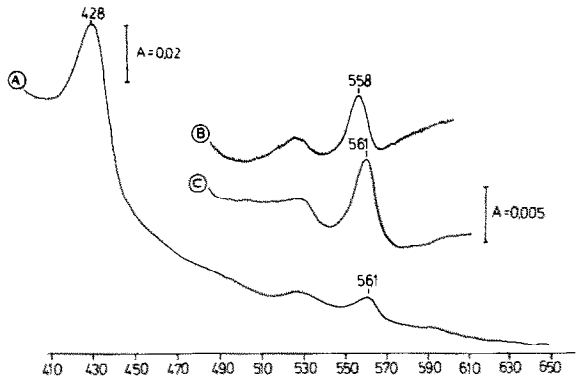


Fig.3. Spectra of the isolated cytochrome *b*. The spectra were recorded with an Aminco DW2 UV/Vis spectrophotometer as in [1,3]. (A) Redox difference spectrum, dithionite minus ferricyanide, at RT: (B) absolute spectrum of the cytochrome reduced with dithionite, at RT; in both cases cytochrome *b* was 290 nM as determined by pyridine hemochrome [10]. (C) Redox difference spectrum, dithionite minus ferricyanide, over liquid $N_2$, 120 nM cytochrome *b*, 0.2 mm cuvette.

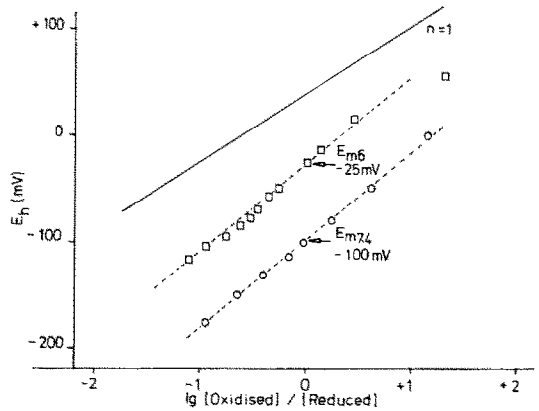


Fig.4. Redox titration of the isolated cytochrome *b* at two pH values. Redox titrations were done as in [1]. The pH was adjusted to either 6.0 or 7.4 in the presence of 20 mM Tris–HCl, 20 mM MES–NaOH and 5 mM phosphate. Cytochrome *b* was 350 nM.

cytochrome *b* isolated from the mitochondrial cytochrome $bc_1$ complex [8,17], and for cytochrome $b_6$ isolated from the cytochrome $b_6f$ complexes from chloroplasts and a cyanobacterium (in preparation). Therefore, cytochrome *b* of these complexes has universal properties (reviewed in [18]).

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