Anomalous Reduction of Cytochrome *b* in Highly Purified Complex III from Baker's Yeast*

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Cytochrome *b*, Anomalous Reduction, "Control Factor", Yeast, Highly Purified *bc*-Complex.

In highly purified bc_1-complex from baker's yeast, the reduction of cyt c_1 and partial reduction of cyt *b* is obtained by catalytic amount of succinate dehydrogenase and succinate in the presence of 7 μM antimycin. After the addition of ferricyanide the c_1 is re-oxidized and a increase in the reduction of *b* is observed. Using stopped-flow we established that the oxidation of c_1 by ferricyanide proceeds as a pseudo-first order reaction and the reduction of *b* is faster and with two phases. Our observation suggests that these two processes are not directly interconected and that other component than c_1 must be the "control factor" in the anomalous reduction of cyt *b*. This component must be, by exclusion, the iron-sulfur protein.

The mitochondrial electron transport chain has been intensively studied, but the mechanism of electron transfer through the cytochrome bc_1-complex is unknown. This problem has a special interest since the bc_1-complex includes the second coupling site of oxidative phosphorylation.

The addition of oxygen to anaerobic mitochondria reduced by substrate in the presence of antimycin causes an increase of the degree of reduction of cytochrome *b* [1–3] while in a resolved succinate-cytochrome *c* reductase complex it is possible to control the reduction of cytochrome *b* by succinate in the presence of ascorbate and antimycin [4, 5].

The reduction of the cytochrome *b* of isolated bc_1-complex by Q_1H_2 (ubiquinol-5) is 80–95% in the presence of antimycin plus added electron acceptors such as oxygen or ferricyanide. However, less than 40% of the cytochrome *b* is reduced in the absence of either antimycin or the electron acceptors [1].

This requirement for both an added electron acceptor and antimycin for the reduction of cytochrome *b* suggests that the state oxidation of a component distant from cytochrome *b* controls the midpoint potential of this cytochrome.

We report here experiments with highly purified bc_1-complex obtained from baker's yeast, which provide information concerning the identity of this "control factor".

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Materials and Methods

The bc_1-complex was prepared from baker's yeast by the method of Siedow *et al.* [6]. The particle catalyses the oxidation of coenzyme Q analogs with ferricytochrome *c* as electron acceptor. For the oxidation of succinate it is necessary to add a catalytic amount of succinate dehydrogenase (SDH) which was obtained as a side product of the preparation of bc_1-complex.

For measurement of cytochrome spectra, the preparation was diluted to the required cytochrome *b* concentration in 0.1 M potassium phosphate, 0.5 mM EDTA, pH 7.4 and difference spectra, as indicated, recorded at 10° on a Cary 17 spectrophotometer scanning the wavelength region 570–535 nm at a scan rate of 0.5 nm per second. Rapid kinetic experiments were performed in a stopped-flow instrument using 550 nm and 563 nm to follow the oxido-reduction of ctochrome c_1 and *b* respectively.

Results and Discussion

Fig. 1 shows the difference spectra of complex III reduced by succinate and catalytic amount of SDH. Cytochrome c_1 was totally reduced by this system but there was partial reduction of *b* despite the presence of antimycin. Addition of ferricyanide to this preparation immediately produced a large increase in absorbance at 562 nm corresponding to the reduction of *b*, together with a disappearance of the peak at 550 nm due to the reoxidation of c_1. Some minutes later cytochrome *b* has become partially oxidized and c_1 re-reduced.

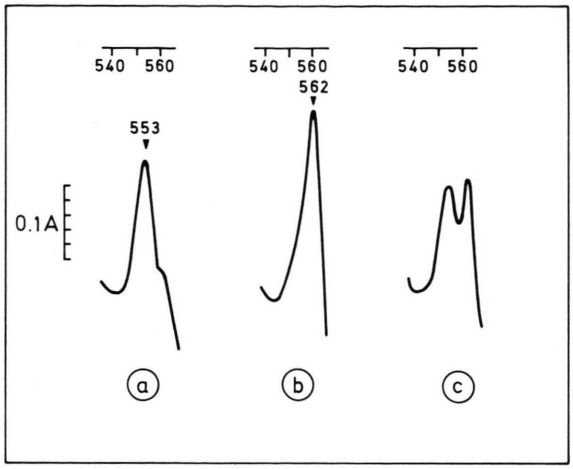

Fig. 1. Asorbance difference spectra of cytochromes in highly purified complex III. The concentration of cytochrome *b* was 23 μM. The spectrum in (a) shows the reduction of cytochrome c_1 and partial reduction of cytochrome *b* by SDH and succinate in the presence of 7 μM antimycin. In (b) the spectrum is of the same sample immediately after the addition of 35 μM ferricyanide. The spectrum in (c) was recorded 5 min later.

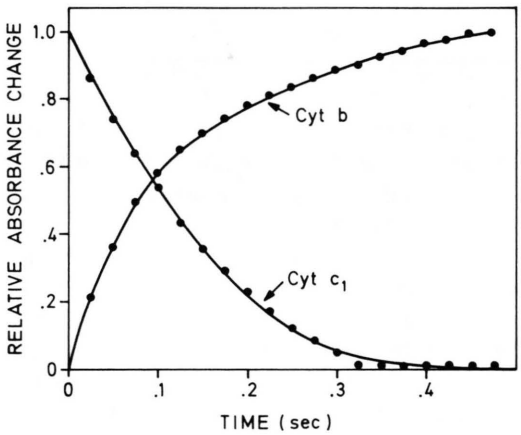

Fig. 2. Time-course of the oxidation of cytochrome c_1 and the induced reduction of cytochrome *b*. The concentration of cytochrome *b* was 40 μM. The bc_1-complex was preincubated with catalytic amount of SDH, 1 mM succinate and 7 μM antimycin for 10 min. After this time the cytochrome c_1 was become totally reduced and cytochrome *b* was only 30% reduced. The reaction was initiated by mixing the sample with the same volume of 200 μM ferricyanide and the absorbance changes at either 550 nm and 563 nm was following in a stopped-flow spectrophotometer.

In Fig. 2, we show the timecourse for the oxidation of c_1 and induced reduction of *b* by ferricyanide, as followed in a stopped-flow spectrophotometer. The oxidation of c_1 proceeds as a pseudofirst order reaction with a rate constant of 7.4 s^{-1} and the reduction of *b* occurs in two phases with rate constants of 9.5 s^{-1} and 5.7 s^{-1} respectively (Fig. 3).

Mechanisms proposed to explain this anomalous reduction have invoked a change in the midpoint potential of *b* and support the existence of a "control factor" whose oxidation state controls the midpoint potential of *b*. Other proposed mechanisms suggest that a component previously reduced, such as ubiquinone, must be oxidized in order to obtain the extra reduction of *b* (Fig. 1).

Our previously published experiments made with the same preparation demonstrates that reduced ubiquinone does not have a role in the induced reduction of cytochrome *b* as reductive titration of this particle showed that the ubiquinone does not become reduced before cytochrome *b* [6].

The kinetic data for the rapid, induced reduction of cytochrome *b* exhibits two phases, the first one

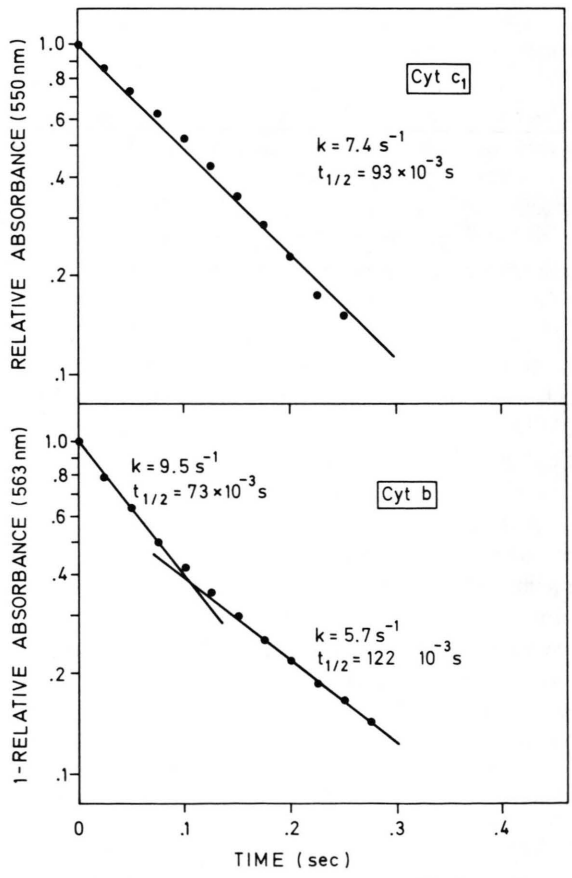

Fig. 3. Semilogarithmic plot of the oxidation of cytochrome c_1 and the reduction of cytochrome *b* by ferricyanide. The data are from the experiments of Fig. 2.

proceeding faster than the rate at which cytochrome c_1 is oxidized. This observation implies that these two processes are not directly interconnected and that a component of bc_1-complex other than c_1 must be the "control factor".

Several reports have appeared demonstrating the presence of unidentified electron transport component in the bc_1-complex which may be responsible for the anomalous reduction of *b* [4, 7–9]. However, the composition of the purified yeast bc_1-complex reported previously [6] and the reductive titration of the complex show no evidence for an additional electron acceptor, with one equivalent of c_1, two of *b* one Rieske iron-sulfur protein and a equivalent of quinone per minimal unit.

From the above data we can conclude that if there is indeed a component whose redox state modulates midpoint potential of cytochrome *b*, this component must be, by exclusion, the iron-sulfur protein [10, 11]. The redox potential of iron-sulfur protein (250 mV) acts on the oxygen side of the antimycin block such as would be expected.

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