ENERGY DEPENDENCE OF OXIDATION-REDUCTION POTENTIALS OF THE b AND c CYTOCHROMES IN BEEF HEART SUBMITOCHONDRIAL PARTICLES*

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SUMMARY

In coupled beef heart submitochondrial particles (Mg-ATP) the addition of ATP causes the measured oxidation-reduction midpoint potential of about 35-40% of the total b-cytochrome complement to assume a value more positive by at least 70 mV. There is no significant effect on the midpoint potentials of cytochromes c + c_1. Hence, submitochondrial particles which many consider to be "inside out" display qualitatively the same behavior as the intact mitochondrion: under the experimental conditions the location of the membrane-bound carriers with respect to the bulk aqueous phase would appear not to be relevant to the chemical events of the energy conserving mechanism.

INTRODUCTION

Oxidation-reduction potential titrations of uncoupled mitochondrial and submitochondrial preparations from beef heart have revealed there are at least three b cytochromes with differing midpoint potentials (1); in 1958, Chance (2) separated the spectra of three b cytochromes from beef heart mitochondria. While this has complicated analysis of oxidation-reduction potential titrations with beef heart mitochondria (in contrast to the pigeon heart) it is apparent that in the presence of ATP they behave analogously (3) to the more extensively studied rat liver (3) and pigeon heart (4), in that on addition of ATP about half the total cytochrome b complement (designated cytochrome b_T) assumes a mid

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point of approximately +240 mV. Because of this energy dependence, cytochrome $\underline{b}_T$ is considered (3, 4) to be directly involved in the transduction of energy at site II in the respiratory chain.

This communication examines the energy dependence of oxidation-reduction midpoint potentials of the b- and c-cytochromes in coupled beef heart submitochondrial particles. The relevance of the study arises from the body of evidence which suggests a) respiratory carriers are arranged anisotropically in the membrane (see ref. 5) and b) that the membranes of particles prepared by sonication are largely inside out with respect to the parent mitochondria (6, 7); thus study of the electrical properties of the cytochromes in coupled submitochondrial particles is of some importance in relation to the measured ATP induced midpoint potential changes of cytochrome $\underline{b}_T$ obtained in the intact mitochondrion.

MATERIALS AND METHODS

The beef heart mitochondria and Mg-ATP submitochondrial particles were prepared by the method of Löw and Vallin (8). The cytochromes were assayed by simultaneous readout of absorbance and oxidation-reduction potential in an anaerobic cuvette (9) as previously described (1, 3). Pertinent details are given in the figure legends.

RESULTS

Figure 1A described the course of oxidation-reduction of the b cytochromes (562-575 nm) in beef heart Mg-ATP submitochondrial particles (MAsp) in the presence of ATP, and in the uncoupled state. It is clear from this plot that ATP induces 35-40% of the total b cytochrome complement (as indicated by absorbance change) to assume a midpoint potential more positive by at least 60-70 mV. Figure 1B presents the absorbance data as the logarithm of the ratio of the oxidized cytochrome to reduced cytochrome [thus for a single one-electron (n=1) carrier a linear expression is obtained with a slope of 59.3 mV per logarithmic decade, (see ref. 3)]. The points derived from the titration in the uncoupled

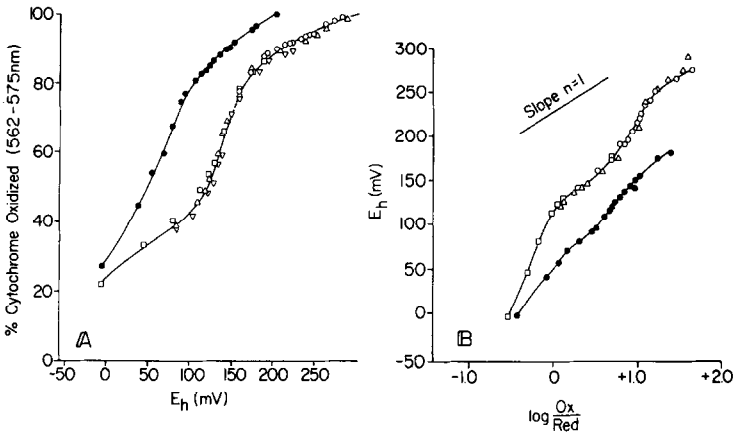

Fig. 1A. The course of oxidation-reduction of the b cytochromes (562-575 nm) in beef heart submitochondrial particles. MAsp. were suspended at 6.65 mg protein/ml (except o , which was 8.7 mg protein/ml) in mannitol-sucrose-morpholinopropane sulphonate (0.22 M, 0.05 M, 0.05 M) buffer at pH 7.2 containing 6 mM $MgSO_4$ and the following oxidation-reduction mediators: 40 µM diaminodurol, 30 µM phenazine methosulphate and phenazine ethosulphate; 5 µM pyocyanine, 20 µM duroquinone, 20 µM 2-hydroxy-1,4-naphthaquinone. The oxidation reduction potential was made more positive with 100 mM potassium ferricyanide and more negative with a fresh solution of sodium dithionite. The open symbols represent separate titrations (duration time of titration limited to 10 min) in the presence of 6 mM ATP; ● represents the titration performed after the ATP was consumed (in Δ) and uncoupled with 0.5 µM 5-chloro,3-(p-phenyl), 2', 4', 5'-trichlorosalicylanilide (S-13). Fig. 1B. Absorbance changes between +300 and -180 mV presented as the logarithm of the ratio of oxidized and reduced forms of the b cytochromes.

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state lie within experimental error of that previously presented in this way (1) which was shown to be composed of three b-type cytochromes, $E_{m7.2}$-103 mV, +38 mV and +125 mV. Presentation in this way accentuates the small amount (12%) which becomes titratable in the presence of ATP with a midpoint potential approaching +250 mV.

Identical experiments were performed but with spectrophotometric assay of cytochromes c + c_1. The submitochondrial preparation used in these experiments produced the usual response in the b cytochromes: the open inverted triangular points in Figure 1 were generated in the presence of ATP by the same preparation as used for the cytochrome c titration shown in Figure 2A and B. The closed points in Figure 2 describe the course of oxidation-reduction (550-540 nm) of cytochromes c + c_1 in the absence of ATP or when ATP hydrolysis is uncoupled or prevented. Under these conditions (Fig. 2A), the reduction of the

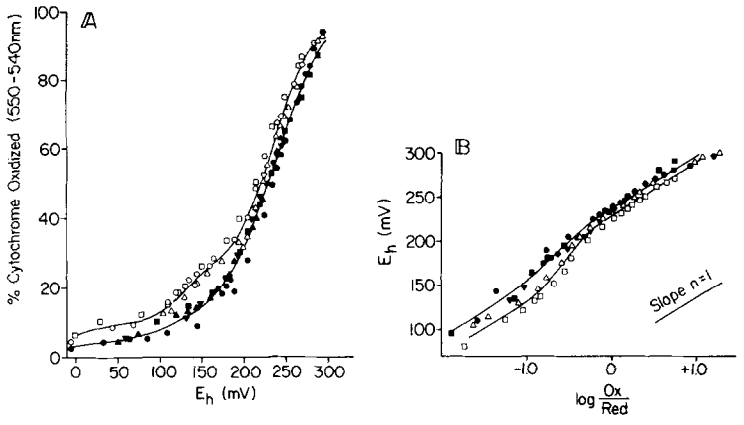

Fig. 2A. The course of oxidation-reduction of the cytochromes c + c_1 (550-540 nm). Basic conditions and oxidation-reduction mediators were as in Fig. 1. Open symbols represent separate titrations (duration time of 10 min) done in the presence of 6 mM ATP; ●, no additions; ■ plus 3 μM S-13 (after □); ▲, plus 2 μg/ml oligomycin; ▼ , plus 2 μg/ml oligomycin and 6 mM ATP (no added $MgSO_4$). Fig. 2B. Absorbance changes between +300 and +50 mV plotted as the logarithm of the ratio of the oxidized and reduced forms of the cytochromes.

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c-cytochromes represents approximately 85% of the total absorbance change from +300 mV to -180 mV; the remainder of components with midpoint potentials of less than 150 mV is attributable to the b-cytochromes which overlap the 550 nm measuring wavelength (see ref. 2). In the presence of ATP, there is no major change in the titration, in contrast to that observed with the b-cytochromes in Figure 1. There are, however, some minor ATP-induced differences apparent at lower potentials.

Figure 2B presents the absorption data as the ratio of cytochrome oxidized to cytochrome reduced, using +50 mV as the 100% reduced endpoint. The slight sigmoidicity of the curve under unenergized conditions reveals 12.5% of lower potential component. On addition of ATP the contribution of titratable material in this potential region increases to about 19%. At present it is not possible to say from where this minor contribution originates, but it seems reasonable to consider that it arises from the b cytochrome which, in the presence of ATP, become titratable in this potential region; in any event it represents less than 10% of the total titratable absorbance change.

DISCUSSION

The measured ATP induced change in the oxidation-reduction midpoint potential of 35-40% of the b cytochrome complement to more electropositive potential is consistent with findings reported for the intact mitochondrion (3, 4). However, only approximately 12% of the total was measured with a midpoint potential approaching +250 mV, the remainder being in the region of +150 mV. Owing to the presence of at least three b cytochromes resolvable in the uncoupled state, an unequivocal explanation for this cannot be given. However, if, as in pigeon heart mitochondria where only one b cytochrome (b_T) appears to undergo a midpoint potential change, it may reflect two distinct levels of coupling in respiratory chains of these particles.

The virtual insensitivity to the presence of ATP of the midpoint potentials of cytochromes c + c_1 in the submitochondrial particles is similar to the behavior of these cytochromes in the intact mitochondria as shown by Hinkle and Mitchell (10) with rat liver and Wilson and Dutton (unpublished observation) with pigeon heart.

Hence, the potentiometric behavior of cytochromes b and c + c_1 in the presence of ATP in both intact mitochondria and submitochondrial particles are qualitatively similar. Since the major structural changes incurred during the preparation of the particles result in relocations and alterations in reactivities of the respiratory components (cf. ref. 7) these results provide more support for the consideration that cytochrome b_T, by undergoing chemical transformations, acts as energy transducer at site II. Further, from our experimental conditions in which combinations of oxidation-reduction reagents have been selected for rapid mediation in electron transfer between membrane-bound electron carriers and the outer aqueous phase, it may be suggested that the anisotropy of the membrane-bound respiratory carriers is not of primary importance to the early events of energy conservation. It is pertinent to add also that the mediators are chosen to minimize the possibility of the measured midpoint changes being a manifestation of ATP-induced reverse electron flow. Some

effects which suggest this problem may have been rendered insignificant are the following: a) The midpoint potentials of cytochromes $\underline{c} + \underline{c}_1$ in submitochondria particles [where it is considered to be located mainly on the inner side of the membrane (11, 7)], as well as in intact mitochondria, are insensitive to the action of coupled ATP hydrolysis; that is, they do not appear to become more electronegative. b) About half the b-cytochrome complement in mitochondria from beef heart, rat liver (3) and pigeon heart (4) also remains unaffected as does (under our conditions) cytochrome a in rat liver (12) (cf. ref. 10). c) A several-fold variation (3) in concentration of the added mediators had no effect on the ATP-dependent behavior of the midpoint potential of cytochrome $\underline{b}_T$.

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