ENERGY DEPENDENT CHANGES IN THE OXIDATION-REDUCTION POTENTIAL OF CYTOCHROME b*

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SUMMARY

The oxidation-reduction midpoint potential measurement of mitochondrial cytochrome b indicate that there are two chemically different species of cytochrome b in the respiratory chain. The midpoint potential of one of these species is energy dependent.

We have previously reported (Wilson and Dutton, 1970) that the measured oxidation-reduction midpoint potential of cytochrome a_3 is dependent on the phosphate potential and hence is "energy dependent." It was further proposed that a similar energy dependence of the midpoint potential of cytochrome is also associated with the energy conservation at site II in mitochondria and with the dark reactions of photosynthetic systems. In this communication we report a) the energy dependence of the midpoint potential of cytochrome b and b) that cytochrome b is actually two chemically different entities.

METHODS

The oxidation-reduction potential of cytochrome b has been measured potentiometrically in rat liver mitochondria prepared as previously described (Wilson, 1969) but washed twice with 0.12 M KCl to remove contaminating hemoglobin (Jacobs et al., 1965). The oxidation-reduction potentials were measured (Wilson and Dutton, 1970) using the redox mediators N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD), phenazine methosulfate (PMS), phenazine ethosulfate

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(PES). The midpoint potentials of these mediators are 250 mV, 80 mV and 55 mV at pH 7.0 (E_o') and they are one, two, and two electron acceptors (n values) respectively (Clark, 1960). Potassium ferricyanide was used as an oxidant and the iron chelate of ethylenediaminetetraacetate (EDTA) (E_o' = 117 mV, n = 1) was used as an optional redox buffer.

The mitochondria were suspended in a mannitol (0.22 M), sucrose (0.05 M) and potassium morpholinopropane sulfonate (15 mM) medium at the indicated pH. Endogenous respiration was inhibited with 5 μM rotenone and reducing equivalents added as aliquots of DPNH. The oxidation and reduction of cytochrome b was measured with a Johnson Foundation dual wavelength spectrophotometer at 430 nm minus 410 nm.

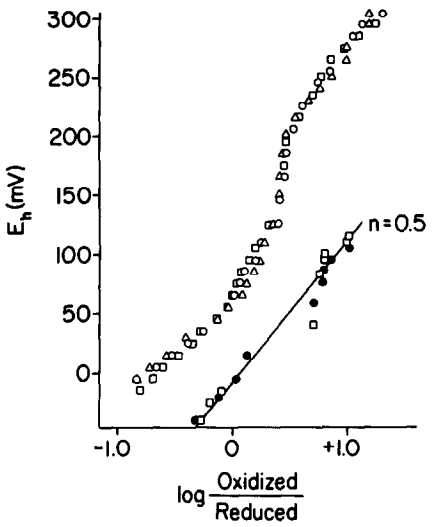

Fig. 1. The dependence of the state of reduction of cytochrome b on the oxidation-reduction potential (E_h). The rat liver mitochondria were suspended at 0.25 μM cytochrome a in a medium containing 0.22 M mannitol, 0.05 M sucrose, 1 mM EDTA and 15 mM morpholinopropane sulfonate, pH 7.3. 5 μM rotenone and the indicated redox mediators were added. Aliquots of dihydroascorbate were added until anaerobiosis was achieved as evidenced by cytochrome reduction and an E_h of less than 300 mV. 1.5 mM ATP was added and the reduction of cytochrome b was effected by the combined slow donation of electrons from endogenous donors and the addition of small aliquots of DPNH. (Δ) 10 μM TMPD, 7 μM PMS, 4 μM PES and 50 μM Fe SO_4. (o) 30 μM TMPD, 21 μM PMS, 12 μM PES and 50 μM Fe SO_4. (□) 45 μM TMPD, 30 μM PMS, 9 μM PES and 50 μM Fe SO_4. The points on the lower curve (n = 0.5) were for the conditions given above for the same symbol shape but measured after the addition of uncoupler [1 μM 5-Cl,3-(p-phenyl),2',4'5'-trichlorosalicylanilide].

RESULTS

As shown in Fig. 1, when the logarithm of the ratio of the oxidized to reduced cytochrome b was plotted against the observed oxidation-reduction potential (E_h) the curve for uncoupled mitochondria is very different from the curve for mitochondria in the presence of excess ATP. In the presence of excess ATP the entire curve is shifted toward more positive potential values and is sigmoid in shape. The sigmoid curve is typical of two components with differing midpoint potentials. Resolution of the curve into two components (Fig. 2) indicates that each has an n value of 1 and that the respective midpoint potentials are approximately +35 mV and +245 mV. The measured midpoint potentials are not changed by increasing the mediator concentrations from 10 μM TMPD, 4 μM PES, and 6.6 μM PMS to 45 μM TMPD, 9 μM PES and 30 μM PMS. Analogous results are obtained for beef heart mitochondria (in collaboration with C. P. Lee).

The cytochrome b has an apparent midpoint potential of approximately 0 mV

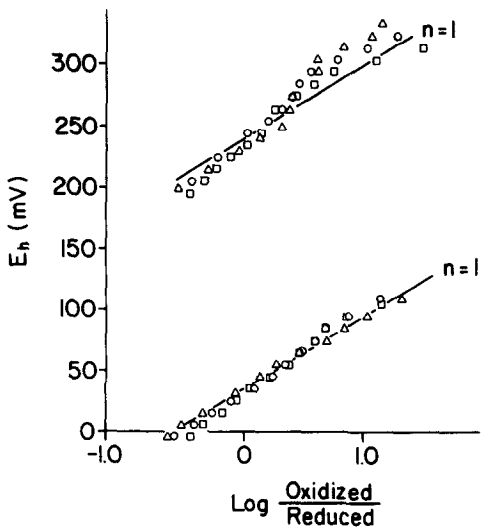

Fig. 2. The oxidation-reduction potentials of the two components of cytochrome b in rat liver mitochondria in the presence of ATP. The logarithm of the oxidized and reduced form of the component was calculated assuming 30% of the absorbance change was from the high potential component and 70% was from the low potential component. The lines are for theoretical one electron acceptors (n = 1). The symbols are as defined in the legend of Fig. 1.

and n value of 0.5 for uncoupled rat liver mitochondria (Fig. 1). The n value of 0.5 is anomalous but can be readily explained on the basis of the two components observed in the presence of ATP. Fig. 3 shows that the mathematical

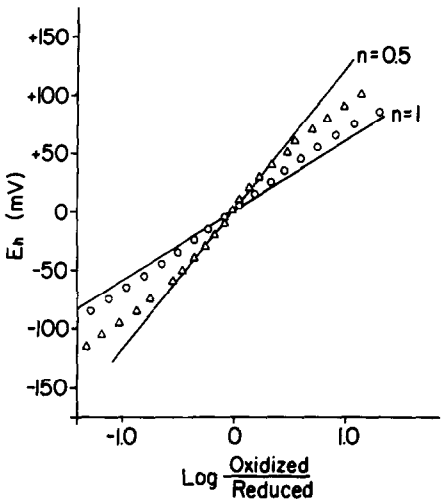

Fig. 3. The theoretical behavior of a two component (n = 1) oxidation-reduction system with two different midpoint potentials. The lines are the single component theoretical curves for n = 0.5 and n = 1. The points were generated by assuming an equal absorbance change for each of two components with midpoint potentials differing by 50 mV (○) and 100 mV (△).

mixing of two components (n = 1) of different midpoint potentials generates curves which have slopes equivalent to n values of less than 1. The theoretical curves are shown for two cases (i.e. 50 mV and 100 mV difference) and the calculated curves show a sigmoid shape. When the midpoint potentials are different by 100 mV, this sigmoidicity is apparent only when the components are more than 80°/o reduced or oxidized. At these extremes it becomes experimentally difficult to make accurate measurements and errors are critical. For example, if the cytochrome is 90°/o ± 2°/o oxidized the ratio of the oxidized to reduced forms is between 7.2 and 11.5. The experimental results for uncoupled rat liver mitochondria are consistent then with the cytochrome b having two forms with n values of 1 and midpoint potentials of approximately +35 mV and -55 mV. In uncoupled beef heart mitochondria the cytochrome b has a midpoint potential of approximately +60 mV at pH 7.2 in good agreement with

Ball (1938) and with Urban and Klingenberg (1969), but our n value is near 0.5 in contrast to 1 as reported by the previous workers. The difference in n values may be related to the different experimental techniques used. These workers used the succinate-fumarate ratio (n = 2) as a measure of the oxidation-reduction potential and were unable to make measurements at potentials in which the cytochrome b was more than 50% oxidized.

DISCUSSION

The observed phosphate potential dependence of the midpoint potentials of one cytochrome b component (this paper) and a_3 (Wilson and Dutton, 1970) is good evidence that this is a general phenomenon associated with energy conservation sites. Energy conservation can be expressed very simply in the general equations;

1) $b^{+2} - L_1 + C_1^{+3} \longleftrightarrow b^{+3} - L_2 + C_1^{+2}$

2) $b^{+3} - L_2 + ADP + Pi \longleftrightarrow b^{+3} - L_1 + ATP$

sum: $b^{+2} - L_1 + C_1^{+3} + ADP + Pi \longleftrightarrow b^{+3} - L_1 + C_1^{+2} + ATP$ where L_1 and L_2 represent different ligands for the heme iron. The equations are correctly written as reversible only if the E_o' for the reaction:

$$b^{+2} - L_1 \longleftrightarrow b^{+3} - L_2 + e^-$$

is approximately +250 mV, and for the reaction:

$$b^{+2} - L_1 \longleftrightarrow b^{+3} - L_1 + e^-$$

it is approximately 0 mV.

The equations are general expressions and do not attempt to give mechanistic detail, i.e. two electrons per ATP. We emphasize that the experimentally observed energy dependence of the cytochrome midpoint potential strongly implies that the cytochrome (b and a_3) iron atom is the chemical entity which forms the first "high energy" intermediate.

Two hypotheses for energy conservation have been published which involve changes in the midpoint potential of the cytochromes (DeVault, 1968; Wang, 1970). Wang (1970) presents a model system for oxidative phosphorylation and has predicted an energy dependence of the cytochrome midpoint potentials.

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