ENERGY DEPENDENCE OF THE HALF-REDUCTION POTENTIAL OF IRON-SULFUR CENTER 1 IN THE SITE I REGION OF THE RESPIRATORY CHAIN IN PIGEON HEART MITOCHONDRIA

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SUMMARY

The half-reduction potential of iron-sulfur Center 1a of intact pigeon heart mitochondria becomes more negative when adenosine triphosphate is added. In contrast, the half-reduction potentials of iron-sulfur Centers 1b, 2 and 3 + 4 are not significantly affected by ATP addition. It is suggested that the iron-sulfur Center 1a is involved in the energy transduction process at Site I of the respiratory chain.

The half-reduction potentials of iron-sulfur centers (Center 1, 2, 3 + 4) in the Site I region of the respiratory chain have been determined in both pigeon heart (1) and in C. utilis (2) mitochondria, by the potentiometric titration procedure of Dutton (3) and Wilson et al. (4). Iron-sulfur Center 1 of uncoupled mitochondria appears to be composed of two centers with similar half-reduction potentials which can not be resolved potentiometrically. In a previous communication (5) we suggested that at least one of five iron-sulfur centers is directly involved in Site I energy conservation from the in vivo induction studies of Site I phosphorylation in C. utilis mitochondria. Wilson and Dutton (6-8) provided evidence that cytochromes b_T and a_3 are directly involved in energy transduction in Site II and III, respectively, from the phosphate potential dependence of their half-reduction potentials. In the present study, this experimental approach was used to provide evidence that iron-sulfur center 1a is directly involved in energy transduction at Site I.

MATERIALS AND METHODS

Pigeon heart mitochondria were prepared by the method of Chance and Hagihara (9).

The oxidation-reduction potentials of iron-sulfur centers were measured potentiometrically according to Dutton (3) and Wilson et al. (4). Oxidation reduction mediators employed were phenazine ethosulfate, duroquinone, pyocyanine, 2 OH-naphthoquinone, phenosafranine, benzyl viologen and methyl viologen. EPR measurements were performed with a Varian Model E-4 spectrometer. The sample temperature for the EPR measurements (30°K) was obtained by cooling the samples with a stream of cold helium gas derived from boiling liquid helium. The temperature was measured by a Au/Co versus Pt. thermocouple. The p-trifluoromethoxyphenylhydrazone of carbonyl cyanide was the generous gift of Dr. P.G. Heytler of the E.I. Dupont Central Research Laboratory, Wilmington, Del.

RESULTS AND DISCUSSION

In order to determine if one of the five iron-sulfur centers in the Site I region (1,2,10) has an energy dependent half-reduction potential, the effect of ATP addition on the oxidation-reduction level of individual iron-sulfur centers of pigeon heart mitochondrial suspension was examined. In these experiments an anaerobic suspension of pigeon heart mitochondria containing suitable mediators was adjusted to specified oxidation-reduction potentials and aliquots were frozen before and after ATP addition. EPR spectra of the frozen aliquots were measured at the sample temperature and instrument power settings required to optimize the spectral contribution of each of the known iron-sulfur proteins in the Site I region of the respiratory chain (1,2,10). In a series of experiments at potentials ranging from +20 mV to -460 mV, almost no effect of ATP was observed on the measured half-reductio potentials of Centers 3 and 4 ($E_{m7.2}$ = -245 mV). Center 2 ($E_{m7.2}$ = -20 mV) was also not markedly affected by ATP but the uncertainties in the measurements permit only the conclusion that the change is less than ±30 mV. When the potential was set at values for which Center 1 ($E_{m7.2}$ = -305 mV) was partially or almost completely reduced (-290 mV to -465 mV) the addition of ATP caused a decrease of the Center 1 signal, presumably due to oxidation of Center 1. The maximal decrease in the Center 1 signal was observed at near -450 mV. The results of a typical experiment

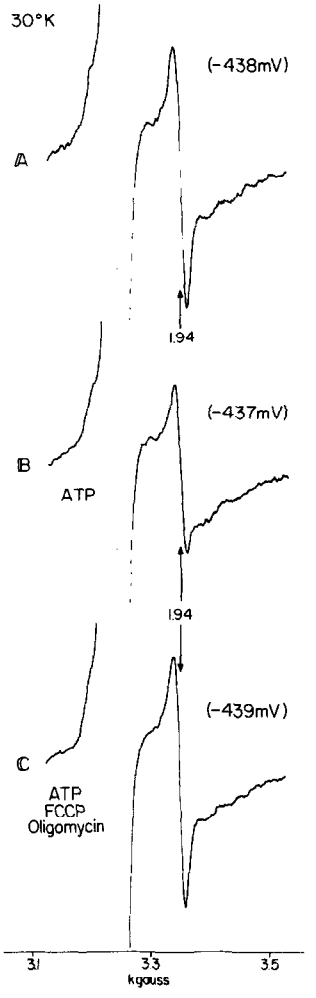

Figure 1. The effect of ATP addition on the EPR signal of iron sulfur Center 1 in pigeon heart mitochondria. Pigeon heart mitochondria were suspended at a protein concentration of 10 mg per ml in a medium containing 0.3 M mannitol, 50 mM Morpholinopropane sulfonate (pH 7.2). To this suspension, 67 µM phenazine ethosulfate, 33 µM duroquinone, 20 µM pyocyanine, 20 µM 2-OH-naphthoquinone, 78 µM phenosafranine, 74 µM benzyl viologen and 133 µM methyl viologen were added to act as oxidation-reduction mediators. The oxidation-reduction potential of the suspension was lowered to -438 mV by the addition of aliquots of freshly prepared dilute solution of dithionite. An aliquot (0.3 ml) of the suspension was transferred anaerobically to an EPR tube and frozen in liquid isopentane at 113°K (Spectrum A). After addition of 3.7 mM ATP, which was previously bubbled with argon to remove oxygen, the oxidation-reduction potential of the suspension was readjusted to the same potential and an aliquot of the suspension was quickly frozen (Spectrum B). P-trifluoromethoxy phenylhydrazone of carbonyl cyanide (0.8 nmoles/mg protein) and oligomycin (0.8 µg/mg protein) were further added to the mitochondrial suspension and an aliquot was frozen in the same fashion (Spectrum C). EPR operating conditions were: modulation amplitude, 12.5 gauss; microwave power, 50 mW; microwave frequency, 9.113 GHz; time constant, 0.3 sec; scanning rate, 500 gauss per min. and the sample temperature was 30°K.

are shown in Figure 1. In this experiment the potential was adjusted to -438 mV and Center 1 was measured to be over 99% reduced in frozen aliquots of the suspension Under the conditions of measurement the "g = 1.94" signal is approximately 65% arising from Center 1 and 35% from the succinate dehydrogenase iron-sulfur Center (1,2,4) (Spectrum A). The addition of 3.7 mM ATP prior to freezing a second aliquot resulted in an approximately 50% decrease in the EPR signal of Center 1. It is consistent with an oxidation of one-half of the iron-sulfur Center 1 (Spectrum B). Subsequent addition of oligomycin and uncoupler caused the signal to return to the level prior to ATP addition, within the time required to remove and freeze an aliquot of the suspension (~40 sec) (Spectrum C). If uncoupler alone, oligomycin alone or oligomycin plus uncoupler were added prior to ATP addition, no ATP induced effect was observed. When no uncoupler nor oligomycin were added, Center 1 remained in 50% oxidized state at least 4 minutes and then gradually returned to the reduction level before ATP addition (corresponding to the time required for hydrolysis of the ATP). ATP additions made at various oxidation-reduction potentials indicate that only 50% ± 10% of the Center 1 signal is involved (hereafter designated Center 1a). The iron-sulfur center responsible for the remaining 50% of the Center 1 which was not affected by ATP is designated as Center 1b. Thus Centers 1a and 1b which constitute the Center 1 signal (1,2,10) have very similar EPR spectra and half-reduction potentials (1,2) but are different in their response to ATP addition. In mitochondrial suspension respiring in the presence of 10 mM succinate and 10 mM glutamate and the absence of added ADP and phosphate, the pyridine nucleotides were highly reduced (greater than 90%). Thus the low potential end of the respiratory chain was at an oxidation-reduction potential of more negative than -350 mV. EPR measurements using frozen aliquots showed that Centers 3 and 4 were more than 90% reduced while Center 1 was approximately 40% reduced. Thus it appears that under these conditions iron-sulfur Center 1 is more difficult to reduce than that expected from its measured half-reduction potential of -305 mV in the non-energized anaerobic state (1). The data are consistent with Center 1b being mostly reduced in state 4 while Center 1a is almost entirely oxidized, in agreement with the potentiometric experiments.

These experimental data are consistent with iron-sulfur Center 1a having a half-reduction potential which is dependent on the phosphate potential. This suggests that Center 1a is involved in energy transduction at Site I, and is consistent with the interpretation of previous data from Site I induction experiments using C. utilis cells grown in iron-deficient medium. ATP induces a shift of the half-reduction potential of cytochrome a_3 to a more negative value (+380 to +160 mV) (6,8) and a shift of the half-reduction potential of cytochrome b_T to a more positive value (-30 mV to +245 mV) (7). ATP appears to shift the half-reduction potential of iron-sulfur Center 1a to a more negative value (-305 to below -450 mV). The ATP induced shift to more negative values is consistent with the multi-reaction mechanisms required for one electron carriers to act as energy transducers in the oxidative phosphorylation with its 2 electrons per ATP stoichiometry (11), although the actual molecular mechanism remains unknown.

Slater and coworkers (12,13) have reported, in the absence of added redox mediators, that the addition of ATP to NADH reduced phosphorylating submitochondrial particles caused partial oxidation of four iron-sulfur centers, including Centers 1 and 2, the succinate dehydrogenase iron-sulfur protein, the Rieske iron-sulfur protein and two additional unidentified species. The authors suggested that the measured oxidations correspond to a "lowering of the effective redox potential" of the six components. A more complete interpretation can now be made. In the absence of mediators, reversed electron transport would be expected to oxidize components which lie on the oxygen side of the first phosphorylation site (all of the measured iron-sulfur proteins except Center 1) but to cause either no change or slight reduction of the components with half-reduction potentials near that of NAD (Center 1) (11). Thus, under the described experimental conditions (12,13) the ATP induced oxidation of Center 1 can be explained by the ATP induced change in the half-reduction potential of Center 1a to a more negative value as described in this paper.

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