INTERACTION OF IONIC DETERGENTS WITH IRON-SULFUR CENTERS IN BEEF HEART MITOCHONDRIAL MEMBRANES

Richard CAMMACK*, John J. MAGUIRE and Lester PACKER

Membrane Bioenergetics Group, Lawrence Berkeley Laboratory and *Department of Physiology-Anatomy, University of California, Berkeley, CA 94720, USA*

Received 26 June 1978

1. Introduction

The mitochondrial respiratory chain consists of a number of membrane-bound electron carriers which include about a dozen iron–sulfur (Fe–S) centers [1] mainly localized in complexes I, II and III of the respiratory chain (fig.1). These Fe–S centers dominate the low temperature electron paramagnetic spectrum (EPR) of mitochondria, just as the absorption bands of the cytochromes dominate the visible absorption spectrum. Spectra of the individual Fe–S centers in mitochondria such as beef heart can be distinguished by their different *g*-values, temperature dependence, midpoint redox potentials and response to various

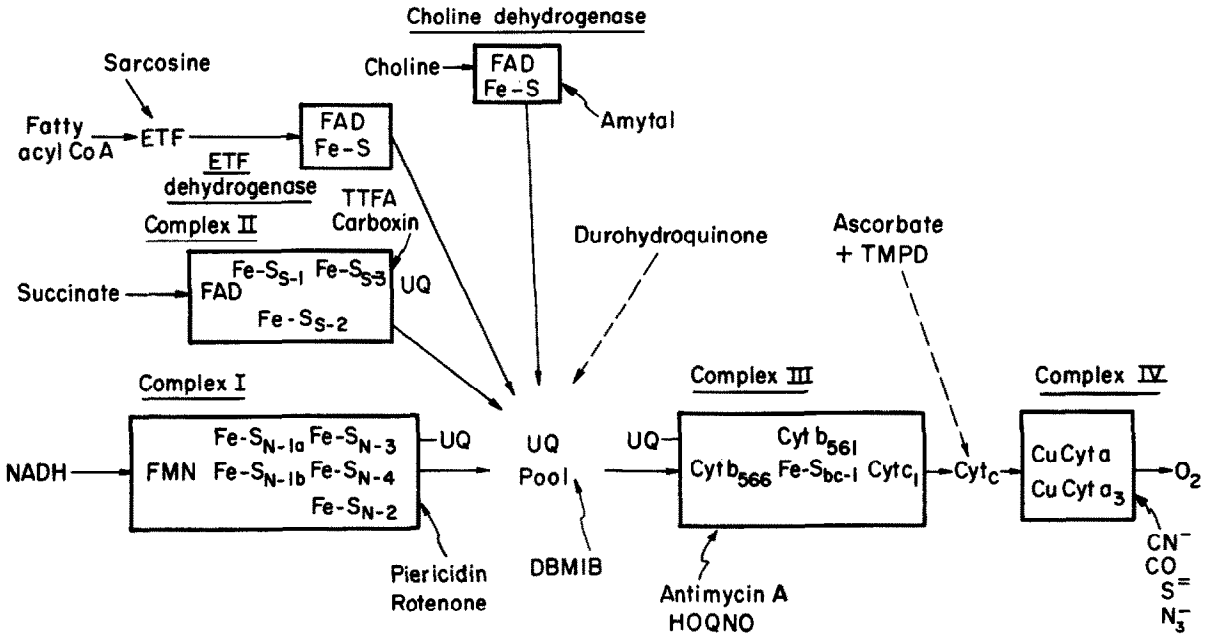

Fig.1. Electron transfer components of the mitochondrial respiratory chain. Nomenclature of Fe–S centers after [14]; Fe–S$_{bc-1}$, is the Rieske center.

* On leave from Department of Plant Sciences, University of London, Kings College, 68 Half Moon Lane, London SE24 9JF, England

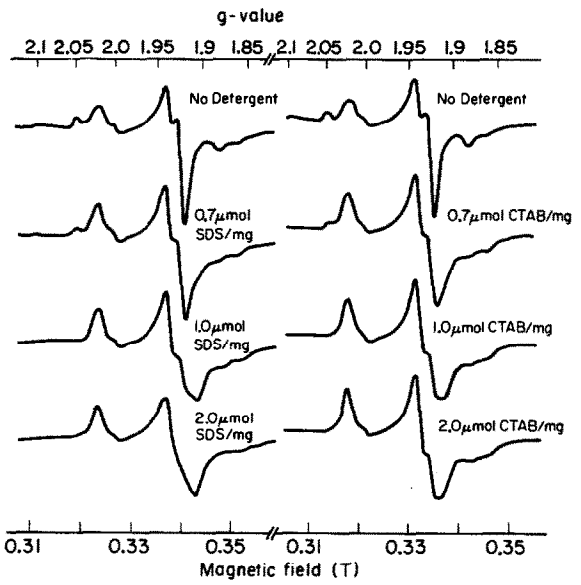

Fig.2. EPR spectra of reduced Fe–S centers in beef heart mitochondria showing the effects of SDS and CTAB. Samples buffered with 30 mM phosphate, pH 7.5 were treated with detergent for 1 min at 293 K, then with 5 mM NADH, 5 mM $Na_2S_2O_4$ for 1 min. before freezing. Final protein concentration was 20 mg/ml. Spectra were recorded at 14 K, with instrument settings; microwave power 10 mW, frequency 9.14 Ghz; modulation amplitude 1 mT, frequency 100 kHz.

substrates and reducing agents.

The cationic detergent cetyl trimethylammonium bromide (CTAB) and the anionic detergent sodium dodecyl sulfate (SDS) have been shown to be potent reversible inhibitors of mitochondrial respiration at $\lesssim 0.7$ μmol/mg protein [2]. In order to assess the mechanism of detergent inhibition we have studied the interaction of charged detergents on the EPR spectra of Fe–S centers, as these spectra can be a sensitive monitor of protein conformational changes [3]. Progressive effects on spectra were observed at 0.7–1.5 μmol/mg protein by these charged detergents, but uncharged detergents did not distort the EPR spectra of Fe–S proteins.

2. Experimental

Beef heart mitochondria were kindly provided by Dr T. P. Singer's laboratory. Submitochondrial particles were prepared by sonication of the mitochondria at 20 mg/ml protein in 50 M Tris–Cl, pH 7.5, and 0.25 M sucrose, with a Branson 350 sonifier, at power 7, with 0.7 pulse/s for 180 pulses, using 45 pulses at a time to keep the temperature below 10°C. The sonicated material was centrifuged at 12 000 × *g* for 15 min and the supernate was centrifuged at 100 000 × *g* for 60 min.

Crosslinking of mitochondria and SMP with dimethyl suberimidate was carried out as in [2].

EPR spectra were recorded on a Varian E 109 spectrometer with an Air Products Heli-Tran refrigerator to cool the sample.

3. Results

3.1. *Effect of charged detergents on EPR spectra of mitochondrial Fe–S proteins*

The lowest levels of detergent concentration that inhibit oxidation by NADH and succinate have no significant effect on the EPR spectra of the Fe–S centers. In order to perturb the EPR spectrum of a 10 mg protein/ml sample of SMP it was necessary to add levels of detergent > 0.7 μmol/mg protein. Figure 2 shows how SDS and CTAB affect the EPR signal in reduced mitochondria. The spectrum at 14 K is the sum of contributions from several Fe–S centers. The principal components are N-1a, N-1b, N2, N-3 and N-4 of complex I (NADH-ubiquinone reductase) [4], and ETF dehydrogenase, and centers S-1 and S-2 of complex II.

On adding 0.7 μmol SDS or 0.7 μmol CTAB/mg protein a decrease in the derivative peak at *g* 2.05 due to the g_{11} component of the N-2 signal. At higher detergent concentrations the spectrum changed more drastically and it became more difficult to recognize the contributions from different centers. Notable changes could be seen in the broadening out of the *g* 1.94 peak, and its seems that SDS was more effective than CTAB. The detergents appear to have two effects on the Fe–S centers:

(i) The lineshape changes which occurred rapidly on adding the detergent are consistent with changes in the protein environment of the Fe–S centers.

(ii) After prolonged treatment with detergent or on addition of > 2 μmol/mg protein, there was a considerable loss of total EPR signal intensity

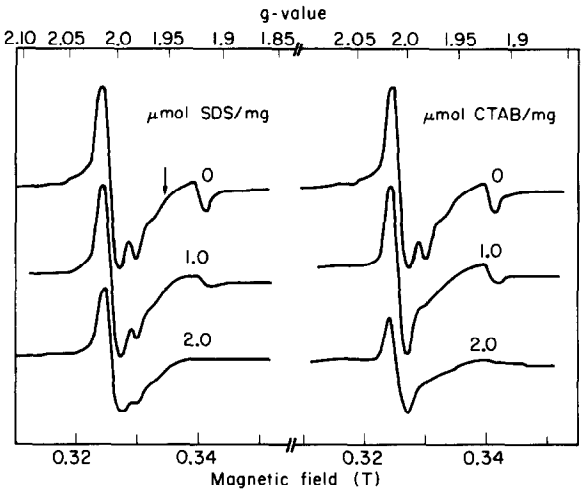

Fig.3. EPR spectra of Fe–S centers in unreduced beef heart mitochondria showing the effects of SDS and CTAB. Concentrations of detergents are in μmol/mg protein; temp., 8 K; other conditions of measurement were as for fig.2.

suggesting that the Fe–S centers were being destroyed.

The effects of detergents on spectra of unreduced mitochondria are shown in fig.3. The signals around g 2.01, in the absence of detergent, have been attributed to the oxidized forms of centers S-3 of succinate dehydrogenase and a soluble Fe–S protein characterized in [5]. These centers are called 'HiPIP-type', by analogy with *Chromatium* HiPIP (high-potential Fe–S protein) which can take a paramagnetic oxidized state corresponding to the $(4\ Fe–4\ S)^{-1}$ state. Additional features around g 2.04, g 1.99 and g 1.96 are due to a ubisemiquinone spin–spin interaction, which appears to be associated with the oxidizing side of complex II [6,7]. This indicates that the membrane system is partially reduced. The signal at g 1.92 is probably due to center N-2, partly reduced by endogenous substrate. On brief treatment with high concentrations of detergents, changes were again observed. In this case, CTAB produced more drastic effects than SDS, as shown by changes in the lineshape of the g 2.01 signal and loss of the features due to the ubisemiquinone interaction. It is not clear whether the g 1.92 center was becoming reduced and the g 1.99 signal oxidized, or both signals were becoming destroyed. The lack of effect of SDS was remarkable in view of the relative instability of center S-3, in purified lipid free soluble succinate dehydrogenase.

3.2. *Effect of detergents on EPR spectra of cross-linked mitochondria*

The lineshape of the EPR spectra reflects the protein environment of the Fe–S centers which are

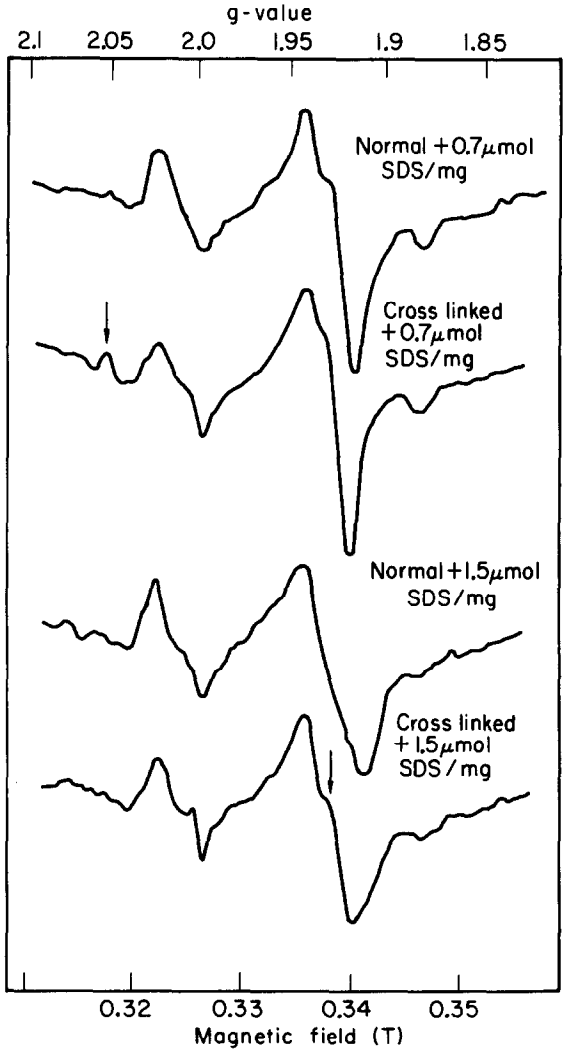

Fig.4. Spectra of reduced Fe–S proteins in SDS-treated cross-linked SMP, 6 mg protein/ml of either normal or preparations crosslinked with 20 mM DMS were treated with various concentrations of SDS. Conditions of measurements as for fig.2.

directly bound to cysteine residues. The detergent might influence this environment indirectly, by changing the membrane structure, or directly by changing the protein folding. Evidence for direct effects on the protein was obtained by the use of the crosslinking agent dimethyl suberimidate (DMS) which reacts with lysine residues of proteins. From fig.4 it can be seen that crosslinking inhibited the effects of detergents although the stabilizing effect of crosslinking could be overcome by adding more detergent. At 0.7 μmol SDS/mg protein, crosslinking prevented the loss of the g 2.05 features and at 1.5 μmol it inhibited the broadening of the g 1.94 signal (features shown with arrows). A monofunctional alkylating agent methyl acetimidate, which binds to lysine residues but does not crosslink them, showed no detectable effects.

4. Discussion

The existence of a charge on a detergent appears to be important for determining the spectra of Fe–S proteins; the neutral detergent Triton X-100 had little effect on the spectra at $\leq$ 10% by vol. However, progressive effects on the spectra were caused by charged detergents at 0.7–1.5 μmol/mg protein. Despite their differences in charge and effectiveness in inhibiting respiration, negatively- and positively-charged detergents had similar spectral effects. No changes were observed at lower concentrations, although respiration was drastically inhibited, particularly by CTAB [2].

The effects of detergents on the Fe–S proteins are analogous to the effects of denaturing solvents. With simple Fe–S proteins it has been found that solvents such as dimethylsulfoxide (DMSO) have little effect until a critical concentration is reached, when the protein undergoes a reversible change in its spectroscopic properties [9]. These changes can be rationalized in terms of the constraints placed on the Fe–S center by the protein around it, which effect EPR properties. When the protein is unfolded these constraints are relaxed and the properties of the Fe–S centers become more uniform. It is noteworthy that the spectrum of reduced mitochondria with 1.5 μmol SDS/mg protein (fig.2) resembles that of reduced (4 Fe–4 S) ferredoxins in 80% DMSO [3]. However, it remains to be determined whether this reflects a similar type of Fe–S center.

These results differ from the observations [10] on the effects of ethanol on Fe–S proteins of pigeon heart mitochondria. In that case, N-2 was relatively little affected and N-3 and N-4 were lost more rapidly. However these results related to relatively long incubations involving slow inactivation of the Fe–S centers, a process that probably involves loss of iron and oxidation of labile sulfur [11], whereas in the present case the immediate effects of adding detergent were examined. These effects appear to go further than simple removal of the Fe–S proteins from their normal membrane environment; purified preparations of complex I, II and III can be prepared with EPR essentially unchanged from those in the mitochondria (e.g. [8,12,13]). It seems likely that these effects are due to an interaction of the detergents either directly with the Fe–S centers or indirectly by causing a protein conformational change that alters the Fe–S center.

Acknowledgement

Research supported by the Department of Energy.

References

[1] Beinert, H. (1977) in: Iron Sulfur Proteins (Lovenberg, W. ed) vol. 3, pp. 61–100, Academic Press, New York.
[2] Mehlhorn, R. J. and Packer, L. (1976) Biochim. Biophys. Acta 423, 382–397.
[3] Cammack, R. (1975) Biochem. Soc. Trans. 3, 482–488.
[4] Albracht, S. P. J., Dooijewaard, G., Leeuwerik, F. J. and Von Swol, B. (1977) Biochim. Biophys. Acta 459, 300–317.
[5] Ruzicka, F. J. and Beinert, H. (1974) Biochem. Biophys. Res. Commun. 58, 556–563.
[6] Ruzicka, F. J., Beinert, H., Schepler, K. L., Dunham, W. R. and Sands, R. H. (1975) Proc. Natl. Acad. Sci. USA 72, 2886–2890.
[7] Ingledew, W. J., Salerno, J. C. and Ohnishi, T. (1976) Arch. Biochem. Biophys. 177, 176–184.
[8] Beinert, H., Ackrell, B. A. C., Vinogradov, A. D., Kearney, E. B. and Singer, T. P. (1977) Arch. Biochem. Biophys. 182, 195–206.

[9] McDonald, C. C., Phillips, W. D., Lovenberg, W. and Molm, R. M. (1973) Ann. NY Acad. Sci. 222, 789–799.
[10] Salerno, J. C. and Ohnishi, T. (1976) Arch. Biochem. Biophys. 176, 757–765.
[11] Petering, D., Fee, J. A. and Palmer, G. (1971) J. Biol. Chem. 246, 643–653.
[12] Ohnishi, T., Leigh, J. S., Ragan, C. I. and Racker, E. (1974) Biochem. Biophys. Res. Commun. 56, 775–782.
[13] Orme-Johnson, N. R., Hansen, R. E. and Beinert, H. (1974) J. Biol. Chem. 249, 1922–1927; 1928–1939.
[14] Ohnishi, T. (1976) Eur. J. Biochem. 64, 91–103.