Biochimica et Biophysica Acta, 635 (1981) 457—469
Elsevier/North-Holland Biomedical Press

BBA 48042

REDUCTION OF EXOGENOUS FMN BY ISOLATED RAT LIVER MITOCHONDRIA

SIGNIFICANCE TO THE MOBILIZATION OF IRON FROM FERRITIN

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(Received October 9th, 1980)

Key words: Ferritin; FMN reduction; Iron mobilization; Iron metabolism; (Mitochondria)

Summary

When FMN is added to rat liver mitochondria or mitoplasts it is reduced at a rate of approx. 0.2 nmol $\cdot$ min^{-1} $\cdot$ mg^{-1} protein. Sonicated mitochondria do not reduce exogenous FMN.

The reduction depends on drainage of reducing equivalents from the respiratory chain at the level of ubiquinone.

The net production of reduced FMN is detectable only at oxygen concentrations less than 4—5 μM.

The mitochondrial ubiquinol-FMN oxidoreductase provides a mechanism for the coupling of FMN-reduction to the reductive mobilization of iron from ferritin.

The results are discussed in relation to the role of ferritin as a donor of iron to the mitochondria.

Introduction

In vitro, iron is readily mobilized from ferritin by free dihydroflavins at a rate which exceeds that obtained with other small molecular weight reductants [1—4]. So far, however, evidence to support flavin induced mobilization of iron from ferritin in situ, are scanty [5—7].

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCIP, 2,6-dichlorophenolindophenol; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

0005-2728/81/0000—0000/$02.50

Recently we have shown that isolated rat liver mitochondria mobilize iron from ferritin by a mechanism which requires exogenously added flavin, a reducing substrate and low oxygen concentrations (<5 μM) [8]. The results were interpreted to mean that external flavins were reduced by the mitochondria, and the dihydroflavins thus produced subsequently penetrated the channels of the ferritin molecules to react with the ferritin iron core. In situ the counterpart to our test system should be a pool of free or non-covalently bound flavin of the cytosol [4]. A cytosolic flavin pool has so far not been characterized, although a number of observations support its existence. According to Fazekas and Sandor [9] injection of radioactive riboflavin into rats distributes to about 17% in the liver with 70—40% (decreasing with time) free in the cytosol, presumably en route from the plasma to its appropriate apoproteins. Essentially the same conclusions were reached by Martinez and McCauley [10] from studies on the incorporation on radioactive riboflavin into flavoproteins of the mitochondrial outer membrane, and by Okuda et al. [11] from the exchange of bound flavins for free flavins in D-amino-acid oxidase.

Reductive mobilization of iron from ferritin has also been ascribed to specific NAD(P)H-FMN oxidoreductases of the cytosol [5,6] or the microsomes [12]. So far, however, these systems have not been characterized in details, and their operation in situ remains uncertain.

The present experiments were undertaken to study the mechanism(s) by means of which isolated rat liver mitochondria reduce exogenously added flavins.

A preliminary account of certain aspects of this work has already appeared [13].

Materials and Methods

Preparation of mitochondria. Rat liver mitochondria and submitochondrial particles were prepared as previously described [14,15]. Mitoplasts were prepared by the digitonin method [16]. The mitoplasts contained >95% succinate dehydrogenase and malate dehydrogenase activity and less than 4% adenylate kinase activity compared to intact mitochondria.

Determination of FMN-reduction. Reduction of external FMN was determined at (near) anaerobic conditions from the decrease in absorbance $\Delta(A_{450nm} - A_{530nm}) = 10.3\ mM^{-1} \cdot cm^{-1}$ [17,18]. The incubation medium contained in a volume of 2.5 ml: 5.5 mg protein, 0.25 M sucrose, 10 mM Hepes buffer (pH 7.40) and 40 μM FMN. Further additions or omissions were as described in the legends to the tables and figures.

The incubations were carried out either in an open cuvette, or in a Thunberg cuvette on an Aminco DW 2 UV/VIS spectrophotometer. Temperature 37°C. The open cuvette was equipped with a vibrating platinum electrode for measuring the oxygen concentration. When indicated oxygenation of the medium was performed by flushing oxygen gently across the surface of the incubation medium. In the Thunberg cuvette, oxygen was removed by repeatedly evacuating and refluxing the cuvette with nitrogen of high purity (better than 99.99%). The efficiency of this procedure as assessed by the dithionite-sulphite assay (19) was better than 99.6%.

Other analytical procedures. Horse spleen ferritin was fractionated as previously described [8] to give apoferritin and fractions of ferritin of varying iron content.

Respiration rates were determined in the closed and thermostated (37°C) chamber of the oxygraph (YSI model 53, Oxygen Monitor).

Photoreduction of FMN in Hepes buffer was carried out by illuminating the incubation mixture in the chamber of the oxygraph for the time period indicated (Fig. 7). The light source was a photochemotherapy unit PUVA (H. Haldmann, D722, Schwenningen, F.R.G.) containing fourteen fluorescence tubes (F8T5/BL PUVA Sylvania) in a bank. The light dose was 66 $W \cdot m^{-2}$ at a distance of 15 cm.

Changes in the redox level of endogenous ubiquinone and cytochrome *b* of the mitochondria were determined from the change in absorbance $\Delta(A_{275nm} - A_{295nm})$ [20] and $\Delta(A_{562nm} - A_{575nm})$ [21].

Malate dehydrogenase [22], succinate phenazine methosulphate oxidoreductase [23], adenylate kinase [24] and glutamate dehydrogenase [25] were assayed as described in the references.

Protein was determined by the Folin-Ciocalteau reagent [26].

Chemicals. ADP, antimycin A, ATP, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2,6-dichlorophenolindophenol (DCIP), FMN (grade I), *N*-2-hydroxyethylpiperazine-*N*-2'-ethanesulphonic acid (Hepes), methylene blue, phenazine methosulphate, rotenone, ruthenium red, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD), tetraphenylboron and Triton X-100 were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Thenoyltrifluoroacetone was from Merck (Darmstadt, F.R.G.). Ferritin (equine spleen, A grade, twice crystallized, Cd^{2+}-free) was purchased from Calbiochem (Luzern, Switzerland).

Other chemicals were of highest purity commercially available. Double quartz-distilled water was used throughout.

Results

Reduction of FMN by whole mitochondria

When rat liver mitochondria were incubated under anaerobic conditions in the presence of FMN and succinate there was a decrease in the absorbance at 450 nm (Fig. 1, spectrum a compared to spectrum b). Upon reoxygenation there was a slight downward displacement of the spectrum due to respiration dependent swelling of the mitochondria [27,28] with an apparent incomplete reversion of the absorbance at 450 nm to its level at the aerobic-anaerobic transition (Fig. 1, spectrum c). When the same experiments were run with solubilized mitochondria, a similar spectral shift, typical of a flavin-dihydroflavin transition [17] occurred, but now the spectrum reverted completely to its form at the aerobic-anaerobic transition when the medium was reoxygenated (Fig. 1, inset). To circumvent the influence of swelling, generation of reduced FMN was determined from the decrease in absorbance $\Delta(A_{450nm} - A_{530nm})$. By this approach also the influence of endogenous chromophores was negligible (Fig. 2a): in the absence of external FMN anaerobiosis coincided with a transient increase in absorbance due to reduction of compo-

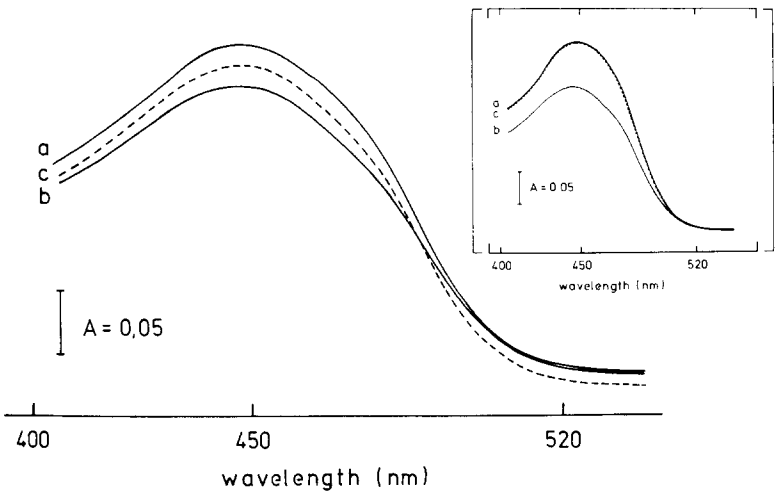

Fig. 1. Reduction of exogenous FMN by whole mitochondria and by solubilized mitochondria (inset). Whole mitochondria (or solubilized mitochondria), approx. 2.2 mg protein/ml, were incubated in an open cuvette (see Materials and Methods) with succinate (1 mM) as respiratory substrate. The spectra were recorded: (a) just prior to anaerobiosis; (b) after 6 min of anaerobiosis; (c) 1 min after reoxygenation of the incubation medium. The mitochondria were solubilized by 0.05% (v/v) Triton X-100.

nents of the respiratory chain [29], whereafter the absorbance remained constant. When FMN was included (Fig. 2b), anaerobiosis was followed by a curvilinear decrease in the absorbance corresponding to a maximum rate of reduction of FMN of 0.2 nmol · min^{-1} · mg^{-1} protein. When the medium was flushed with oxygen the absorbance rapidly reverted to its original level. As to be expected [1,4] with ferritin, but not with apoferritin net production of $FMNH_2$ was markedly reduced (Fig. 2c). Steady-state equilibrium between FMN-reduction by the mitochondria and its oxidation by ferritin was reached after 3—5 min incubation at a concentration of $FMNH_2$ of 0.6 nmol · mg^{-1} protein (Fig. 2c).

No dihydroflavin was generated in the absence of mitochondria, neither did FMN influence the turbidity of the mitochondria, and there was no photoreduction of FMN by the monochromatic light of the spectrophotometer as assessed by the method of Radda and Calvin [30].

The efficiency of the mitochondria to reduce external FMN parallelled the ability of the mitochondria to undergo respiration dependent swelling, i.e. a 4-fold increase in the rate of FMN-reduction was found in maximally swollen mitochondria compared to that of contracted mitochondria (Fig. 3). During swelling and the subsequent experimental period less than 5% of the total glutamate dehydrogenase activity of the mitochondria leaked to the medium, i.e. swelling was not synonymous with lysis [31]. The enhanced generation of reduced flavin by swollen mitochondria was a function, not of energy-dissipation, but of the swelling itself (Table I). Thus, in uncoupled mitochondria treated with ATP plus Mg^{2+} to inhibit the respiration-dependent swelling [27] reduction of FMN was essentially as that of tightly coupled mitochondria. By contrast, when added to swollen, anaerobic mitochondria in which FMN-reduction already had reached steady-state rate,

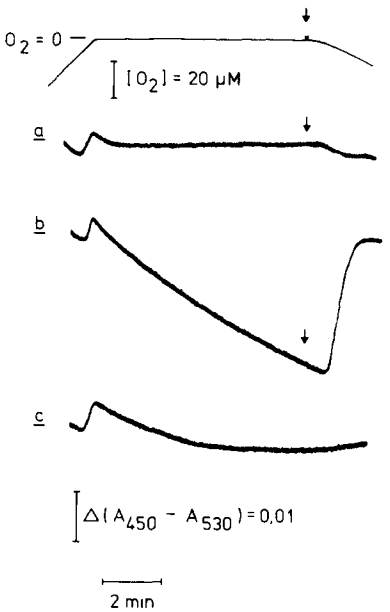

Fig. 2. Reduction of exogenous FMN by whole mitochondria. Mitochondria were incubated as described in Table I. (a) control without FMN; (b) with 40 μM FMN; and (c) with 40 μM FMN plus 0.22 μM ferritin (saturated with 1400 atoms FE(III)/mol ferritin). At the points indicated (arrows) the medium was reoxygenated. The oxygen concentration of the medium is shown by the oxygraphic trace at the top.

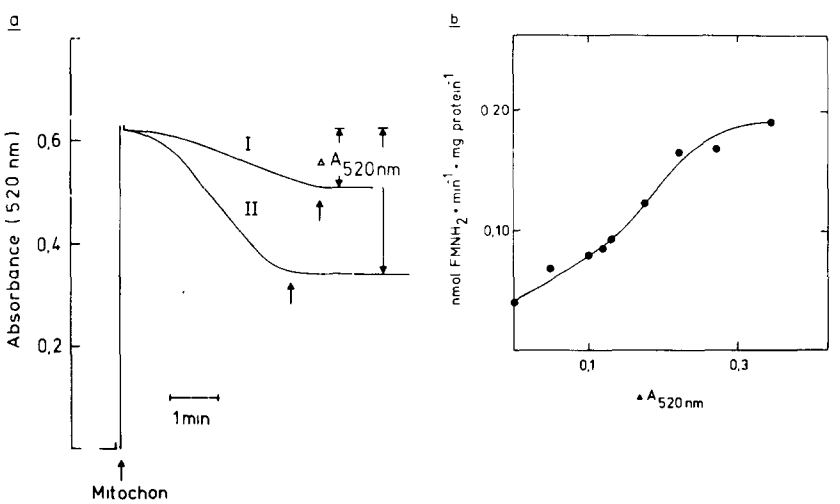

Fig. 3. Effect of swelling of the mitochondria on the rate of reduction of exogenous FMN. Mitochondria, approx. 2.2 mg protein/ml, were incubated as described in Fig. 2b. (a) Swelling of mitochondria aged at room temperature to a respiratory control ratio (ADP) of 3 (I) or <2 (II). Anaerobiosis is indicated by arrows. (b) Reduction of exogenous FMN by mitochondria at varying degrees of swelling. ΔA_{520nm} was determined as in (a). The time to reach anaerobiosis in each experiment was kept within 2—5 min. Reduction of FMN was determined as in Fig. 2.

TABLE I

EFFECT OF ATP, Mg^{2+}, CCCP AND P_i ON THE REDUCTION OF EXOGENOUS FMN BY MITOCHONDRIA OF DIFFERENT ENERGY COUPLING

Mitochondria, approx. 2.2 mg protein/ml, were incubated in an open cuvette (see Materials and Methods) with 1 mM succinate as substrate. The rate of $FMNH_2$ generation was determined from the initial decrease in the absorbance $\Delta(A_{450nm} - A_{530nm})$ (see Fig. 2). Energy dissipation was by storage at room temperature. The compounds tested were added at the start of the experiments (unless stated otherwise). The results are the means and the ranges (in parentheses) from three separate experiments. R.C., respiration control ratio.

Addition	nmol $FMNH_2 \cdot min^{-1} \cdot mg^{-1}$ protein generated by mitochondria of	
	$R.C._{ADP} > 5$	$R.C._{ADP} < 2$
None	0.05 (0.04—0.06)	0.20 (0.18—0.23)
1 mM ATP + 1 mM $MgCl_2$	0.04 (0.03—0.05)	0.04 (0.03—0.05)
1 mM ATP + 1 mM $MgCl_2$ (added at anaerobiosis)		0.21 (0.19—0.22)
20 μM CCCP	0.05 (0.04—0.06)	0.21 (0.19—0.23)
10 mM P_i (pH 7.4)	0.19 (0.18—0.21)	0.22 (0.21—0.23)

ATP plus Mg^{2+} had no effect. Furthermore, CCCP added to mitochondria with respiratory control ratio >5 had virtually no effect on the reduction of external FMN. However, when the same mitochondria were swollen in hypotonic phosphate buffer, FMN was reduced at the same rate as in mitochondria swollen by aging.

The rate of reduction increased with the concentration of FMN to reach a level of about 0.2 nmol $\cdot$ min^{-1} $\cdot$ mg^{-1} protein at 40 μM FMN (Fig. 4).

Reduction of FMN by mitoplasts and by mitochondria modified by freezing and thawing, treatment with Triton X-100 or sonication

Mitochondria, either intact or modified by freezing and thawing, or treated with Triton X-100 all readily reduced FMN under anaerobic conditions (Table

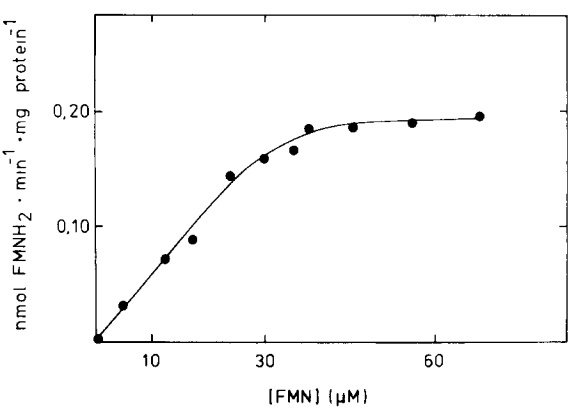

Fig. 4. Effect of increasing concentrations of exogenous FMN on the rate of reduction of FMN by whole mitochondria. Experimental conditions were as in Fig. 2b with increasing concentration of exogenous FMN.

II). Essentially similar results were obtained with mitoplasts. By contrast, with sonicated mitochondria there was no net reduction of FMN following anaerobiosis, neither did admixture of sonicated mitochondria influence the rate of reduction by whole mitochondria (data not shown).

The ability of solubilized mitochondria to reduce FMN was about 2-fold that of intact mitochondria. These results, however, were highly dependent on the time interval from the addition of Triton X-100 to the addition of FMN (Fig. 5). Thus, if the mitochondria were preincubated with Triton X-100 beyond 5 min the ability to reduce FMN was lost, and this was not caused by back diffusion of oxygen or impaired respiratory activity of the solubilized particles.

Effect of respiratory substrates, respiratory inhibitors, redox dyes, ruthenium red and tetraphenylboron on the reduction of FMN

Table III summarizes the results obtained with a number of respiratory substrates and respiratory inhibitors. To rule out differences in the rate of FMN reduction caused by different degrees of swelling of the mitochondria during the experimental period (see Fig. 3), all the experiments were done on solubilized mitochondria. Succinate was by far the most effective respiratory substrate for the process. With succinate the reaction was virtually completely inhibited by malonate, less so by thenoyltrifluoroacetone and not at all by antimycin A. Pyruvate plus malate with (or without) malonate was only half as effective as succinate with rotenone, and ascorbate with TMPD was virtually ineffective compared to the control without exogenous substrate.

Reduced flavins in a protein-free solution are readily oxidized by phenazine methosulphate, DCIP and methylene blue [32]. Combined with protein, however, the acceptor specificity of the flavins might be quite different [33,34]. As seen from Fig. 6, reduced FMN was completely and rapidly oxidized by DCIP, less so by phenazine methosulphate and not at all by methylene blue.

When $FMNH_2$ is reoxidized by molecular oxygen, oxygen radicals which may be deleterious to the mitochondria are generated [35,36]. However, the

TABLE II

REDUCTION OF EXOGENOUS FMN BY MITOCHONDRIA, MITOPLASTS, SUBMITOCHONDRIAL PARTICLES AND MITOCHONDRIA DISINTEGRATED BY FREEZING AND THAWING OR TREATED WITH TRITON X-100

Experimental conditions were as in Table I with a protein concentration of 2.0—2.4 mg/ml. The mitochondria and the mitoplasts were aged at room temperature to a respiratory control ratio (ADP) < 2. Triton X-100 (0.05%, v/v) was added at the onset of anaerobiosis. The results are the means and the ranges (in parentheses) from three separate experiments.

	nmol $FMNH_2 \cdot min^{-1} \cdot mg^{-1}$ protein
Mitochondria	0.21 (0.17—0.24)
Mitochondria following freezing and thawing	0.19 (0.16—0.21)
Mitochondria treated with Triton X-100	0.37 (0.34—0.39)
Mitoplasts	0.22 (0.17—0.25)
Sonicated mitochondria	0

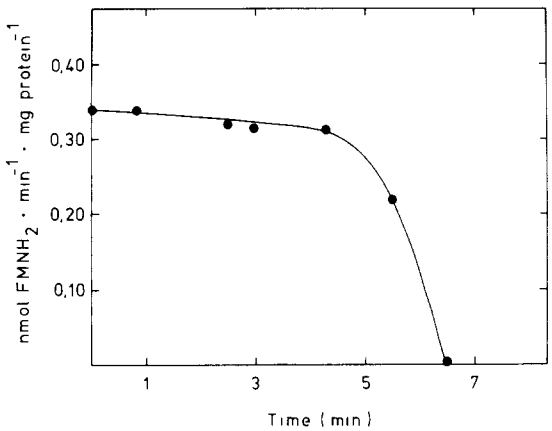

Fig. 5. Reduction of exogenous FMN by mitochondria preincubated with Triton X-100. Mitochondria, approx. 2.2 mg protein/ml were incubated as described in Fig. 1 without FMN. At anaerobiosis the mitochondria were solubilized by adding 0.05% (v/v) Triton X-100 followed at the time interval indicated by 40 μM FMN. Reduction of FMN was determined as in Fig. 2b.

nature and the yield of oxygen radicals differ, depending on the protein binding of the flavins [37]. As seen from Fig. 7, reoxygenation of reduced FMN in amount far in excess of that produced in our system, had no effect on the energy coupling of the mitochondria.

The polycation ruthenium red [38] at a concentration of 10 μM, completely abolished the formation of $FMNH_2$. On the other hand the polyanion tetraphenylboron [39] at concentration as high as 80 μM had no effect on the reduction of FMN. Mitoplasts behaved as whole mitochondria (data not shown).

TABLE III

EFFECT OF RESPIRATORY SUBSTRATES AND INHIBITORS ON THE REDUCTION OF EXOGENOUS FMN BY SOLUBILIZED MITOCHONDRIA

Mitochondria, approx. 2.2 mg protein/ml, were incubated in a Thunberg cuvette with the substrate and inhibitor to be tested. The reaction was started by adding 30 μM FMN and 0.05% (v/v) Triton X-100 from the short arm of the cuvette to the deaerated medium (see Materials and Methods). The results are the means and the ranges (in parentheses) from three separate experiments. TTFA, thenoyltrifluoroacetone.

Addition	nmol $FMNH_2 \cdot min^{-1} \cdot mg^{-1}$ protein
None	0.08 (0.06—0.09)
1 mM succinate	
+ 8 μM rotenone	0.43 (0.41—0.46)
+ 12 mM malonate	0.04 (0 —0.07)
+ 0.4 mM TTFA	0.13 (0.09—0.16)
+ 2 μg/ml antimycin A	0.44 (0.40—0.49)
1 mM pyruvate + 1 mM malate	
+ 12 mM malonate	0.18 (0.12—0.24)
+ 8 μM rotenone	0.02 (0 —0.03)
1 mM ascorbate + 3 μM TMPD	0.08 (0.06—0.11)

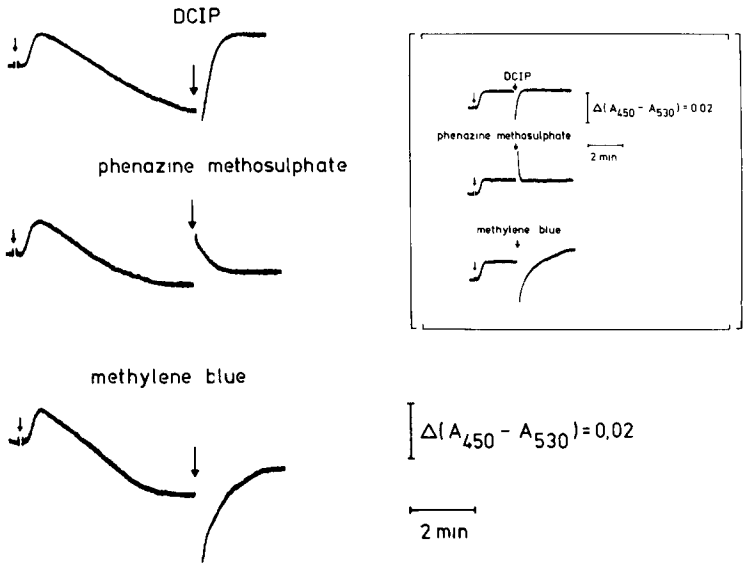

Fig. 6. Reoxidation of $FMNH_2$ by redox dyes. Mitochondria were incubated as described in Fig. 1. At anaerobiosis 0.05% (v/v) Triton X-100 was added (arrows). At the time indicated a small volume of deaerated redox dye was added to a final concentration of 20 μM. Inset: control experiments without FMN.

Effect of endogenous FMN on the redox level of ubiquinone and cytochrome b

The results of Table III and Fig. 6 suggest that FMN drains reducing equivalents from the respiratory chain at the level of ubiquinone-cytochrome *b*. Ubiquinone has been characterized as a mobile carrier, shuttling reducing

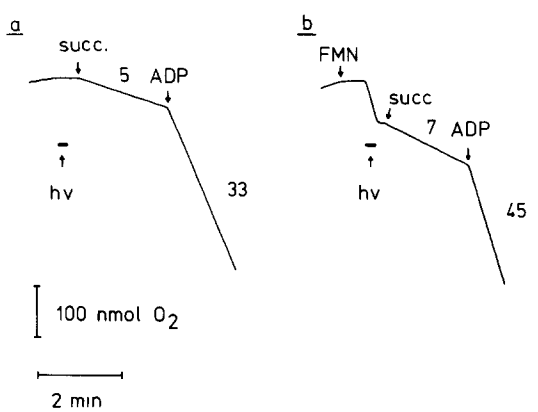

Fig. 7. Effect of reoxidation of photoreduced FMN on ADP-stimulated respiration of tightly coupled mitochondria. Mitochondria, approx. 1.5 mg protein/ml were incubated in the chamber of the oxygraph in a medium of 4 ml: 50 mM glucose, 175 mM sucrose, 5 mM $MgCl_2$, 10 mM KCl, 5 mM P_i, 2 μM rotenone and 10mM Hepes buffer (pH 7.40). Further additions were: 2.5 mM succinate (succ.), 0.6 mM ADP and 30 μM FMN. At the time period indicated (arrows) the incubation medium was irradiated as described (see Materials and Methods). The figures above each trace represent the respiration rates (natoms oxygen · min^{-1} · mg^{-1} protein).

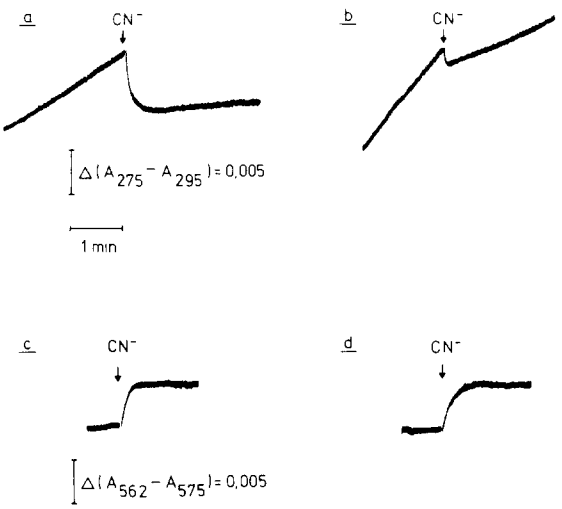

Fig. 8. Effect of exogenous FMN on the redox level of ubiquinone and cytochrome *b* in whole mitochondria. Mitochondria, approx. 0.8 mg protein/ml, were incubated in an open cuvette in a medium of 0.25 M sucrose, 10 mM Hepes buffer (pH 7.40), 2 μM CCCP and 2 mM succinate. Reduction of ubiquinone was measured from the decrease in absorbance $\Delta(A_{275nm} - A_{295nm})$ following the addition of 1.8 mM cyanide in the absence (a) or the presence of 20 μM FMN (b). Reduction of cytochrome *b* was measured from the increase in absorbance $\Delta(A_{562nm} - A_{575nm})$ following the addition of 1.8 mM cyanide to a medium of approx. 2.5 mg protein/ml, 4 μM CCCP and 4 mM succinate in the absence (c) and the presence of 40 μM FMN (d).

equivalents between the dehydrogenases and cytochrome *b* [18,41]. Moreover, ubiquinone appears to subserve a pool function, i.e. it occurs in the membrane in a homogenous pool rather than as part of separate functional compartments [42]. From this point of view ubiquinone should be well suited to react with exogenous FMN, as actually found (Fig. 8): FMN reduced the cyanide-induced reduction of ubiquinone by about 80% and it also counterbalanced the effect of cyanide on the production of fumarate [20] to about 60%. On the other hand, external FMN had no effect on the redox level of cytochrome *b* (Fig. 8, c and d).

Discussion

The ability of mitochondria to reduce exogenous FMN is of relevance to the removal of iron from ferritin [1—8]. Thus, whereas the NAD(P)H-FMN oxidoreductase of the cytosol has been incriminated in the mobilization of iron from ferritin to outside the cell [7], the mitochondrial $FMNH_2$-ferrireductase may represent a linkage between ferritin of the cytosol and production of heme within the mitochondria [8,13,15,43].

Generation of $FMNH_2$ is evident from the spectral shift (Fig. 1) which is that of a flavin-dihydroflavin transition [17]. The rate of reoxidation of $FMNH_2$ by ferritin equalized the rate of generation of $FMNH_2$ only after a delay of 3—5 min (Fig. 2c). A similar delay has been found for the FMN-dependent mobilization of iron from ferritin [4,8,44]. The delay can not be

explained by iron heterogeneities of the ferritin molecule [4,8]. More likely the delay reflects the building up of $FMNH_2$ to concentrations necessary to initiate iron mobilization across the electrostatic hindrance of the ferritin channels [4]. This interpretation is in accordance with the results of Crichton et al. [44] and by the finding that when $FMNH_2$ is generated by dithionite there is no delay in the iron release process [1,8].

According to Zaman and Verwilghen [12] the microsomal NADH-FMN ferrireductase operates only at strict anaerobic conditions. By comparison, the rate of net production of $FMNH_2$ by mitochondria suspended in an open cuvette where oxygen readily diffuses into the medium (Table II) is about 85% of the rate obtained under strict anaerobic conditions (Table III). The limiting concentration of oxygen for net production of $FMNH_2$ by the mitochondria is about 4—5 μM [8] (Fig. 2), or close to the oxygen concentration of the mitochondrial environment in situ [45].

Mitoplasts are as effective as whole mitochondria to reduce exogenous FMN (Table II), and as for the mitochondria, the reaction rate increases in parallel to the swelling of the particles. The outer membrane is therefore neither a permeability barrier nor a prerequisite to the operation of the mitochondrial FMN-reductase.

The inability of sonicated mitochondria to reduce exogenous FMN readily explains why sonicated mitochondria do not mobilize iron from ferritin [8]. Apparently structures necessary for the reduction of FMN might be prevented from interacting with FMN by the inside-out topography of the sonicated particles [46], or they may be released from the membrane [47] or inactivated by the sonication as they are by prolonged treatment with Triton X-100 (Fig. 5). Possible candidates are glycoproteins of the outer surface of the inner membrane [47] as supported also by the results obtained with ruthenium red.

Exogenous FMN is reduced by the respiratory chain at the substrate side of the antimycin A sensitive site (Table III) with ubiquinol as the most likely immediate reductant: exogenous FMN effectively counterbalances the cyanide-induced reduction of endogenous ubiquinone (Fig. 8); thenoyltrifluoroacetone which inhibits the reduction of semiquinone to ubiquinol [48], depresses the reduction of FMN by about 70% (Table III) and FMN added to mitochondria has a more favourable midpoint potential (Fig. 6) for the reduction by ubiquinol than FMN in solution [32—34].

Consistent with this interpretation are also the concept of ubiquinone as an independent, diffusible redox component in the mitochondrial inner-membrane [41,42], and the findings that the polarity of ubiquinone increases upon reduction [40] and that the rate of FMN reduction increases upon swelling of the mitochondria with enhanced access of FMN to the ubiquinol pool (Fig. 3).

As to the association of FMN with the mitochondria it seems to be rather weak since $FMNH_2$ generated by the mitochondria readily mobilizes iron from ferritin [8] and therefore should easily gain access to within the ferritin channels. However, the association is of sufficient strength to change the redox potential of FMN linked with the mitochondria from that of FMN in solution (Fig. 6) [32—34]. One possible way of binding could be a reversible electro-

static interaction between the electrodeficient isoalloxazine ring of FMN [49] and negatively charged glycoproteins [47].

How then do the present experiments fit the concept of mobilization of iron from ferritin to heme synthesis in situ? Quantitatively the rate of FMN reduction as reported here is highly sufficient to cope with the need for iron to the ferrochelatase of liver mitochondria [50]. The questions remain, however, if ferritin is on the main path of iron from the plasma membrane to the mitochondria [51,52], and if there is a need for the mitochondria to accumulate iron directly from ferritin.

Acknowledgements

The technical assistance of Mrs. A. Iden and Mr. H. Henriksen is gratefully acknowledged. The study was supported in part by the Norwegian Research Council for Science and Humanities.

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