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ENERGY-DEPENDENT ACCUMULATION OF IRON BY ISOLATED RAT LIVER MITOCHONDRIA

V. EFFECT ON FACTORS CONTROLLING RESPIRATION AND OXIDATIVE PHOSPHORYLATION

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SUMMARY

1. Depending on the metabolic state, the addition of iron(III)-sucrose induces an inhibition or a stimulation of the respiration rate when added to isolated rat liver mitochondria.

2. Under conditions identical to those used in the accumulation studies (Romslo, I. and Flatmark, T. (1973) Biochim. Biophys. Acta 305, 29–40), the ferric complex induces a decrease in the oxygen uptake concomitant to an oxidation of cytochromes c $(+c_1)$ and a $(+a_3)$. These results suggest that ferric iron is reduced to ferrous iron by the respiratory chain prior to or simultaneously with its energy-dependent accumulation.

3. On the other hand, the addition of iron(III)-sucrose induces a stimulation of respiration in State 4 and State 3 provided Mg^{2+} is present in the suspending medium. In contrast to Ca^{2+}, iron stimulates State 4 respiration in a cyclic process only within narrow concentration limits; at concentrations of iron above 100 μM the respiration remains in the activated state until anaerobiosis. The stimulation of State 4 respiration is more pronounced with succinate than with NAD-linked substrates, a difference which partly may be attributed to a stimulation of the succinate dehydrogenase complex.

4. The stimulation of respiration by iron is approx. 3 times higher in State 3 than in State 4 and this difference can be attributed to a stimulation of the adenine nucleotide exchange reaction in State 3 with a concomitant increase in the rate of oxidative phosphorylation, although the P/O ratio is slightly diminished.

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

INTRODUCTION

In previous studies from this laboratory [1–3], rat liver mitochondria were shown to accumulate iron partly by an energy-dependent and partly by an energy-independent mechanism with different time, pH and temperature dependencies [1]. The mitochondria were shown to possess two classes of iron-binding sites with low and high affinity, respectively [2]. The 'high-affinity binding' of iron was linked to an energy-dependent inner membrane and matrix loading with iron [3], and revealed similarities to the energy-dependent [4] high-affinity loading with Ca^{2+} [5]. Nevertheless, the accumulation of iron differed in several aspects from that of Ca^{2+} [6]. Notably, P_i, carboxylates and ADP, which stimulate the accumulation of Ca^{2+} [7], all inhibited the energy-dependent as well as the energy-independent accumulation of iron [8], presumably through the formation of metal-ligand complexes with unfavourable stability constants. Thus, one would indeed expect the effect of iron on mitochondrial respiration to be different from that of Ca^{2+} [9] and Sr^{2+} [10].

So far, however, the results from studies on the effect of iron on mitochondrial respiration are rather conflicting. Thus, Cederbaum and Wainio [11] found that ferric as well as ferrous iron inhibited the oxygen uptake when added as 8-hydroxyquinoline complexes to mitochondria respiring on exogenous substrates, whereas Strickland and Goucher [12] found that a Fe(III)-ADP complex stimulated the respiration rate. Therefore, in the present study, the effect of iron(III)-sucrose on the respiration rate of rat liver mitochondria in different metabolic states has been studied in more detail.

Preliminary accounts of certain aspects of this work have already appeared [13, 14].

MATERIALS AND METHODS

Preparation of rat liver mitochondria

Rat liver mitochondria were prepared essentially as previously described [2]. The final mitochondrial pellet was resuspended and stored at 0–2 °C in 0.25 M sucrose containing 5 mM HEPES buffer, pH 7.4 at a concentration of 30–40 mg protein/ml.

Respiration rates

Mitochondrial respiration rates were determined by a Clark oxygen electrode (Yellow Springs Instrument's Biological Oxygen Monitor) with appropriate polarization circuitry. The instrument was calibrated as described by Grav et al. [15]. The mitochondria (2–4 mg of protein) were incubated at 25 °C in a medium containing in a final volume of 2.5 ml: 50 mM glucose, 175 mM sucrose, 10 mM HEPES buffer, pH 7.4, 5 mM $MgCl_2$ and 5 mM P_i. Omissions and other additions were made as indicated in the text or in the legends to figures. The respiratory States 1, 3 and 4 are metabolic states as defined by Chance and Williams [16].

Oxidative phosphorylation

Oxidative phosphorylation was assayed in the presence (Method A) as well as in the absence (Method B) of an ADP-regenerating system essentially as described by Grav et al. [15].

Method A. The mitochondria (2–4 mg of protein) were incubated at 25 °C in a reaction medium containing in a final volume of 2.5 ml: 50 mM glucose, 175 mM sucrose, 10 mM HEPES buffer, pH 7.4, 5 mM $MgCl_2$, and 2 mM ATP. Recording of oxygen consumption was started after a preincubation period of 2–3 min. Subsequent additions of substrate, 1 mM P_i (pH 7.4) containing 100 nCi $^{32}P_i$ per μmol P_i plus 25 units hexokinase and iron(III)-sucrose were made in that order, at intervals of 2–3 min. The total reaction period was approx. 10 min. The reaction was stopped by transferring duplicate samples (0.5 ml each) to a mixture of sulphuric acid and silicotungstic acid, and the incorporated P_i was estimated according to the distribution method of Lindberg and Ernster [17].

Method B. The mitochondria (2–4 mg of protein) were preincubated at 25 °C in a medium containing in a final volume of 2.5 ml: 50 mM glucose, 175 mM sucrose, 10 mM HEPES buffer, pH 7.4, 5 mM $MgCl_2$ and 1 mM P_i (pH 7.4) containing 100 nCi $^{32}P_i$ per μmol P_i. After a preincubation period of 2–3 min, the recording of the oxygen consumption was started, and susequent additions of substrates, ADP and iron(III)-sucrose were made. The further procedure was as described for method A. ADP was added in excess to insure a linear State 3 respiration until anaerobiosis was reached.

Adenine nucleotide exchange

The adenine nucleotide exchange was measured in the backward direction by the 'inhibitor stop' method essentially as described by Pfaff and Klingenberg [18].

Prelabeling of the mitochondria was carried out with ^{14}C-labeled adenine nucleotides as described by Souverijn et al. [19]. Unless otherwise stated the reactions were performed in thermostated vessels at 0–2 °C in a medium containing in a final volume of 8 ml: mitochondria (4–5 mg protein/ml), 225 mM sucrose, 10 mM HEPES buffer, pH 7.4, 5 mM $MgCl_2$ and 10 mM KCl. Other additions were made as indicated in the legends to Fig. 10A and B and Table II. At different time intervals, aliquots of 1 ml were withdrawn, the exchange reaction terminated by atractyloside (50 μM), and the mitochondria were rapidly sedimented by centrifugation in an Eppendorf microcentrifuge (type 3200).

The radioactive adenine nucleotides were analyzed for radiochemical purity by thin-layer chromatography as described by Flatmark and Pedersen [20].

Adenine nucleotides were determined enzymically [21, 22] using a Shimadzu MPS-50L recording spectrophotometer thermostated at 25 °C.

The data were analyzed as described in the literature [18, 23].

Succinate: phenazine methosulfate oxidoreductase activity

The succinate: phenazine methosulfate oxidoreductase activity (EC 1.3.99.1) was assayed by a modification of the method described by Arrigone and Singer [24] on mitochondria respiring at steady-state in the presence of 4 mM succinate and a variable concentration of iron(III)-sucrose. At timed intervals, samples of 0.1 ml were transferred from the reaction chamber of the oxygraph to cuvettes containing in a final volume of 2.5 ml: 225 mM sucrose, 10 mM HEPES buffer, pH 7.4, 5 mM P_i, 5 mM $MgCl_2$, 1 mM KCN, 2 mM phenazine methosulfate, 50 μM DCIP, 25 μg phospholipase A and 20 mM succinate. The reduction of phenazine methosulfate was followed in a Shimadzu MPS-50L recording spectrophotometer thermostated at 25 °C.

Succinate transport

The kinetics of the uptake of succinate were studied by the 'inhibitor stop' method essentially as described by Meisner et al. [25]. Mitochondria with approx. 4 mg of protein were preincubated in 1 ml of medium for 1 min under conditions specified in the legend to Fig. 8. Iron(III)-sucrose was added, followed at 30 s by the labeled substrate. The reaction was stopped at 10 s with 20 mM phenylsuccinate, and the mitochondria separated by the silicone oil technique [26] in an Eppendorf microcentrifuge (type 3200). Controls were incubated with phenylsuccinate present before the labeled substrate was added, and the amount taken up subtracted from the experimental values with succinate [25]. In parallel experiments, variations in the adherent supernatant and the total water space were determined from the distribution of ^3H_2O and [^{14}C]carboxyldextran.

Redox level of flavoproteins and cytochromes

The measurements of the oxidation-reduction level of flavoproteins and cytochromes *b*, $c(+c_1)$ and $a(+a_3)$ were performed in cuvettes of 10-mm light path using an Aminco Chance dual wave-length spectrophotometer with both monochromators calibrated with reduced cytochrome *c* at an accuracy of ± 0.2 nm [27] (for wavelength settings, see Results). The temperature was thermostatically controlled at 25 °C. Protein was determined using the Folin-Ciocalteau reagent [28].

Chemicals

ADP, ATP, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2,6-dichlorophenol-indophenol (DCIP), glucose-6-phosphate dehydrogenase (grade II from yeast), hexokinase (type III from yeast), lactic dehydrogenase (type II from rabbit muscle), oligomycin, phenazine methosulfate, pyruvate kinase (type III from rabbit muscle) and rotenone were obtained from the Sigma Chemical Co. (St. Louis, Mo., U.S.A.), HEPES (A grade) was purchased from Calbiochem (Lucerne, Switzerland), phospholipase A from C. F. Boehringer & Soehne GmbH (Mannheim), phenylsuccinic acid from Aldrich-Europe (Beerse, Belgium), [1,4−^{14}C]succinic acid, [^{14}C]dextran-carboxyl mol. wt. 60–90 000, 8-[^{14}C]ATP and 8-[^{14}C]ADP from New England Nuclear (Boston, Mass.), and ^3H_2O from Institutt for Atomenergi, Kjeller, Norway.

Iron(II/III)-sucrose was prepared as described [1]. The iron(II) complex was prepared immediately before use and in order to minimize autoxidation, the solution was flushed with N_2 and stored under paraffin in the dark.

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

RESULTS

Effect of added iron(III)-sucrose on the respiration rate

From Fig. 1A–F it is seen that the addition of iron(III)-sucrose induced at least three responses in respiring mitochondria depending on their metabolic state. In State 1 (Fig. 1A) the addition of iron lowered the oxygen uptake in contrast to Ca^{2+} which induced a transient increase in respiration rate. The inhibition by iron was found to be slightly more pronounced in the absence than in the presence of

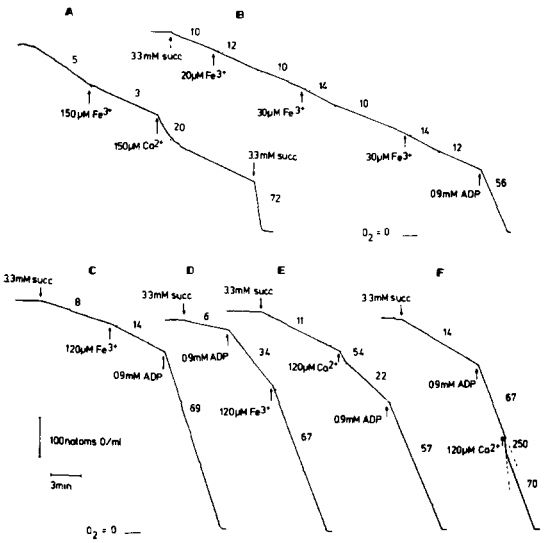

Fig. 1. Effect of added iron(III)-sucrose on the respiration rate of rat liver mitochondria. The mitochondria were suspended in the standard incubation medium (see Methods) at 6 mg protein/ml (A) and 1.0–1.5 mg protein/ml (B–F) in the absence (A) and presence (B–F) of 3.0 μM rotenone. Other additions were as indicated. The numbers immediately above each trace represent the specific respiration rate (natoms oxygen · min^{-1} · mg $protein^{-1}$) in the time interval indicated.

P_i (figure not shown). On the other hand, in State 4 (Figs 1C and 2) the addition of iron(III)-sucrose increased the oxygen uptake and the degree of stimulation was highly dependent on the presence of Mg^{2+} and P_i (Fig. 3A and B). Furthermore, the stimulation was smaller with NAD-linked substrates than with succinate (Table I); the stimulation reached an optimum at an iron concentration of 80–100 μM with the latter substrate (Fig. 2) and a pH optimum of 7.2–7.4 (Fig. 4). Finally, the iron-induced stimulation of respiration was approx. 3-fold higher in State 3 than in State 4 (Fig. 1D, Fig. 2 and Table I); a maximal stimulation was found at a concentration

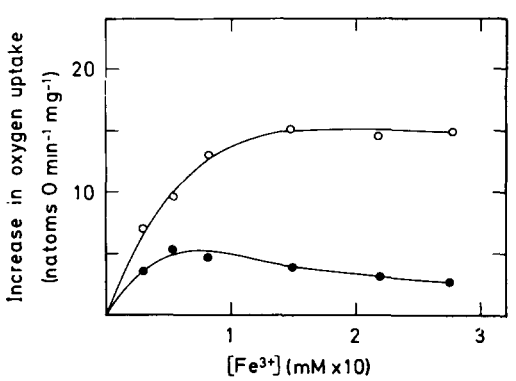

Fig. 2. Effect of adding increasing concentrations of iron(III)-sucrose on State 4 (●) and State 3 (○) respiration in rat liver mitochondria with succinate as the substrate. Experimental conditions as in Fig. 1C and D. All the points represent the mean of three different experiments.

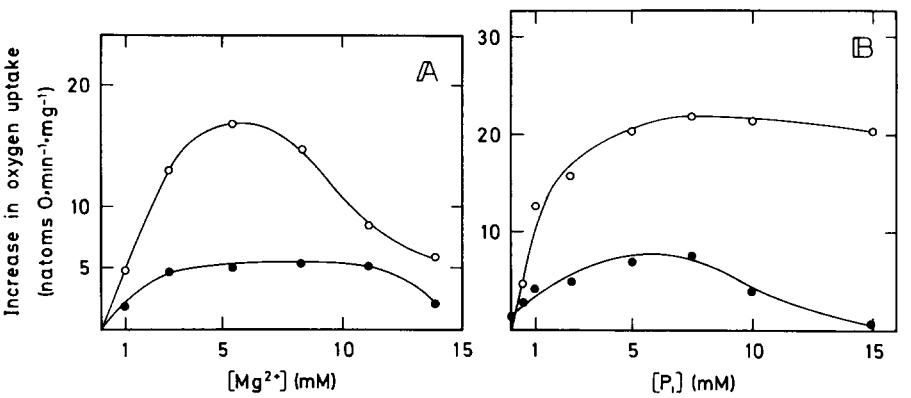

Fig. 3. Effect of Mg^{2+} (A) and P_i (B) on the stimulation by added iron(III)-sucrose of State 4 (●) and State 3 (○) respiration of rat liver mitochondria. Experimental details as described in the Materials and Methods section and Fig. 1C and D except that 5 mM Mg^{2+} was added in B and 5 mM P_i was added in A.

of 150–200 μM Fe^{3+} (Fig. 2) with a pH optimum at 7.2–7.4 (Fig. 4) and the stimulation could only be elicited in the presence of Mg^{2+} and P_i (Fig. 3A and B).

In contrast to the effect of Ca^{2+} (Fig. 1E and F), respiratory control could be elicited with iron only within narrow concentration limits; i.e. additions of 20–40 μM iron induced a transient increase in the oxygen uptake (Fig. 1B). At concentrations of iron $>$ 100 μM the respiration remained in the activated state until anaerobiosis.

Effect of added iron(III)-sucrose on the redox level of cytochromes c $(+c_1)$ *and a*$(+a_3)$ *in State* 1

As shown above, the difference in the accumulation pattern of Ca^{2+} and iron [1, 2, 9] is reflected in the respiratory responses elicited by these ions, which should

TABLE I

STIMULATION OF STATE 4 AND STATE 3 RESPIRATION BY ADDED IRON (III)-SUCROSE

For experimental details, see legend to Fig. 1. The substrate concentrations were 3.3 mM and 0.93 mM ADP was used to induce State 3 respiration.

Respiratory state	Increase in oxygen consumption*				**Respiratory control with Fe^{3+}	
	(natoms O/min/mg of protein)		(%)			
	mean	range	mean	range	mean	range
State 4 (succinate)	9.9 (n = 14)	5.4–16.4	51	27–84	1.51	1.27–1.84
State 4 (pyruvate/malate)	2.5 (n = 5)	0.9–4.9	30	19–47	1.30	1.19–1.47
State 3 (succinate)	26.6 (n = 14)	15.2–42.7	37	26–48		
State 3 (pyruvate/malate)	8.9 (n = 10)	4.5–15.3	27	18–31		

* The concentration of iron was 112 μM.

** Respiratory control with iron is defined as the ratio between the respiratory rate in the presence of iron and the rate obtained before the addition of iron.

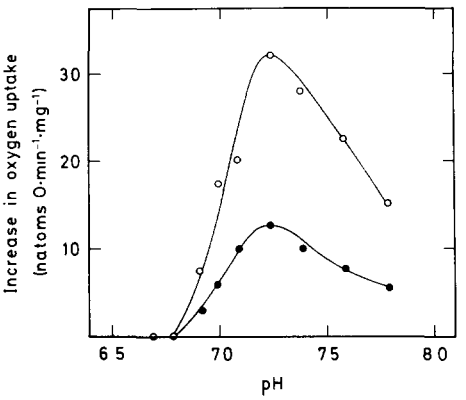

Fig. 4. Effect of pH on the stimulation by added iron(III)-sucrose of State 4 (●) and State 3 (○) respiration (succinate) in rat liver mitochondria. Experimental conditions as in Fig. 1C and D.

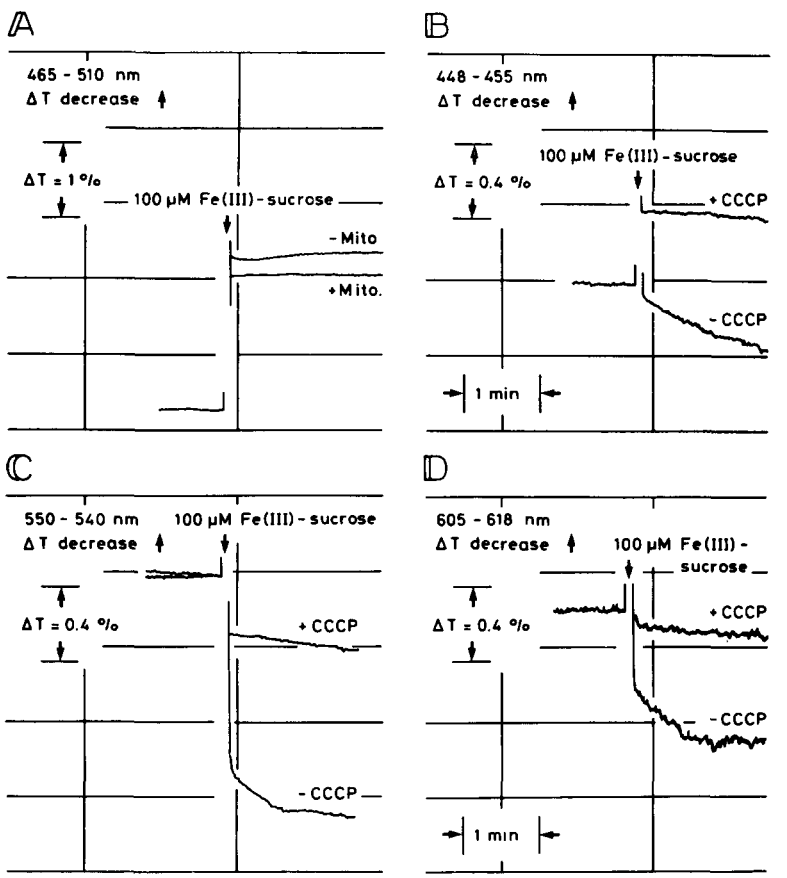

Fig. 5. Effect of added iron(III)-sucrose on the redox level of flavoproteins (A), cytochromes a_3(B), c ($+c_1$) (C) and a (D) of rat liver mitochondria respiring on endogenous substrates. The mitochondria were suspended in the standard incubation medium (see Methods) at 4.1 mg protein/ml. The change in transmission ΔT (%) was recorded at the indicated wavelength settings of the dual-wave length spectrophotometer. The ΔT (%) values measured when the iron complex was added in the presence of 20 μM of the uncoupler CCCP (B–D) represent the contribution of the colour of the iron complex to the total spectral change in the absence of uncoupler.

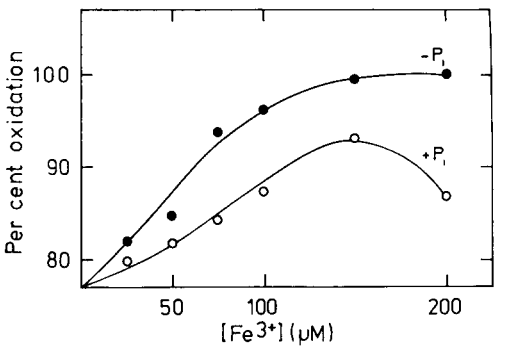

Fig. 6. Effect of adding increasing concentrations of iron(III)-sucrose on the steady-state redox level of cytochrome *c* ($+c_1$) in the absence (●) and presence (○) of 5 mM P_i. For experimental details, see Fig. 5C.

be considered primarily in view of the redox activity of iron ions and their possible interactions with components of the respiratory chain [30, 31]. Thus, ferric iron may function as an alternate electron acceptor and thereby modify the respiratory responses.

From Fig. 5 it is seen that the addition of iron(III)-sucrose to rat liver mitochondria in the absence of P_i and exogenous substrate induced an oxidation of all the measured components of the terminal part of the respiratory chain. In the absence of P_i, approx. 150 μM iron was sufficient to obtain an almost complete oxidation of cytochrome *c*, whereas in the presence of P_i the effect of the ferric complex was markedly reduced (Fig. 6).

The data of Figs 5 and 6 also rule out the fact that the stimulation of State 4 and State 3 respiration was due to impurities of ferrous iron in the ferric complex. This was confirmed polarographically where the ferrous complex could be seen to be readily oxidized by dissolved molecular oxygen in the absence of mitochondria (data not shown).

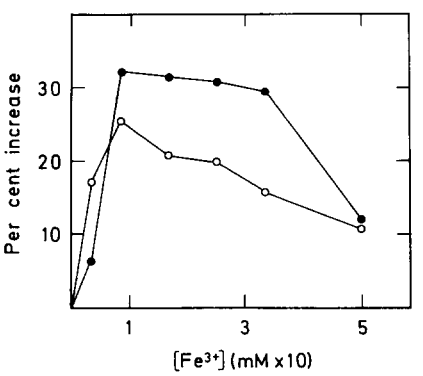

Fig. 7. Effect of added iron(III)-sucrose on State 4 respiration (●) and the succinate: phenazine methosulfate oxidoreductase activity (○) in rat liver mitochondria. For experimental details, see Materials and Methods and legend to Fig. 1C. The 100 % values were (●) 16.8 natoms oxygen · min^{-1} · mg $protein^{-1}$ and (○) 104.5 nmol succinate · min^{-1} · mg $protein^{-1}$.

Effect of added iron(III)-sucrose on succinate: phenazine methosulfate oxidoreductase activity

From the more pronounced stimulation of the oxidation of succinate than of pyruvate plus malate by addition of iron(III)-sucrose (Table I), and the diversity of substances known to modulate the succinate dehydrogenase activity [32–37], it was of interest to study the effect of the iron complex on this activity in intact mitochondria. From Fig. 7 it is seen that preincubation of the mitochondria respiring on 4 mM succinate, with increasing concentrations of iron(III)-sucrose resulted in a stimulation of succinate: phenazine methosulfate oxidoreductase activity which closely parallelled the stimulation of the succinate oxidase activity.

Effect of added iron(III)-sucrose on succinate transport

That the results given in Fig. 7 may reflect a stimulation of the dicarboxylate carrier by iron is suggested by several observations. Thus, experimental evidence has been presented that the rate of respiration with succinate plus rotenone as the substrate is controlled mainly by the uptake of succinate [38] which in turn is affected by cations, notably metal ions [25, 39]. From Fig. 8, however, it is seen that the addition of iron(III)-sucrose has no significant effect on the succinate uptake.

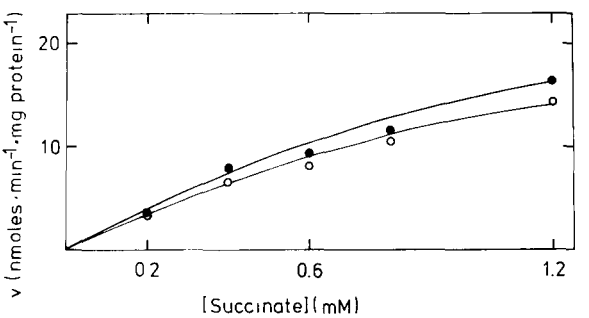

Fig. 8. Effect of succinate concentration on the rate of succinate uptake in rat liver mitochondria in the absence (●) and presence (○) of 150 μM iron (as a ferric-sucrose complex). The mitochondria (3.5–4.1 mg protein/ml) were incubated in a medium containing in a volume of 1.0 ml: 0.25 M sucrose, 5 mM HEPES-Tris buffer, pH 7.4; 10 °C. For further details, see Methods.

Effect of added iron(III)-sucrose on the oxidative phosphorylation and the adenine nucleotide exchange reaction

Since the stimulation of respiration by iron was approx. 3-fold higher in State 3 than in State 4, it is suggested that iron somehow interacts with the mitochondrial energy-transducing system.

When oxidative phosphorylation was measured in the presence of an ADP-regenerating system using succinate or pyruvate plus malate as the substrates, the addition of iron(III)-sucrose had no effect on the respiration rate. On the other hand, when oxidative phosphorylation was measured in the absence of hexokinase, the addition of iron(III)-sucrose stimulated respiration as well as oxidative phosphorylation (Fig. 9A and B). Both effects were slightly more pronounced with succinate (Fig. 9A) than with NAD-linked substrates (Fig. 9B), and with both substrates a slight decrease in the P/O ratios was observed.

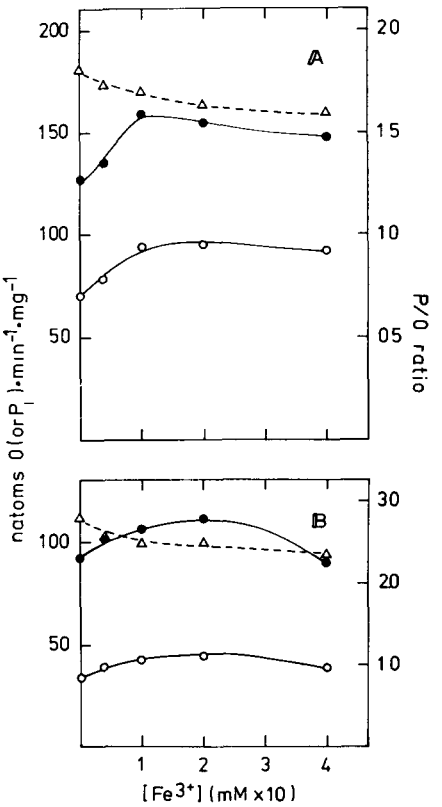

Fig. 9. Effect of adding increasing concentrations of iron(III)-sucrose on the oxygen uptake (○), oxidative phosphorylation (●) and the P/O ratio (△) of rat liver mitochondria. Experimental conditions as described in Method B of the Methods section. The substrates were 3.3 mM succinate (A) and 3.3 mM pyruvate plus 3.3 mM malate (B).

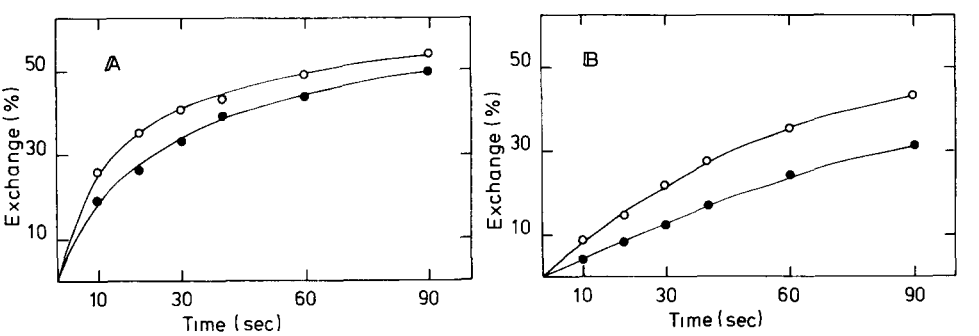

Fig. 10. Effect of added iron(III)-sucrose on the exchange by external ADP (A) or ATP (B). The mitochondria were preloaded with [^{14}C]ATP and the back exchange measured as described (for details, see Methods). External ADP (or ATP) and iron (when added) were present at concentrations of 200 μM and 150 μM, respectively. The mitochondria were preincubated for 1 min, and the exchange was started by the addition of the nucleotide (ADP or ATP) alone (●), or simultaneously to the iron(III)-sucrose (○). Temperature 0–2 °C.

TABLE II

EFFECT OF RESPIRATORY AND ENERGY TRANSFER INHIBITORS ON THE ADENINE NUCLEOTIDE EXCHANGE REACTION IN THE PRESENCE OF IRON

Mitochondria with approx. 2.5 mg of protein were incubated as described in the Materials and Methods section in a total volume of 1.5 ml. The concentrations of the inhibitors were 3.5 μg oligomycin/ml, 17 μM CCCP, 0.5 μg antimycin A/ml and 3.3 μM rotenone. The mitochondria were preincubated with the inhibitors for 10 min before 200 μM ATP and 150 μM iron (as a ferric-sucrose complex) were added simultaneously. Temperature 0–2 °C. The data represent the means of two different experimental series.

Inhibitor	Rate of adenine nucleotide translocation (nmol ATP · min^{-1} · mg $protein^{-1}$)	
	Iron absent	Iron present
None	2.1	3.7
Oligomycin	3.3	5.0
Antimycin A+rotenone	5.5	10.7
CCCP	7.2	11.7
CCCP+oligomycin	6.6	11.5

From Fig. 10A and B it is seen that the rate of the adenine nucleotide exchange reaction was significantly stimulated in the presence of added iron. It should be noted that the leakage of adenine nucleotides from the prelabeled mitochondria remained at a low and constant level of 6–7 % for at least 15 min in the absence as well as in the presence of 150 μM iron. Finally, from Table II it is seen that the stimulation of ATP translocation by added iron(III)-sucrose was obtained even in the presence of respiratory and energy transfer inhibitors.

Effects of added iron(III)-sucrose on the mitochondrial energy state

The inhibition of the energy-dependent accumulation of iron [1] and the decrease in respiratory control with ADP values (Fig. 1C and D) as well as P/O ratios (Fig. 9A and B) observed at iron concentrations above 100–150 μM point to the induction of a slight loose-coupling of the rat liver mitochondria at these experimental conditions. Of the many probes used to estimate the energy state of the mitochondrial inner membrane, measurement of the redox state of the cytochrome *b*-complex in terminally inhibited mitochondria [27] is one of the more well-established methods [40]. Using this parameter it is seen from Fig. 11A that the addition of iron(III)-sucrose lowered the energy potential by approx. 30 % in the concentration range 25–200 μM of iron, whereas at concentrations above 200 μM the energization is markedly reduced. Furthermore, upon maximal energization of the mitochondria with ATP [20], the rate of energy dissipation induced by the subsequent addition of oligomycin [20] was largely increased at these high concentrations of iron (Fig. 11B).

DISCUSSION

In a preliminary study of tightly coupled rat liver mitochondria respiring in State 4 [41], an apparent respiratory control was found to be induced by the addition of iron(III)-sucrose, and it was suggested that ferric iron behaved principally as

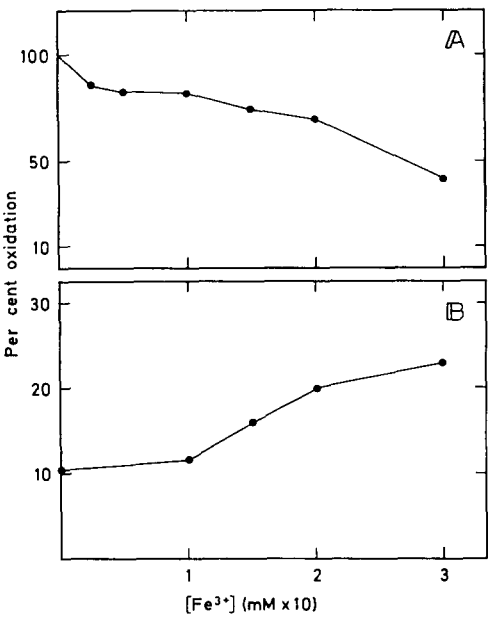

Fig. 11. Effect of adding increasing concentrations of iron(III)-sucrose on the steady-state redox level of the cytochrome *b*-complex (A), and the rate of the oligomycin induced dissipation of the "energy potential" of rat liver mitochondria (B). (A) The mitochondria were suspended in the standard incubation medium (see Methods) at 3.2 mg protein/ml and supplemented with 3.3 mM KCN, 3 μM rotenone, 4.2 mM ascorbate and 90 mM TMPD. After a preincubation for 5 min, iron(III)-sucrose was added, and when the steady-state redox level was reached, uncoupler (30 μM CCCP) was added to induce complete deenergization. 100 % oxidation represents the difference in ΔT (%) = $\Delta(T_{564\ nm}-T_{575\ nm})$ values in the presence of ascorbate/TMPD and CCCP. (B) The mitochondria (3.2 mg protein/ml) were incubated as described in Fig. 11A. Subsequent to ascorbate/TMPD, maximal energization was induced by 0.5 mM ATP. At steady-state redox level, 150 μM iron was added followed (15 s later) by the addition of 10 μg oligomycin. 100 % oxidation represents the difference in ΔT (%) = $\Delta(T_{564\ nm}-T_{575\ nm})$ values in the presence of 0.5 mM ATP and 30 μM CCCP. The rate is the degree of oxidation in 60 s.

reported for other cations accumulated by energy-requiring processes (for review, see ref. 42). More detailed studies, however, have revealed that iron induces a series of responses in respiring mitochondria, responses which in certain aspects differ from those given by e.g. Ca^{2+} [9] and Sr^{2+} [10]. Of particular interest, from a mechanistic point of view, is the effect of iron on State 1 respiration, i.e. the metabolic state selected for our accumulation studies [1–3, 8, 13, 14]. Although the respiratory rate in State 1 is rather low, the observed inhibition of respiration (Fig. 1A) is highly significant and reproducible. Furthermore, it is parallelled by an oxidation of the terminal part of the respiratory chain (Figs 5 and 6) suggesting that ferric iron in this case functions as an alternate electron 'sink' in the same way as previously proposed by Barnes et al. [43] for the ferrioxamine G complex. For this reason, polarographic measurements possess obvious limitations when used to calculate energy expenditure during accumulation of this cation, and it is further complicated by the inhibition of the energy-dependent accumulation by P_i [1] and substrates [8]. On the other hand, an apparent respiratory control with iron, similar in extent to that reported for Mn^{2+}

[10], Ba^{2+} [10] and La^{3+} [44], could only be elicited in the presence of P_i and substrates (Fig. 1B), suggesting that the stimulation of respiration is related to reactions other than utilization of conserved energy. Hence, the calculation of an Fe/O-activation ratio has no meaning. The stimulation of State 4 respiration by the addition of the iron complex is related mainly to an increase in the rate of succinate oxidation (Table I) which in turn is primarily governed by (1) the rate of succinate uptake [38] and (2) the degree of activation of the succinate dehydrogenase [33]. That the effect is not mediated via the dicarboxylate carrier is supported by the data of Fig. 8 as well as kinetic studies of the succinate transport [45, 46]. Thus, the stimulation of State 4 respiration may be attributed primarily to a positive modulation of the succinate dehydrogenase activity (Fig. 7), in agreement with the findings of Thorn [47], Kimura et al. [48] and of Gutman et al. [33]. Finally, the effect of added iron(III)-sucrose on State 4 respiration with NAD-linked substrates should be noted (Table I). Although quantitatively small, the results indicate that the effect of iron in State 4 is not confined solely to activation of the succinate dehydrogenase. Although the mechanism of this effect has not been explored in detail it should be mentioned in this context that added iron(III)-sucrose enhances the rate of endogenous energy dissipation (Fig. 11) and lowers the respiratory control values with ADP as well as the P/O ratios. This energy dissipation is certainly partly linked to the energy-dependent accumulation of iron (i.e. true respiratory control), but may also partly be attributed to a deleterious effect of the iron-sucrose complex or iron ions on the integrity of the mitochondrial inner membrane (i.e. loose-coupling). From the negligible effect of the ferric complex on the transmembrane pH-gradient, the passive proton conductance, and the finding that under the present experimental conditions ferric iron had no effect on lipid peroxide formation (unpublished observations), the noxious effect of iron probably plays only a minor role, at least at iron concentrations below 150–200 μM.

Ferric iron reacts with adenine nucleotides [49], and the addition of an iron(III)-ADP complex to rat liver mitochondria was shown by Strickland and Goucher [12] to enhance succinate respiration 60 % more than Mg(II)-ADP. It was assumed that the stimulation by Fe(III)-ADP was due to displacement of some hypothetical inhibitory ion (e.g. Ni^{2+}), although their experiments did not provide sufficient proof for this conclusion. In the overall reaction of oxidative phosphorylation. adenine nucleotide translocation is a rate limiting step [50], and by consequence, an increase in the translocase activity would increase the rate of oxygen uptake and oxidative phosphorylation within the maximal capacity of the mitochondria for oxidative phosphorylation. Thus, the effect of iron on State 3 respiration and oxidative phosphorylation may at least in part be attributed to a stimulation of the adenine nucleotide exchange reaction (Fig. 10A and B), essentially as recently reported for Ca^{2+} [51].

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