ENERGY-DEPENDENT ACCUMULATION OF IRON BY MITOCHONDRIA ISOLATED FROM DIFFERENT TISSUES AND SPECIES

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1. Introduction

Rat liver mitochondria accumulate iron partly by an energy-dependent and partly by an energy-independent mechanism [1]. The energy-dependent accumulation has several features in common with the uncoupler-sensitive high-affinity binding of Ca^{2+} [2,3], and the iron thus accumulated is partitioned between the matrix and inner membrane [4]. Thus, the ability of rat liver mitochondria to carry out energy-dependent accumulation of iron seems hardly less remarkable than their capacity for energy-dependent accumulation of other cations (for review, see ref. [15]). Nevertheless the iron accumulation process reveals some peculiarities related to the strong complex-binding properties of the iron ions, e.g. the energy-dependent accumulation is inhibited by addition of substrates, ATP ($> 50\ \mu M$) or inorganic phosphate [6]. On the other hand, the energy-dependent accumulation can be increased by approx. 50% when coupled to valinomycin-induced K^+-efflux [6].

Furthermore in a recent paper it was shown that energy-dependent accumulation of iron by mitochondria isolated from rabbit reticulocytes amounts to 2–3 times that taken up by rat liver mitochondria [7]. This observation is of considerable importance in view of the specialized function of reticulocytes in iron metabolism and heme synthesis [8,9].

In the present paper these studies have been extended to mitochondria isolated from other tissues and species to determined whether differences exist that might be relevant to the biological role of mitochondria in iron metabolism [7–10], and it is concluded that whereas no species differences exist, there are marked tissue differences, i.e. the energy-dependent accumulation of iron by kidney and liver mitochondria amounts otherwise identical conditions. The implication of these findings is discussed in view of the demand of iron for heme-protein synthesis in different types of mitochondria.

2. Materials and methods

The animals were of ordinary laboratory strains [1,7,11]. For preparation of heart mitochondria 0.5 mM EDTA (sodium salt) was included in the initial homogenization medium [12]. The further procedure was a described for kidney and liver mitochondria [2]. The iron accumulation experiments and the measurement of oxygen uptake were as previously described [2].

3. Results

Table 1 shows the results of representative experiments of the rates of oxygen uptake before and after addition of small amounts of iron(III)–sucrose to various types of mitochondria respiring in State 4 as well as State 3. Whatsoever the species, the rate of succinate oxidation was highest in heart mitochondria, i.e. 2–4 times that obtained with kidney and liver mitochondria. On the other hand, the degree of coupling in liver mitochondria.

As to the effect of adding iron(III)–sucrose whereas no significant species differences could be observed, the increase in oxygen uptake was most pronounced

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Table 1
Effect of iron(III)–sucrose on respiratory rate of mitochondria isolated from different tissues and species

Source of mitochondria	State 4 rate (natoms oxygen min^{-1} mg $protein^{-1}$)	$R.C._{ADP}$	Per cent increase in resp. rate after iron	
			State 4	State 3
Rat heart	74	2.6	18	3
Rat kidney	49	2.3	21	19
Rat liver	22	3.9	27	26
Guinea pig heart	73	2.3	14	4
Guinea pig kidney	48	2.7	19	13
Guinea pig liver	27	5.1	19	17
Rabbit heart	94	2.9	22	10
Rabbit kidney	23	3.3	17	13
Rabbit liver	21	2.9	27	22

Mitochondria, 3–5 mg of protein were incubated at 25°C in a medium containing in a final volume of 2.5 ml : 175 mM sucrose; 50 mM glucose; 10 mM HEPES buffer, pH 7.4; 5 mM $MgCl_2$; 5 mM P_i and 2.5 μM rotenone. State 4 respiration was initiated by adding 4 mM succinate, and at steady-state respiration 0.25 mM iron(III)–sucrose and/or 1 mM ADP were added at timed intervals. The results represent the mean of four different experiments.

in experiments with liver mitochondria in all the species tested, in State 4 as well as State 3. Furthermore, with kidney and liver mitochondria the per cent stimulation of iron(III)–sucrose on State 3 was only slightly less than that of State 4. On the other hand, in heart mitochondria, compared to State 4 both the relative and absolute degree of stimulation was markedly decreased in State 3.

Accumulation of iron, energy-dependent as well as energy-independent, are given in table 2. The most important findings were: (i) no species differences in the ability to accumulate iron by either of the two mechanism, energy-dependent as well as energy-independent, and (ii) a marked tissue-selectivity towards iron accumulation, i.e. whereas heart mitochondria accumulated 50–100 nmoles of iron per mg of protein by some energy-independent mechanism, the energy-dependent accumulation amounted to about 1 nmole iron per mg of protein on the average. Vice versa, in liver and kidney mitochondria, energy-independent accumulation amounted to 10–30 nmoles iron per mg of protein, and energy-dependent accumulation to 6–8 nmoles iron per mg of protein.

Table 2
Accumulation of iron by mitochondria isolated from different tissues and species

Source of mitochondria	Iron accumulation (nmoles/mg protein)	
	Energy-dependent	Energy-independent
Rat heart	0.2	63.1
Rat kidney	6.5	28.9
Rat liver	5.9	24.6
Guinea pig heart	1.3	70.7
Guinea pig kidney	7.7	29.4
Guinea pig liver	6.5	19.3
Rabbit heart	0.6	79.7
Rabbit kidney	6.2	13.5
Rabbit liver	5.4	13.3

Mitochondria, 3–5 mg of protein were preincubated for 10 min at 25°C in a medium containing in a final volume of 1.5 ml: 225 mM sucrose; 10 mM HEPES buffer, pH 7.4; 10 mM KCl and 5 mM $MgCl_2$. 0.25 mM iron(III)–sucrose was added (for details, see Materials and methods and ref. [2]). Energy-dependent accumulation was taken as the difference in accumulation in the absence and presence of 17 μM CCCP. The results represent the mean of four different experiments.

4. Discussion

The data presented demonstrate that the ability to accumulate iron is a property common to mitochondria independent of species, but highly dependent on tissue of origin. Thus, whereas mitochondria isolated from heart tissue possess very scarce properties of energy-dependent accumulation of iron, mitochondria from liver and kidney accumulate significant amounts if iron. This difference could be related neither to degree of coupling (table 1 and ref. [6]) nor to differences in the ratio iron : protein [6]. Neither could the results be ascribed to loose-coupling from accumulation of endogenous Ca^{2+} during preparation [12]. On the other hand, as there appears to be an inverse relationship between energy-dependent and energy-independent iron accumulation within certain limits [6,7], the negligible energy-dependent accumulation in heart mitochondria could in part be ascribed to a very marked energy-independent accumulation, and thereby to a damaging of the mitochondrial membrane system.

A more satisfactory explanation, however, can be proposed on the basis of differences in demand of iron by the mitochondria, i.e. the amount accumulated reflects the relative rate of mitochondrial iron-protein turnover. This suggestion is supported by the findings of (i) Jones et al. [13] of the submitochondrial localization of ferrochelatase, (ii) Flatmark and Sletten [14] that labeling of isocytochrome *c*-I occurs far more rapidly in kidney than in heart, (iii) Kadenbach [15] that $T_{1/2}$ of cytochrome *c* of heart tissue is four times that of liver and kidney, and finally (iv) energy-dependent accumulation of relatively large amounts of iron by mitochondria isolated from rabbit reticulocytes [7]. Thus, the iron accumulation process may reflect a mechanism by which mitochondria could meet their requirements for iron at any moment.

From a comparative point of view, it should be mentioned that Carafoli and Lehninger [16] in a study of mitochondria from different tissues and species found that uncoupler sensitive accumulation of Ca^{2+} by heart mitochondria was approx. half that obtained with mitochondria from kidney and liver, and vice versa uncoupler-insensitive accumulation five times higher in heart than in liver and kidney mitochondria, i.e. there is a parallellism between the uptake patterns of Ca^{2+} and iron by mitochondria from different tissues.

However, whereas the iron accumulation may reflect the rate of intramitochondrial heme protein synthesis, Ca^{2+} accumulation is thought to represent a regulatory mechanism of the Ca^{2+} concentration in the cytosolic environment [17].

The stimulating effect of iron ions on oxygen uptake by isolated rat liver mitochondria has been repeatedly observed [18,19], and this has been ascribed to a positive modulation of the succinate dehydrogenase complex and an increase in the rate of oxidative phosphorylation and adenine nucleotide exchange [19]. Although quantitative differences exist, qualitatively similar effects on oxygen uptake are found also in mitochondria from other tissues and species (table 1).

Another point of considerable importance is the dissociation between the extent of energy-dependent accumulation of iron and the effect of iron on oxygen uptake. From the results with heart mitochondria (tables 1 and 2) it can be seen that the effect of iron on oxygen consumption is not necessarily linked to the energy-dependent accumulation of iron, and as discussed elsewhere [19], this paradoxical finding is presumably due to the redox activity of the iron ions together with the formation of stable iron-complexes with a series of mitochondrial components.

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