Qualitative difference in mitochondria of endothermic and ectothermic animals

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A significantly higher rate of respiration in the absence of added ADP has been revealed in mitochondria of endotherms as compared with that of the ectotherms with similar rates of respiration during phosphorylation. A high rate of ADP-independent (non-coupled with ATP synthesis) respiration is observed during oxidation of succinate, NADH and ascorbate + cytochrome *c*, but not with NAD-dependent substrates. It increases in the presence of cytochrome *c* during oxidation of succinate and NADH and is also revealed during oxidation of NAD-dependent substrates in the presence of NAD^+ and cytochrome *c*. ADP-independent respiration is not affected by oligomycin, however, it is essentially inhibited in the presence of GDP. It is suggested that the significant difference in the value of ADP-independent respiration of endo- and ectotherms is due to the existence in endotherms of a non-coupled population of mitochondria which generates heat without preliminary synthesis of ATP.

Mitochondria *Phosphorylation* *ADP independence* *Respiration* *Endotherm* *Ectotherm*

1. INTRODUCTION

It is accepted that the respiration of mitochondria from endo- and ectothermic animals does not differ qualitatively [1-6]. This assumption is based on two groups of data. On the one hand, in the process of phosphorylation, the respiration rates are similar in isolated mitochondria from endo- and ectotherms [1-4], which demonstrates their similarity. On the other, a higher activity of NADH, succinate and cytochrome oxidases in tissue homogenates of endotherms has been shown [5,6] which could testify to their difference. However, it has also been found that the volume density and surface area of mitochondria in tissues of endothermic animals are larger [6], which made it possible to explain the observed differences in terms of the greater amount of mitochondria in their tissues [5,6]. It seemed to us that the assumption on the absence of qualitative differences in mitochondria from animals with a different temperature status could not be accepted as being final. In [1-4], investigations were mainly made of the phosphorylatory respiration with NAD-dependent substrates, comparison was made with the literature data and not under the experimental conditions used by those authors. Less attention was paid to the ADP-independent respiration. Meanwhile, analysis of the available data shows that mitochondria of endothermic animals oxidize some substrates at a high rate of state 4 respiration in the absence of added ADP. ADP-independent respiration is observed during oxidation of succinate [7-10] and NADH [11-15]. The insufficient consideration of ADP-independent respiration, in particular of heart mitochondria, is likely due to the viewpoint that it may be the result of damage to mitochondria during isolation [16]. However, many authors assume that it is a native property of mitochondria which accomplishes oxidation of cytoplasmic NADH [11-14]. It should be noted that the high rate of V_4 respiration and low respiratory control ratio during succinate oxidation in mitochondria are also observed in isolated cardiocytes [17,18]. Consequently, ADP-independent succinate oxidation is not the result of the

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procedure used for isolating mitochondria.

According to our previous data [19,20], ADP-independent respiration does not change significantly upon variation of the tissue homogenization time from 10 s to 3 min and is not abolished with albumin, EDTA, ATP or α-tocopherol. These data are compatible with the assumption that ADP-independent respiration is a native property of tissues. As ADP-independent respiration proceeds at a high rate in tissues of endotherms [19,20], it seems likely that it participates in heat production.

The aim of this paper is to compare under identical conditions the rates of different substrate oxidation in mitochondria of endo- and ectotherms in the absence and presence of ADP.

2. MATERIALS AND METHODS

Rat and frog heart and liver mitochondria were isolated according to [21]. Animals were decapitated. Tissues were placed in a cooled isolation medium containing 300 mM sucrose, 10 mM Tris-HCl, pH 7.5. After preliminary grinding the tissues were homogenized in a homogenizer with a teflon pestle [22] in a 10-fold volume of the isolation medium. The homogenate was centrifuged at $700 \times g$ for 7 min. Mitochondria were precipitated from the supernatant at $6000 \times g$ for 20 min. The mitochondrial pellet was suspended in the same isolation medium (about 30–40 mg protein per ml) and stored in the cold at 0–2°C. Mitochondrial protein was determined according to Lowry et al. [23].

Oxidation of different substrates in mitochondria was measured polarographically with the use of a rotating platinum electrode [26]. The incubation mixture contained 120 mM KCl, 5 mM KH_2PO_4, 5 mM EDTA, 10 mM Tris-HCl, pH 7.5, the incubation temperature being 25°C. The following substrates were used: 5 mM succinate, 5 mM glutamate, 1 mM NADH, 1 mM NAD^+, 20 mM ascorbate + 2.5 mg cytochrome *c* per ml. ADP was added in 100 μM portions. Oxidative phosphorylation was followed according to Chance and Williams [24]. The following symbols are used: V_3, respiration during phosphorylation; V_4, respiration after phosphorylation; ΔV_3, increment of respiration induced by ADP.

Rats kept at 22–28°C and frogs kept at 20–22°C were used.

3. RESULTS AND DISCUSSION

Our results on oxidation of different substrates in heart and liver mitochondria are shown in fig.1 as polarographic records. It is seen that in the absence of ADP the rate of NADH and ascorbate oxidation is high and is not influenced by DNP and oligomycin. Consequently, oxidation of these substrates is ADP-independent. Oxidation of NADH in heart mitochondria proceeds through the main respiratory chain as is inhibited by rotenone. In liver mitochondria, such oxidation of NADH, proceeding rapidly in the presence of cytochrome *c*, is not very sensitive to rotenone. In accordance with literature data [15], it proceeds mainly along the redox chain with cytochrome b_5, and to a lesser extent (by 15%) through the main respiratory chain.

Oxidation of succinate is characterized by a higher V_4 and a lower level of respiratory control ratio as compared with oxidation of glutamate, which is more pronounced in heart mitochondria. This respiration is additionally increased by cytochrome *c*, but is not changed in the presence of

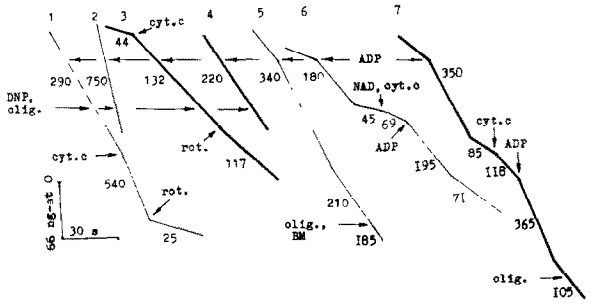

Fig.1. Polarographic recording of oxidation of different substrates in heart and liver mitochondria. The following substrates were used: (1,3) NADH, (2,4) ascorbate + cytochrome *c*; (5,7) succinate; (6) glutamate. Additions: 200 μM ADP, 5 μM DNP, 250 μM cytochrome *c* (cyt. *c*), 1 mM NAD, 5 mM butylmalonate (BM), 1.5 μg rotenone (rot.), 1.5 μg oligomycin (olig.). Thin lines show respiration of heart mitochondria and thick lines the respiration of liver mitochondria. Volume of polarographic cell, 1 ml; protein of heart mitochondria, 2.1 mg; protein of liver mitochondria, 4.2 mg. Incubation temperature, 25°C. Other conditions are given in section 2.

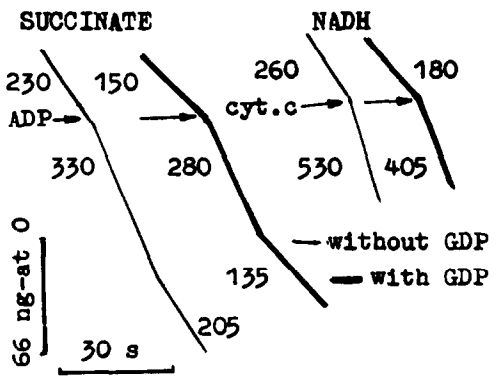

Fig.2. GDP inhibition of ADP-independent succinate and NADH oxidation in heart mitochondria. GDP addition 0.5 mM. Experimental conditions as in fig.1.

oligomycin and butylmalonate. Consequently, V_4 with succinate can also be ascribed to ADP-independent respiration. An increase in V_4, connected with the ADP-independent respiration, is also observed during oxidation of glutamate in the presence of NAD^+ and cytochrome *c*.

The above demonstrates that ADP-independent respiration of mitochondria is revealed upon oxidation of different substrates. This respiration is more rapid during oxidation of ascorbate, NADH and succinate.

ADP-independent oxidation of NADH and succinate, detected in heart mitochondria, is considerably inhibited in the presence of GDP (fig.2). The extent of the inhibitory action of GDP is comparable with that for oxidation of succinate in mitochondria of brown adipose tissue [25]. This indicates a certain similarity in respiration of heart mitochondria and mitochondria of a special thermogenic organ - brown adipose tissue.

To clarify the role of ADP-independent respiration in heat production, we studied V_4 and ΔV_3 in mitochondria of ectothermic frogs in comparison with endothermic rats (tables 1 and 2). As seen from table 1, the level of NADH oxidation in frog mitochondria is about 4–10-times lower than that in rat mitochondria. With succinate as a substrate, it was found that mitochondria from tissues of both animals differ only slightly in the ADP-induced ΔV_3 increment of respiration, whereas they exhibit considerably different values of V_4. The level of the latter is 5- and 2-times lower in frog heart and liver mitochondria, respectively, as compared with the corresponding values for rat mitochondria (table 2). At the same time, in glutamate oxidation there is no significant difference between mitochondria of endo- and ectotherms in either V_4 or ΔV_3 (table 2). The present results show that mitochondria from frog tissues, showing no essential differences from rat mitochondria in phosphorylatory respiration, exhibit a significantly lower rate of ADP-independent respiration. This demonstrates the existence of qualitative differences in the mitochondria of endo- and ectotherms.

A possible explanation for the described properties of endotherm respiration is given in fig.3. ADP-independent respiration of endotherms may be realized by a separate non-coupled population of mitochondria in which the inner is highly permeable to NADH, ascorbate and cytochrome *c*.

Table 1

ADP-independent respiration in rat and frog tissue mitochondria during oxidation of different substrates

	Without substrates	NADH	NADH + cyt. *c*	NADH + cyt. *c* + rotenone	Ascorbate + cyt. *c*
Rat HM	18.18 ± 1.69	129.70 ± 4.84	229.70 ± 5.04	24.71 ± 1.34	316.02 ± 33.21
LM	12.56 ± 1.32	13.56 ± 0.43	40.45 ± 3.77	34.52 ± 2.16	57.58 ± 1.18
Frog HM	13.17 ± 0.18	21.36 ± 2.36	32.25 ± 3.11	14.21 ± 0.36	42.36 ± 3.18
LM	8.24 ± 1.18	8.24 ± 1.18	14.86 ± 1.39	–	16.31 ± 0.58

Experimental conditions are described in section 2. Mean of 10–19 determinations ± SE. HM, heart mitochondria; LM, liver mitochondria; cyt. *c*, cytochrome *c*. Oxidation rates of the given substrates in the absence of added ADP are given in ngatom O/min per mg mitochondrial protein

Table 2

Relation of phosphorylatory respiration in rat and frog tissue mitochondria during oxidation of succinate and glutamate

Preparations and substrates	V_3	V_4	ΔV_3	RCR	ADP/O
Rat HM succinate	169.25 ± 8.34	106.83 ± 3.34	62.42	1.59	1.38 ± 0.14
glutamate	83.24 ± 5.13	20.02 ± 1.12	63.22	4.12	2.56 ± 0.14
LM succinate	80.01 ± 1.84	19.41 ± 1.12	63.22	4.53	1.79 ± 0.24
glutamate	69.92 ± 1.39	14.39 ± 0.38	55.53	4.58	2.79 ± 0.26
Frog HM succinate	108.42 ± 5.80	28.24 ± 3.17	80.18	3.83	1.68 ± 0.16
glutamate	74.28 ± 3.26	16.43 ± 1.85	57.85	4.52	2.72 ± 0.13
LM succinate	68.24 ± 3.17	16.14 ± 2.18	52.10	4.23	1.72 ± 0.34
glutamate	52.14 ± 2.14	11.21 ± 1.46	40.94	4.65	2.68 ± 0.16

Experimental conditions as in table 1. Mean of 12–25 determinations ± SE. RCR, respiratory control ratio (V_3/V_4). RCR and ADP/O were determined according to Chance and Williams [24]. Respiratory rates V_3, V_4 and ΔV_3 are given in ngatom O/min per mg mitochondrial protein

Fig.3 gives a schematic representation of two populations of mitochondria. NAD-dependent substrates are oxidized in phosphorylatory mitochondria. They can also be oxidized by a non-coupled population, but for this it is necessary to add NAD^+ and cytochrome *c*, which are lost by the population during isolation. Succinate is oxidized simultaneously in both populations, which provides for a high V_4, while ΔV_3 remains close to that at oxidation of NAD-dependent substrates. NADH and ascorbate do not penetrate into phosphorylatory mitochondria and are oxidized by a non-coupled population of mitochondria.

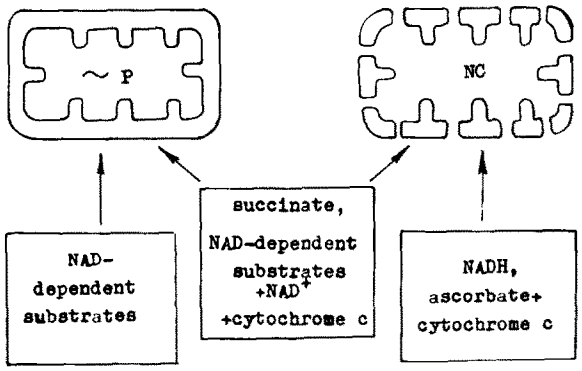

Fig.3. Schematic representation of two populations of mitochondria. ~P, phosphorylatory mitochondria; NC, non-coupled mitochondria realizing ADP-independent respiration. Boxes and arrows show substrates oxidizing in the corresponding population of mitochondria.

The above literature and our data allow us to regard ADP-independent respiration of mitochondria as a native property of tissues which is revealed during oxidation of different substrates and enables heat production. A significant inhibition of ADP-independent respiration by GDP indicates that this respiration may be due to high proton conductivity in mitochondria which switches the respiration from ATP synthesis to heat production.

Endothermy appeared at a later period of evolution of organisms and could be attributed to the formation of a new metabolic system - the thermorespiratory one. In tissues of endotherms it can be presented by a population of mitochondria which are transformed into 'thermosomes' and carry out ADP-independent respiration.

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