INTERMEMBRANE ELECTRON TRANSFER IN MITOCHONDRIAL AND MICROSOMAL SYSTEMS

A.I. ARCHAKOV, A.V. KARYAKIN and V.P. SKULACHEV

Central Research Laboratory of the N.I. Pirogov Moscow Second Medical Institute, Moscow 119435, and Department of Bioenergetics of the Laboratory of Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

Received 12 November 1973

Original figures received 13 December 1973

1. Introduction

It is generally accepted that electron transfer reactions take place in different types of the membranes of the living cell. However, information about the intermembrane exchange of reducing equivalents is scanty.

The electron transfer between outer and inner membranes of isolated mitochondria was found to be catalyzed by added cytochrome *c* [1, 2] and some artificial carriers [2, 3]. It is not known whether this is the same in the intact cell.

In this work an attempt was made to investigate intermembrane electron transfer in rat liver mitochondria, microsomes and hepatocytes. It has been shown that added cytochrome *c* can shuttle between rotenone- and antimycin A-insensitive electron transfer chains localized in microsomal (as well as outer mitochondrial) membranes, and the cyanide-sensitive (cytochrome oxidase) system localized in inner mitochondrial membranes. Evidence of the functioning of such an intermembrane electron transfer shuttle in the intact hepatocytes has been obtained.

2. Materials and methods

Microsomal and mitochondrial fractions were obtained as described previously [4]. Liver cells were isolated with EDTA as a dissociating agent [5]. The rate of oxygen consumption was measured polarographically by a platinum electrode covered with a Teflon film. The protein concentration was measured by the method of Lowry et al. [6] in the presence of 0.1% sodium deoxycholate.

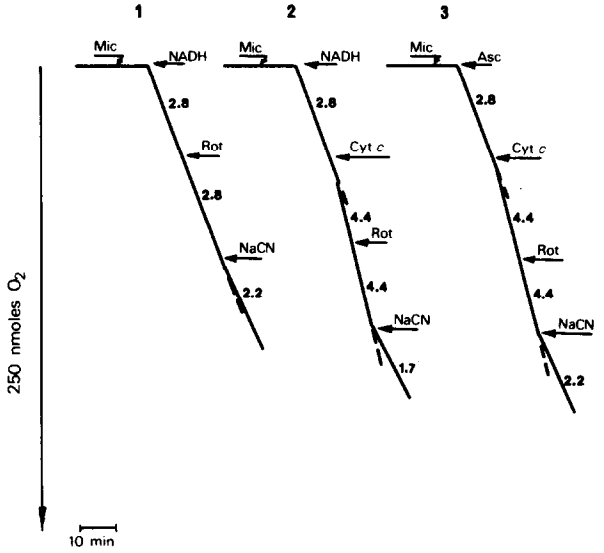

Fig. 1. The effect of cytochrome *c* on the NADH oxidation rate by microsomes. Incubation mixture contained 100 mM Tris–HCl buffer, pH 7.4; 0.2 mM EDTA, 4 mg of microsomal protein per ml (Mic). Additions: NADH – 1 mM, cytochrome *c* (Cyt *c*) – 10 μM, rotenone (Rot) – 5 μM, NaCN - 2 mM, sodium ascorbate (Asc) – 5 mM. Figures above the curves indicate the oxygen consumption rate in nmol · min^{-1} · mg^{-1} protein.

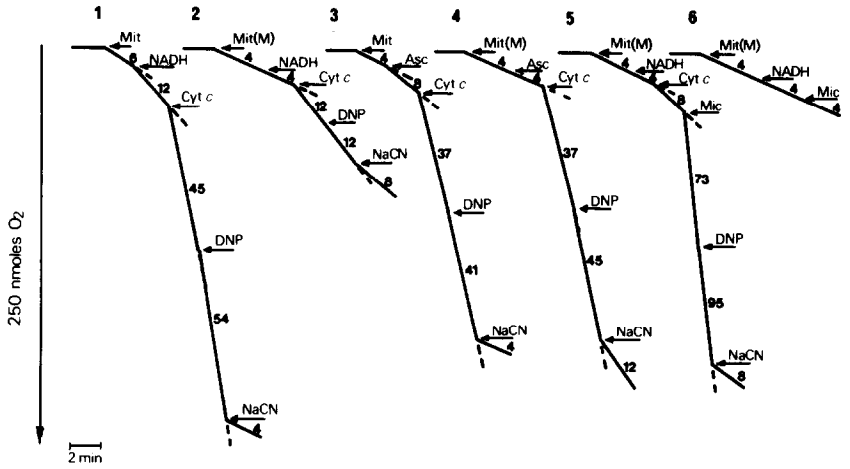

Fig. 2. The effect of cytochrome *c* on the NADH oxidation rate in the 'hybrid system containing microsomes and mitochondria. Incubation mixture contained 100 mM Tris–HCl buffer, pH 7.4; 0.5 mM EDTA. Additions: microsomes (Mic) – 50 μg of protein/ml, mitochondria (Mit) – 0.5 mg of protein/ml, mitochondria treated with mersalyl – Mit (M) – 0.5 mg of protein/ml, NADH – 1 mM, cytochrome *c* (Cyt *c*) – 10 μM, DNP – 0.2 mM, NaCN – 2 mM.

3. Results and discussion

The first indication that electron transfer occurs between the NADH-specific microsomal chain and mitochondrial cytochrome oxidase was obtained when it was found that NADH oxidation in the microsomal fraction can be stimulated by cytochrome *c*. It was shown that addition of cytochrome *c* to microsomes increases the rate of NADH oxidation via a cyanide-sensitive pathway (fig. 1, curve 2). Similar data were obtained with ascorbate as hydrogen donor (fig. 1, curve 3). These effects could be due to the mitochondrial cytochrome oxidase contamination of the microsomal fraction.

In further experiments mersalyl-treated mitochondria were used. It turned out that the pre-incubation of mitochondria (10 mg of protein/ml) with 1 mM mersalyl for 30 min at 4°C results in strong inhibition of the NADH-cytochrome b_5 reductase system of the outer mitochondrial membrane without the activity of cytochrome oxidase being affected. As one can see from fig. 2, NADH does not stimulate oxygen consumption in mersalyl-treated mitochondria (cf. curves 1 and 2), whereas oxidation of ascorbate in the presence of cytochrome *c* remains unaffected (cf. curves 3 and 4). Addition of catalytic amounts of microsomes (50 μg/ml) to mersalyl-treated mitochondria greatly

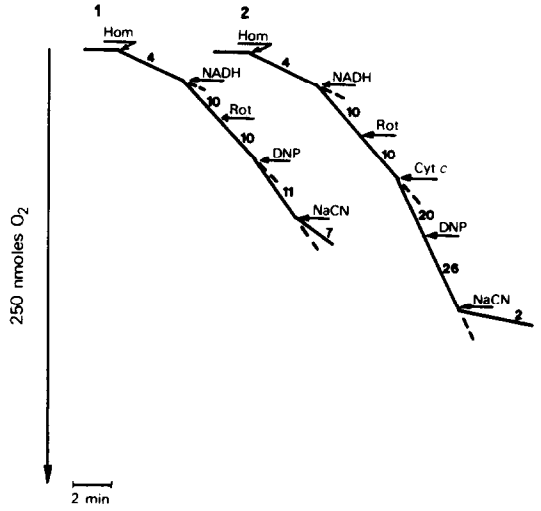

Fig. 3. NADH oxidation in rat liver homogenate. For incubation mixture see fig. 4. Additions: Homogenate (Hom) – 3 mg of protein/ml, NADH – 1 mM, rotenone (Rot) – 5 μM, cytochrome *c* (Cyt *c*) – 10 μM, DNP – 0.2 mM, NaCN – 2 mM.

stimulates NADH oxidation (curve 5). The effect of microsome demonstrates that added cytochrome *c* is indispensable (cf. curves 5 and 6). Cytochrome *c*-induced NADH Oxidation in both intact mitochondria

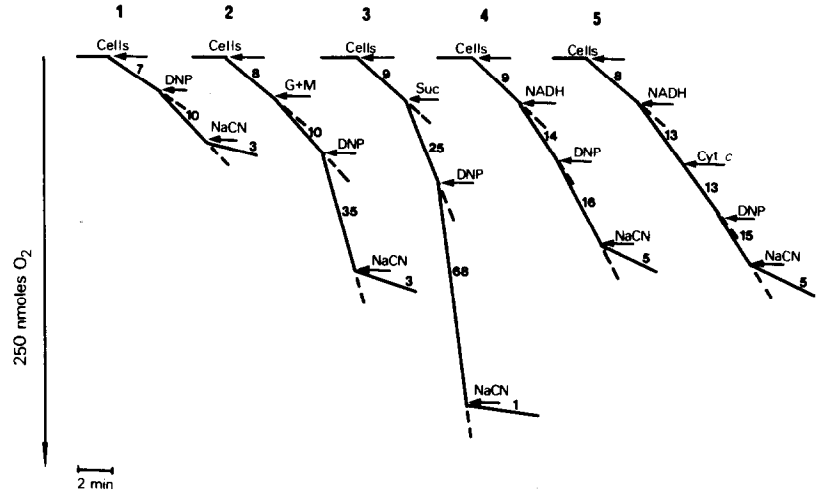

Fig. 4. The respiration of isolated hepatoxytes. Incubation contained contained: 1% albumin (fraction V, Sigma), 0.25 M sucrose, 5 mM phosphate buffer, pH 7.4; 1.0 mM EDTA; 1.0 mM $MgCl_2$; 0.2 mM dinitrophenol, 50 mM KCl. Additions: cells – 3 mg of protein/ml. DNP – 0.2 mM, glutamate + malate (G + M) – 5 mM, succinate (Suc) – 10 mM, NADH – 2 mM, cytochrome *c* (Cyt *c*) – 10 μM.

and mersalyl-treated mitochondria+microsomes was found to be slightly stimulated by 2,4-dinitrophenol (DNP) (curves 1,3–5); rotenone and antimycin A were without effect (not shown in fig. 2).

Similar data were obtained in experiments with rat liver homogenate (fig. 3). It is noteworthy that addition of cytochrome *c* to the homogenate, not only stimulated NADH oxidation (curve 2), but also decreased the rate of cyanide-resistant oxygen consumption (cf. curves 1 and 2). The latter effect, which may be of regulatory nature, will be considered elsewhere.

The data on the respiration of a suspension of isolated rat liver cells is shown in fig. 4. One can see that added substrates (glutamate+malate or succinate) stimulate oxygen consumption by the cells. Subsequent addition of DNP induces further and strong stimulation of respiratory activity (curves 2 and 3) Addition of NADH to the cell suspension also induces some increase in the respiration rate which cannot be enhanced significantly by DNP. Addition of cytochrome *c* does not change the rate of oxygen consumption either in the absence or in the presence of cyanide, this result differs from that obtained in experiments with homogenate. Cyanide induces a three-fold decrease in NADH oxidation rate. The level of cyanide-resistant respiration is higher in the samples with NADH than in those with glutamate+malate or succinate.

Fig. 5 shows the effect of antimycin A and rotenone on the respiration rate of hepatocytes. It is seen that oxidation of glutamate+malate and succinate is inhibited by antimycin A and rotenone whereas NADH oxidation is not.

The above results can be summarized as follows:

(1) Isolated rat liver mitochondria can oxidize added NADH via added cytochrome *c* by rotenone- and antimycin-insensitive, mersalyl- and cyanide-sensitive mechanisms;

(2) Mersalyl (but not cyanide) block can be overcome by addition of rat liver microsomes and cytochrome *c*;

(3) Rat liver cell suspensions oxidize added NADH. The rate of this process is not affected by added rotenone, antimycin A and cytochrome *c* and is decreased 3-fold by cyanide;

(4) NADH oxidation in homogenates can be stimulated by added cytochrome *c*.

It is most probable that oxidation of extramitochondrial NADH by mitochondria or homogenates in

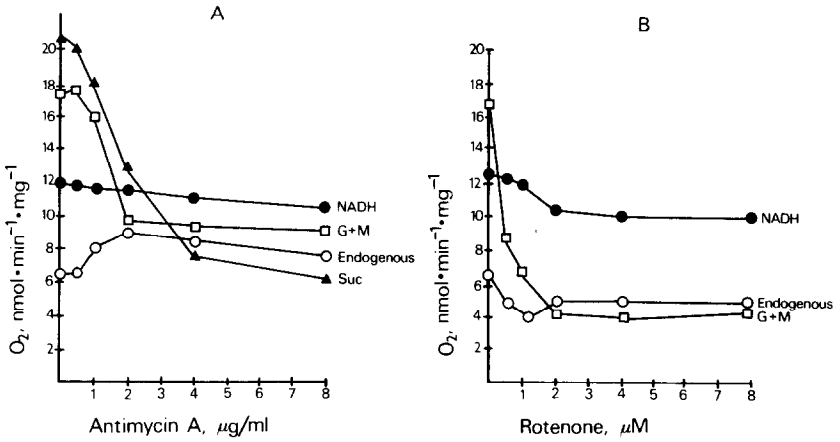

Fig. 5. The effect of antimucin A (A) and rotenone (B) on respiration of isolated hepatocytes. For incubation mixture, see fig. 4.

the presence of added cytochrome *c* is via NADH-cytochrome b_5 reductase and cytochrome b_5. This rotenone- and antimycin A-sensitive pathway localized in the outer membrane of mitochondria and in the microsomal membrane, can reduce added cytochrome *c* which is oxidized by the cyanide-sensitive mitochondrial system, i.e. cytochrome oxidase of the inner mitochondrial membrane. It should be emphasized that the rate of these NADH oxidations involving the step of intermembrane electron transfer is very high being of the same order of magnitude as that of the cytochrome oxidase reaction.

The respiration of the liver cell suspensions did not respond to added cytochrome *c*. Rotenone- and antimycin A-insensitive cyanide sensitive oxidation of NADH by cells should be catalyzed by some endogenous intermembrane electron carrier(s). Cytochrome *c*, dissolved in the intermembrane water of mitochondria may be such a carrier [1]. Respectively, changes in the intermembrane cytochrome *c* concentration as a result of its reversible desorption from outer surface of the inner mitochondrial membrane might be a mechanism of regulation of the two pathways of NADH oxidation, one of which (outer) is resistant to agents inhibiting the initial and middle parts of phosphorylating respiratory chain, which are very sensitive to the action of foreign hydrophobic compounds of very different structures [7]. It is important that electron transfer via cytochrome b_5, intermembrane cytochrome *c* and cytochrome oxidase of the inner mitochondrial membrane is still competent in membrane potential generation and oxidative phosphorylation at the level of the third energy coupling site of the respiratory chain.

It seems likely that not only mitochondrial but also microsomal cytochrome b_5 may be involved in the poison-resistant respiratory chain shunt, as is the case in vitro in the 'hybrid' mitochondrial–microsomal system described above.

References

[1] Skulachev, V.P. (1969) Energy Accumulation in the Cell, Cell,Nauka Press, Moscow.
[2] Archakov, A.I., Karjakin, A.V. and Skulachev, V.P. (1973) Dokl. Akad. Nauk. SSSR 209, 221.
[3] O'Brien, P.J. (1971) in: Energy Transduction in Respiration and Photosynthesis (Qugliariello E. et al., eds), p. 361, Adriatica, Bari.
[4] Archakov, A.I., Panchenko, L.P., Kapitanov, A.B., Efron, I.I., Knayzeva, T.A. and Zherebkova, N.S. (1973) Anal. Biochem. 54, 223.
[5] Hommes, F.A., Draiama, M.J. and Malenar, Z. (1970) Biochim. Biophys. Acta 222, 361.
[6] Lowry, O.H., Rosenbraugh, H.J., Faur, A.Z. and Randall, R.J. (1951) J. Biol. Chem. 193, 265.
[7] Jaguzhinsky, L.S., Smirnova, E.G., Ratnikova, L.A., Krasinskaja, I.P., Asarenkova, N.A. (1972) Dokl. Akad. Nauk SSSR 205, 734.