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Deoxyadenine nucleotides and oxidative phosphorylation

In 1963 LÖW, VALLIN AND ALM[1] demonstrated that the specificity of oxidative phosphorylation for adenine nucleotides found with intact mitochondria does not exist in submitochondrial particles. The specificity of the system in intact mitochondria is caused by a specific transport mechanism for adenine nucleotides, as was demonstrated by PFAFF[2] and by BRIERLEY AND O'BRIEN[3]. PFAFF[2] as well as BRIERLEY AND O'BRIEN[3] showed that mitochondria labelled with radioactive adenine nucleotides lost their radioactivity on incubation not only with unlabelled ADP or ATP, but also, to some extent, with dADP or dATP. In both cases the diphosphate was the more effective.

Since these experiments suggest that the deoxy compounds can pass the mitochondrial membrane system, while oxidative phosphorylation *per se* is not specific for the adenine nucleotides, we examined the possibility that dADP can function as phosphate acceptor in the process of oxidative phosphorylation.

TABLE I

SPECIFICITY OF PHOSPHATE ACCEPTOR FOR OXIDATIVE PHOSPHORYLATION IN INTACT RAT-LIVER MITOCHONDRIA

The reaction medium contained 30 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 15 mM KCl, 6 mM $MgCl_2$, and 75 mM sucrose. In Expt. 1, 10 mM glutamate and 6.9 mg mitochondrial protein were used in a volume of 3.0 ml. Temperature, 20°. In Expt. 2, 20 mM succinate and 2.3 mg mitochondrial protein were used in a volume of 2.0 ml. Temperature, 25°.

Expt. No.	*Additions*	*Oxygen uptake (natoms/min per mg)*
1	None	7
	ADP (0.33 mM)	52
	dADP (0.33 mM)	15
	dADP (0.33 mM) + ATP (3.7 mM)	56
2	None	39
	CDP (0.17 mM)	38
	CDP (0.17 mM) + ATP (2.5 mM)	62
	UDP (0.22 mM)	45
	UDP (0.22 mM) + ATP (2.5 mM)	115
	GDP (0.21 mM)	37
	GDP (0.21 mM) + ATP (2.5 mM)	179

Table I demonstrates that the addition of dADP gives only a slight stimulation of the rate of oxygen uptake in a system where phosphate acceptor is limiting, in comparison with that obtained by addition of ADP. In the presence of ATP, however, dADP was effective, half maximal stimulation being given by 0.13 mM ATP. This effect can be explained by the phosphotransferase reaction:

$$dADP + ATP \rightleftharpoons ADP + dATP$$

(*cf.* ref. 1). The increase after addition of dADP alone is probably due to reaction with endogenous ATP that has leaked out from the mitochondria.

Table I, Expt. 2, shows that other nucleotides can also serve as phosphate acceptor in the presence of ATP. This (these) phosphotransferase(s) must then be localized outside the specific adenine nucleotide translocase (*cf.* ref. 4).

Since, however, the experiments described in Table I did not answer the question whether the inability of dADP on its own to act as phosphate acceptor is due to its inability to act as substrate for the translocase or for oxidative phosphorylation, the 2,4-dinitrophenol-induced dATPase activity of intact rat-liver mitochondria was compared with the activity after sonication. From Fig. 1, it is clear that dATP (Fig. 1B) is very slowly broken down by intact rat-liver mitochondria. After sonication,

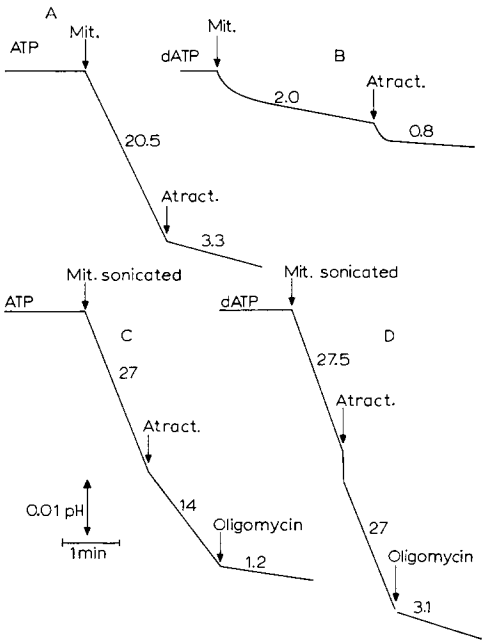

Fig. 1. ATPase and dATPase activity in intact and sonicated rat-liver mitochondria. The reaction medium contained 75 mM KCl, 10 mM Tris–HCl buffer (pH 7.4), 60 mM sucrose, 1 mM EDTA, 0.1 mM 2,4-dinitrophenol, 3 mM $MgCl_2$, 3 mM ATP or dATP and 1.5 mg mitochondrial protein. Atractyloside (34 μg/mg protein) and oligomycin (3.4 μg/mg protein) added where indicated. Reaction volume, 2.5 ml. Temperature, 25°. The mitochondria were sonicated twice for 1 min at 0° in the Mullard Ultrasonic power unit at maximal output. The reaction was followed by registration of the pH. The numbers against the tracings give the rate of ATP breakdown in arbitrary units.

however, it is as rapidly hydrolysed as ATP (*cf.* Figs. 1C and 1D). The hydrolysis of ATP by the sonicated particles was inhibited by atractyloside to the extent of 50 %, whereas the hydrolysis of dATP was insensitive to atractyloside. The hydrolysis of both ATP and dATP is sensitive to oligomycin. The soluble ATPase isolated from beef-heart mitochondria, F_1 (ref. 5), hydrolyses dATP equally well as ATP.

The behaviour of dATP is analogous to that of GTP, because with both compounds the reaction rate increases after disintegration of the mitochondria, as was already demonstrated for ITP by KLINGENBERG AND PFAFF[4]. It cannot be decided if the low rate of hydrolysis of dATP by intact mitochondria is due to a direct hydrolysis of dATP or occurs *via* a reaction with endogenous ADP.

In conclusion we can say that with respect to deoxyadenine nucleotides, the specificity of the overall process of the oxidative phosphorylation also resides at the level of the translocase. The transport of the deoxyadenine nucleotides demonstrated previously[2,3] must be one or two orders of magnitude slower than that of the adenine nucleotides.

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Electron microscopic evidence for volumetric changes in heart mitochondria

Electron microscopy of mitochondria in various metabolic states has recently been reported[1,2], and changes in contractile states induced by ATP have been observed[3,4]. CHAPPELL AND CROFTS[5,6] have shown that oxidation of electron transfer substrates will cause the contraction of mitochondria swollen with Ca^{2+} provided the Ca^{2+} is previously removed. We have recently reported[7] that oxidizable substrates (*e.g.* succinate) will contract beef heart mitochondria which have been swollen by exposure to hypotonic conditions. The substrate-induced contraction is blocked by electron transfer inhibitors (antimycin), but is not affected by inhibitors of terminal phosphorylation (oligomycin), and is prevented by 2,4-dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone[8].

The spectrophotometric and gravimetric evidence for substrate-induced contraction of heart mitochondria is now well documented. This evidence substantiates the inclusion or requirement of a "contractile" process in energy transducing functions