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THE ADENINE NUCLEOTIDE TRANSLOCATOR AND THE NUCLEOTIDE SPECIFICITY OF OXIDATIVE PHOSPHORYLATION

J. H. M. SOUVERIJN, P. J. WEIJERS, G. S. P. GROOT AND A. KEMP JR.

Laboratory of Biochemistry, B. C. P. Jansen Institute, University of Amsterdam, Amsterdam (The Netherlands)*

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SUMMARY

By studying P/O ratios, proton translocation and morphology of different mitochondrial preparations, it was shown, in agreement with previous work but in disagreement with a recent report, that the nucleotide specificity of the oxidative phosphorylation resides at the level of the adenine nucleotide translocator.

PFAFF *et al.*[1] have proposed that in intact mitochondria the specificity of oxidative phosphorylation for ADP as phosphate acceptor is due to the nucleotide specificity of the adenine nucleotide translocator in the inner membrane. This proposal was based on the results of experiments in which the exchange of intra- and extramitochondrial nucleotides was measured. Additional evidence in favour of this proposal was obtained by KEMP AND GROOT[2], who showed that nucleotides other than adenine nucleotides can be phosphorylated in intact mitochondria only when extramitochondrial ATP is also present.

However, DUÉE AND VIGNAIS[3] have recently suggested that the adenine nucleotide translocator is not the only factor governing the specificity of oxidative phosphorylation in intact mitochondria for ADP (see also ref. 4). They found that the specificity for ADP is still present in digitonin particles, in which it was presumed that the adenine nucleotide translocator does not play a role.

In order to throw some light on this discrepancy we have compared the properties of rat-liver mitochondria, digitonin particles and sonic (Mg^{2+}–ATP) particles from beef-heart mitochondria.

The results of determinations of the P/O ratio in intact mitochondria, digitonin particles of the same type as used by DUÉE AND VIGNAIS[3], and Mg^{2+}–ATP particles are summarized in Table I. The P/O ratios with GDP and IDP were corrected for the phosphorylation found without added nucleoside diphosphate (see lines 1 and 5). It can be seen that (1) GDP is better phosphorylated by Mg^{2+}–ATP particles than by intact rat-liver mitochondria or digitonin particles; (2) the P/O ratio is lowered by atractyloside when intact rat-liver mitochondria or digitonin particles are used. The fact that the inhibition is only partial is due to the high ADP (5 mM) concentration present. Atractyloside has no effect on the P/O ratio in Mg^{2+}–ATP particles.

* Postal address: Plantage Muidergracht 12, Amsterdam, The Netherlands.

TABLE I

P/O RATIOS DETERMINED WITH DIFFERENT MITOCHONDRIAL AND SUBMITOCHONDRIAL PREPARATIONS IN THE PRESENCE OF DIFFERENT PHOSPHATE ACCEPTORS

The values are the means of different experiments. The values given for GDP and IDP are corrected for the phosphorylation found without *added* nucleoside diphosphate. The reaction mixture (final volume 1.7 ml) contained 20 mM phosphate buffer (pH 7.4), 5 mM succinate, 30 mM glucose, 1 mM glucose 6-phosphate, 1 mM EDTA, 5 mM nucleoside diphosphate, rotenone (0.13 μg/mg protein), and, when indicated, about 20 Units hexokinase, 4 mM $MgCl_2$, and 80 μg atractyloside. The amount of protein was 1–2 mg. Temperature, 25°.

Nucleoside diphosphate added	*P/O ratio in*		
	Intact rat-liver mitochondria	*Digitonin particles from rat-liver mitochondria*	*Mg^{2+}–ATP particles from beef-heart mitochondria*
Mg^{2+} and hexokinase present			
None	0.36	0.09	0.00
ADP	1.54	0.46	0.47
GDP	0.08	0.03	0.12
IDP	0.01	0.01	—
Mg^{2+}, hexokinase and atractyloside added			
None	0.00	0.04	0.08
ADP	0.82	0.19	0.53
GDP	0.04	0.02	0.16
IDP	0.02	0.02	—

When an anaerobic suspension of mitochondria is supplied with oxygen, the pH of the medium is lowered. This is thought to be due to a movement of H^+ through the mitochondrial inner membrane from the inside to the outside of the mitochondrion[5]. This phenomenon was studied in the three preparations used in the experiments of Table I and in sonicated digitonin particles. Fig. 1 shows that the direction of proton movement in digitonin particles is the same as that in intact rat-liver mitochondria, and opposite to that in Mg^{2+}–ATP particles or sonicated digitonin particles.

Fig. 2 shows an electron micrograph of a negatively stained preparation of rat-liver mitochondria. Fig. 3 shows that morphologically different sorts of particles are present in a digitonin-treated rat-liver mitochondria preparation. The particles marked P_1 and P_2 have dimensions approaching those of intact mitochondria (*cf.* Fig. 2) and no outer membrane can be seen on these particles. An enlargement of particle P_2 is shown in Fig. 4. Structures like P_1 and P_2 are not seen in electron micrographs of Mg^{2+}–ATP particles (Fig. 5) or sonicated digitonin particles (Fig. 6).

The following lines of evidence suggest that digitonin particles of the type used by DUÉE AND VIGNAIS[3] have a functionally intact inner membrane. First, in electron micrographs particles are seen that look like mitochondria divested of the outer membrane. Secondly, the direction of proton movement is the same as that in intact mitochondria. Thirdly, oxidative phosphorylation in the particles is sensitive to atractyloside. Fourthly, a decreased nucleotide specificity of oxidative phosphorylation is correlated with a decreased atractyloside sensitivity. We therefore conclude that the nucleotide specificity of oxidative phosphorylation in intact mitochondria resides

at the level of the adenine nucleotide translocator, and that the oxidative phosphorylation system *per se* is much less specific (see refs. 1, 2).

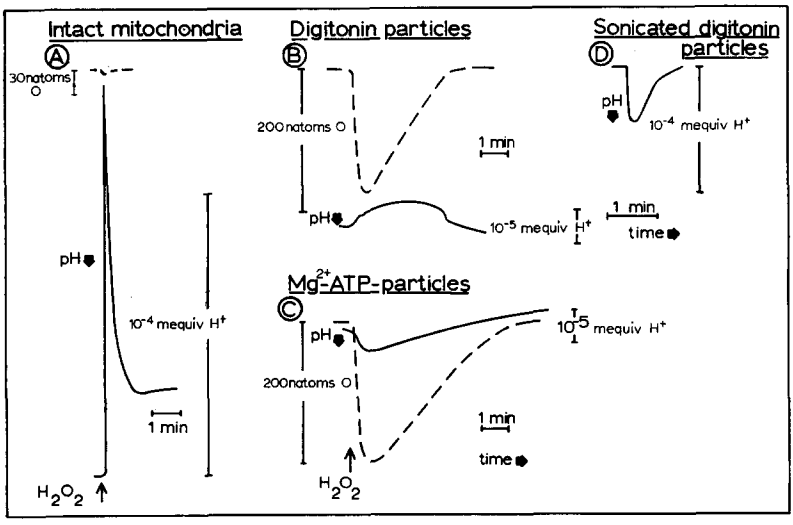

Fig. 1. Changes in pH on the addition of oxygen to different preparations in anaerobic media. A. Intact rat-liver mitochondria. B. Digitonin particles. C. Mg^{2+}–ATP particles. D. Sonicated digitonin particles. The reaction mixture (final volume 1.2 ml) contained 25 mM sucrose, 150 mM KCl, 3.3 mM glycylglycine buffer (pH 7.2), 10 mM succinate, 0.13 μg/mg protein rotenone, and catalase (except in D). The amount of protein was 1.1–2 mg. After the reaction mixture had become anaerobic 0.2% H_2O_2 (5 μl in A, 20 μl in B, and 20 μl in C) or 100 μl of a solution saturated with O_2 (D) was added. Temperature, 25°; ———, pH; – – – –, oxygen concentration.

EXPERIMENTAL

Rat-liver mitochondria were prepared according to MYERS AND SLATER[6], digitonin particles according to DEVLIN AND LEHNINGER[7] or ELLIOTT AND HAAS[8], and Mg^{2+}–ATP particles according to LÖW AND VALLIN[9].

O_2 consumption was measured polarographically and phosphate esterification by the method of ERNSTER *et al.*[10].

The proton movement experiments were carried out according to MITCHELL AND MOYLE[5].

Preparations for electron microscopy were negatively stained with 2% phosphotungstate solution (pH 6.9).

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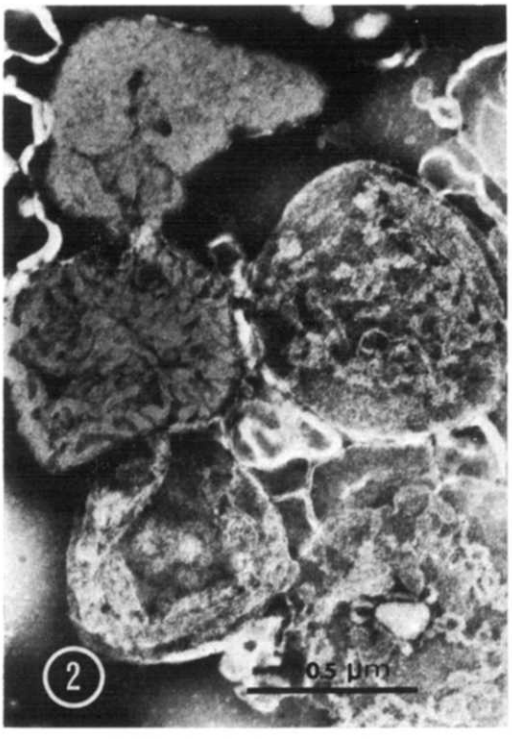
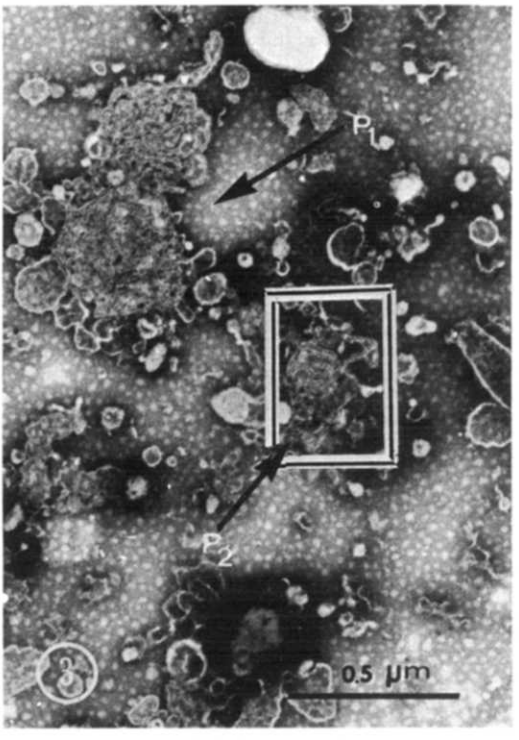
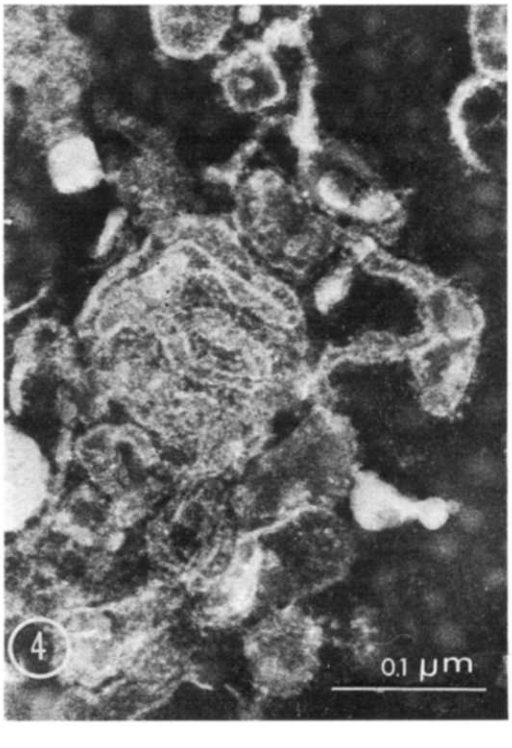

2
0.5 µm


P1
P2
3
0.5 µm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0.1 µm

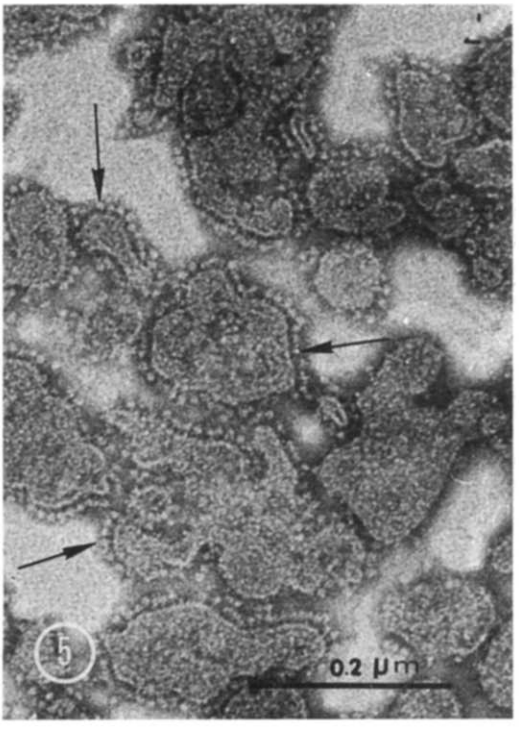
5
0.2 µm

Fig. 2. Electron micrograph of negatively stained rat-liver mitochondria.

Fig. 3. Electron micrograph of digitonin-treated rat-liver mitochondria. Particle P_1 can be interpreted as a mitochondrion devoid of outer membrane. Smooth and rough surfaced particles can be seen.

Fig. 4. Enlargement of particle P_2 from Fig. 3. At the outside of the slender structure sub-units can be seen. These sub-units are identifiable with the inner membrane sub-units of mitochondria.

Fig. 5. Electron micrograph of a negatively stained preparation of Mg^{2+}–ATP particles. Inner membrane sub-units are indicated at the arrows.

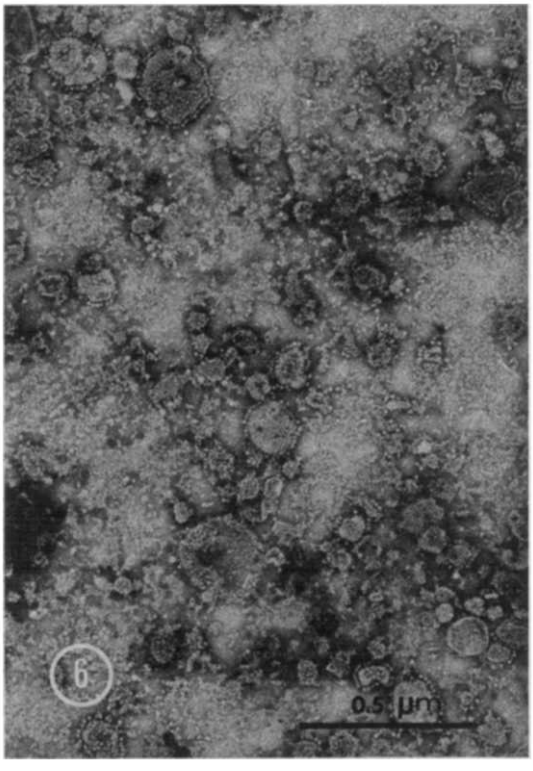

Fig. 6. Electron micrograph of a preparation of sonicated digitonin particles from beef-heart mitochondria. The digitonin particles were prepared according to ELLIOTT AND HAAS[8].

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