Biochimica et Biophysica Acta, 459 (1977) 612—616

BBA Report

BBA 41291

UNCOUPLING OF MITOCHONDRIAL RESPIRATION BY ADP

T.A. OUT, K. KRAB, A. KEMP, Jr. and E.C. SLATER

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, Amsterdam (The Netherlands)

(Received October 15th, 1976)

Summary

Even when oxidative phosphorylation is blocked completely by addition of high concentrations of oligomycin plus aurovertin, the addition of ADP to a suspension of mitochondria containing a high concentration of ATP inside the mitochondria induces a stimulation of respiration and oxidation of nicotinamide nucleotide.

It is concluded that transport of ADP into mitochondria with a high endogenous ATP/ADP ratio requires energy.

As first shown by Klingenberg et al. [1] and confirmed by others [2,3], a respiring mitochondrial suspension in 'State 4' [4] can maintain a higher [ATP]/[ADP] ratio and consequently a higher phosphorylation potential (which is a function of the ratio $[ATP]/([ADP][P_i])$ in the extramitochondrial space than inside the mitochondria. Since it seems unlikely that this discrepancy, which can be as high as 4.5. kcal/mol [2], can be quantitatively accounted for by differences in activity coefficients or of liganding of the components of the phosphorylation reaction [2,5], it is possible (as suggested by Klingenberg [1]) that the energy made available by mitochondrial respiration is utilized, not only for the synthesis of ATP within the mitochondria, but also for the transport of the synthesized ATP out of the mitochondria in exchange for ADP. As recently pointed out [5], this implies that ADP, under certain conditions, could act as an uncoupler in the sense that it could cause a stimulation of mitochondrial respiration in the presence of oligomycin. The uncoupling activity of ADP is demonstrated in this paper.

In the experiment shown in Fig. 1, two inhibitors, oligomycin and aurovertin, were added to block mitochondrial ATP synthesis. Trace 1 shows that when ADP is added to a concentrated suspension of rat-liver mitochondria oxidizing glutamate and malate in the presence of phosphate, magnesium, oligomycin and aurovertin, there is a temporary stimulation of the rate of respiration. (The plateau in the Oxygraph trace immediately after adding the

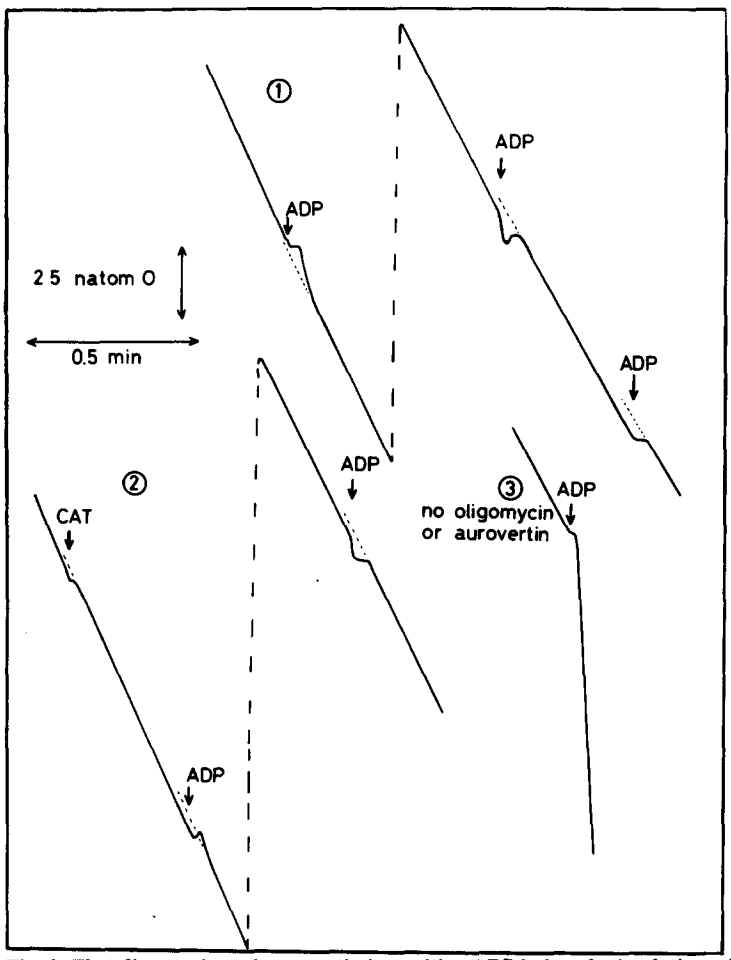

Fig. 1. The oligomycin and aurovertin-insensitive ADP-induced stimulation of mitochondrial respiration. Rat-liver mitochondria (13 mg protein) were added to 1.4 ml (final volume) of an incubation medium containing 50 mM triethanolamine-HCl buffer, 15 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, 5 mM L-glutamate, 5 mM L-malate, 10 mM potassium phosphate and 30 mM sucrose at pH 7.2 and 25°C. Oligomycin (15 μg/mg protein) and aurovertin (15 μg/mg protein) were added except in Trace 3. Where indicated 16 μl of an air-saturated solution containing 1.4 μmol ADP were added. Carboxyatractyloside (CAT) was added where indicated to a final concentration of 28 μM.

ADP is due to the addition of oxygen with the ADP solution). The stimulation is not clearly present after a second or third addition of ADP. Trace 2 shows that it is absent in the presence of an inhibitor of the adenine nucleotide translocator: carboxyatractyloside. Trace 3 shows the normal stimulation of the respiration by the addition of ADP in the absence of oligomycin or aurovertin.

The ADP-induced extra oxygen uptake, calculated from traces similar to Trace 1 in Fig. 1, amounted to 0.59 ± 0.12 (S.D.) natom O/mg protein in 4 experiments.

This effect of ADP could also be demonstrated by following the redox state of the nicotinamide nucleotide, either spectrophotometrically (Fig. 2) or fluorimetrically (Fig. 3). Trace 1 of Fig. 2 shows the usual State 4—State 3—

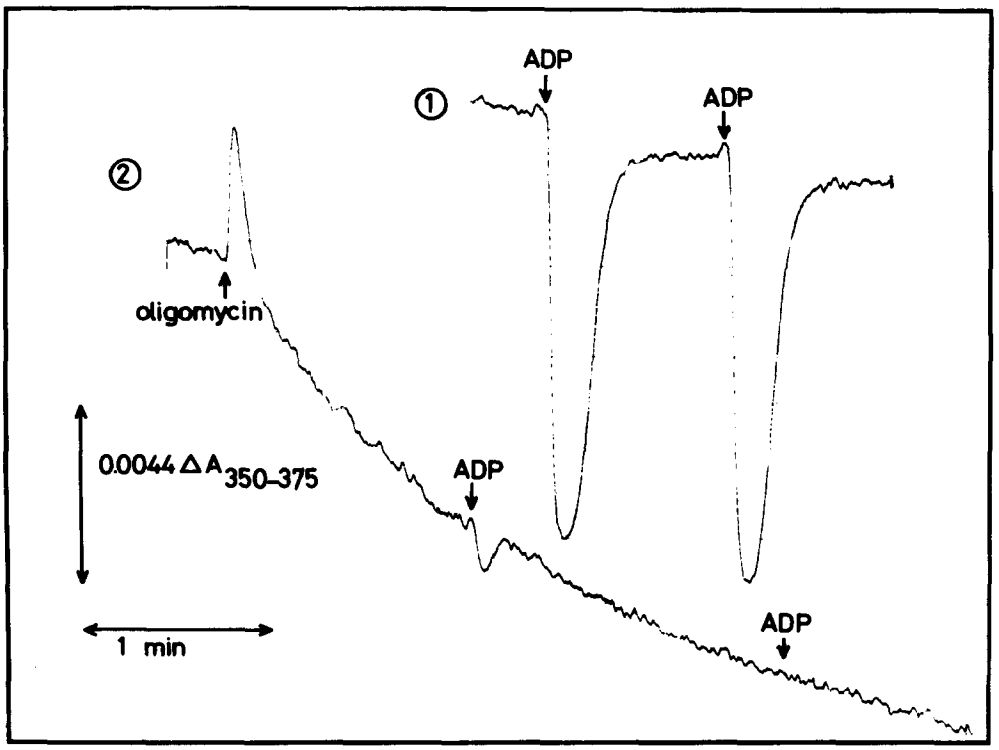

Fig. 2. The oligomycin-insensitive ADP-induced oxidation of reduced nicotinamide-adenine nucleotide, measured spectrophotometrically. Rat-liver mitochondria (4.2 mg protein) were added to 2.5 ml (final volume) of the incubation medium described in Fig. 1. In Curve 1, 0.2 μmol ADP was added where indicated. In Curve 2 oligomycin (47 μg/mg protein) and 1.5 μmol ADP were added where indicated. The incubations were performed in a cuvette adapted for continuous stirring.

State 4 transition when ADP is added in the absence of inhibitors of the phosphorylation reaction. Trace 2 shows that, superimposed on the slow oxidation of nicotinamide nucleotide induced by oligomycin, ADP induces a rapid transitory oxidation. A second addition of ADP is without effect. In other experiments (not shown) carboxyatractyloside was shown to prevent the ADP-induced oxidation.

Fig. 3 shows that similar results are obtained when the redox state of the nicotinamide nucleotide is followed fluorimetrically. In this case, according to measurements in parallel experiments, the mitochondria contained 5.6 nmol ATP per mg protein just before the first addition of ADP in Trace 2, and 1.6 nmol before the second addition. Trace 3 shows that carboxyatractyloside prevents the ADP-induced oxidation of nicotinamide nucleotide. In another experiment (not shown) rotenone (8.6 nmol/mg protein) was also found to prevent this oxidation. Fig. 4 shows that the ADP-induced decrease of fluorescence is roughly proportional to the concentration of mitochondria used (and therefore to the amount of intramitochondrial ATP).

Since the incubation with 7 mM arsenite for 5 min did not affect the transient oxidation of NADH, oxidative phosphorylation of 2-oxoglutarate at the substrate level can be excluded as contributing to the effect described.

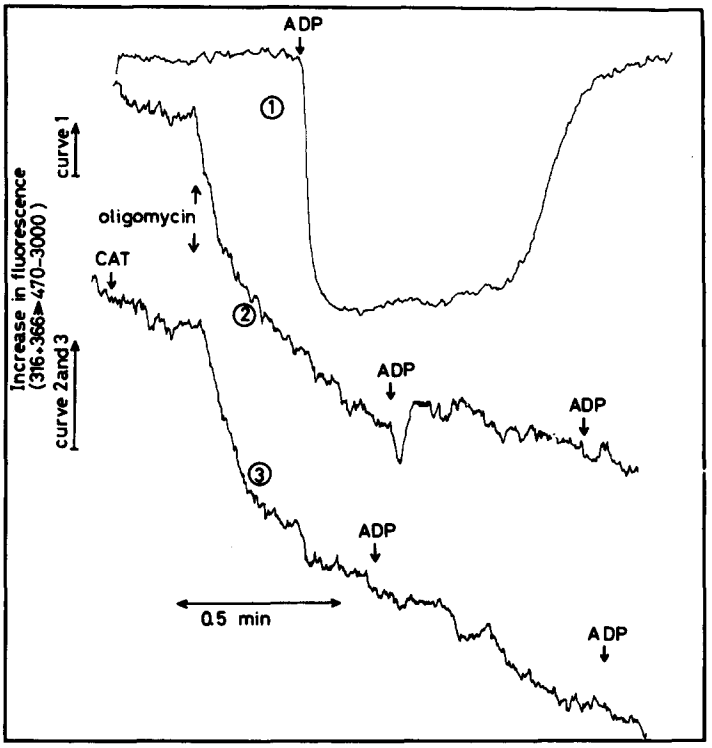

Fig. 3. The oligomycin-insensitive ADP-induced oxidation of reduced nicotinamide-adenine nucleotide as measured fluorimetrically. Rat-liver mitochondria (1.45 mg protein) were added to 1.5 ml (final volume) of an incubation medium containing 33 mM KCl, 10 mM $MgCl_2$, 6 mM EDTA, 66 mM Tris•HCl buffer, 5 mM L-glutamate, 5 mM L-malate and 16 mM potassium phosphate at pH 7.4 and 25°C. In Curve 1, 0.5 μmol ADP was added where indicated. In Curves 2 and 3, oligomycin (69 μg/mg protein), carboxyatractyloside (CAT, 17 nmol/mg protein) and 1 μmol ADP were added where indicated. The fluorescence was measured with an Eppendorf fluorimeter using a cuvette adapted for continuous stirring.

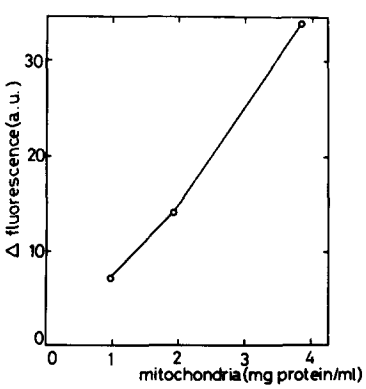

Fig. 4. The extent of the oligomycin-insensitive ADP-induced decline of fluorescence, measured in arbitrary units, as in Fig. 3, as a function of concentration of mitochondria.

The results shown here clearly demonstrate that the exchange of intramitochondrial ATP for added ADP, catalysed by the carboxyatractyloside-sensitive adenine nucleotide translocator, is an energy-requiring process as suggested by Klingenberg. The amount of energy used for the translocation may be calculated from the amount of ADP-induced extra oxygen uptake (0.59 ± 0.06 (S.E.) natom O/mg protein), the amount of ATP translocated out of the mitochondria (5.6 - 1.6 = 4.0 nmol/mg protein), and the Gibbs-energy difference in the redox reaction (50.8 kcal/atom O [2]). This equals 50.8 × 0.59/4.0 = 7.5 ± 0.7 (S.E.) kcal/mol ATP. This is, in fact, even higher than the 4.5 kcal/mol difference in phosphorylation potential between the intra- and extramitochondrial spaces [2].

This work was supported in part by grants from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).

References

1 Klingenberg, M., Heldt, H.W. and Pfaff, E. (1969) in The Energy Level and Metabolic Control in Mitochondria (Papa, S., Tager, J.M., Quagliariello, E. and Slater, E.C., eds.), pp. 237—253, Adriatica Editrice, Bari
2 Slater, E.C., Rosing, J. and Mol, A. (1973) Biochim. Biophys. Acta 292, 534—553
3 Vignais, P.V., Vignais, P.M., Lauquin, G. and Morel, F. (1973) Biochimie 55, 763—778
4 Chance, B. and Williams, G.R. (1955) J. Biol. Chem. 217, 409—427
5 Slater, E.C. (1976) in Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies (Tager, J.M., Söling, H.D. and Williamson, J.R., eds.),pp. 65—77, North-Holland, Amsterdam