INFLUENCE OF AUROVERTIN ON AFFINITY OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE FOR ATP AND ADP

Peter MITCHELL and Jennifer MOYLE
Glynn Research Laboratories, Bodmin, Cornwall, England

Received 2 January 1970

1. Introduction

The phosphorylation of ADP by the reversible mitochondrial ATPase system was shown by Lardy and coworkers [1–3] to be inhibited as effectively by aurovertin as by oligomycin, whereas the hydrolysis of ATP by the same system appeared to be unaffected. This interesting property of aurovertin was confirmed by others [4–7], and it was suggested that "the apparent discrepancy of aurovertin sensitivity between forward and reverse energy transfer reactions may eventually find its explanation in kinetic considerations" [6].

Following a preliminary report [8], we describe here observations on the kinetics of the ATPase of rat liver mitochondria showing that ATP and ADP react competitively and that aurovertin causes at least a hundredfold increase in the affinity of the reversible ATPase system for ATP relative to that for ADP (given by the ratio $(K_i)_{ADP}/(K_m)_{ATP}$). These observations provide a clue to the mechanism by which aurovertin kinetically promotes the forward ATPase reaction but inhibits its reversal. They also indicate that aurovertin may prove to be a useful tool for hastening completion of ATP hydrolysis by the proton-translocating ATPase system.

2. Materials and methods

Rat liver mitochondria were isolated as described preiously [9]; and proton translocation by the intact mitochondria was measured as before [10]. Sonic particles were prepared from mitochondrial suspensions (30 mg of protein/ml) at 4°C in 250 M sucrose containing 20 mM glycylglycine-KOH buffer at pH 7.5, using an MSE 60 W sonicator operated at full power for 2 min. The particles were isolated by differential centrifugation at 4°C, and were finally suspended in 250 mM sucrose at a concentration corresponding 80 mg of protein/ml. The ATPase activity was measured at 25°C by the pH method of Nishimura, Ito and Chance [11] as before [10], or by the luciferin-luciferase method [12], using a medium containing 150 mM KCl, 3.3 mM glycylglycine and 5 mM $MgCl_2$ near pH 7. The ATPase measurements were routinely done in presence of 1 μM carbonylcyanide *p*-trifluoromethoxy phenylhydrazone (FCCP) to avoid possible effects of proton translocation. Aurovertin was generously provided by Dr. H.A.Lardy (Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin, U.S.A.) and by Dr. R.B.Beechey (Shell Research, Sittingbourne, Kent).

3. Results and discussion

Fig. 1 shows the time-course of ATP hydrolysis (initial ATP concentration 60 μM) by sonic particles in presence and absence of aurovertin, using the pH method. The initial rate of hydrolysis was the same whether aurovertin was present or not; but whereas in absence of aurovertin the rate steadily decreased as ATP was hydrolysed to ADP, in presence of aurovertin the rate increased at first and subsequently decreased after hydrolysis was some 80% complete. This type of behaviour was observed over a wide range of

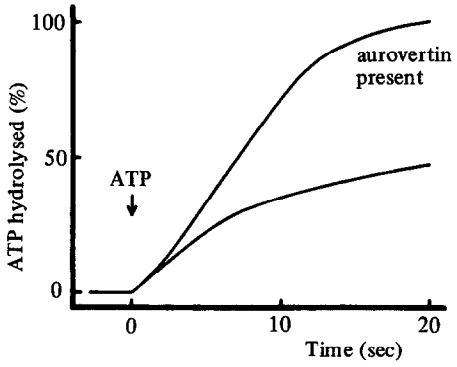

Fig. 1. Time-course of ATP hydrolysis by sonic particles. Sonic particles (8 mg protein) were suspended in 3.3 ml of a medium containing 150 mM KCl, 3.3 mM glycylglycine, 5 mM $MgCl_2$ at pH 7.0–7.1 at 25°C. At the arrow, pulses of ATP (0.2 μ-moles) were added and ATP hydrolysis was followed by the pH method [10, 11]. Lower curve, in absence of aurovertin; upper curve, in presence of aurovertin (2.5 mg/g of particle protein).

initial ATP concentration. In particular, the initial rate of hydrolysis at initial ATP concentrations from 15 μM to 1 mM was unaffected by aurovertin. Omission of the FCCP did not change the results significantly. The same time-course of hydrolysis was observed when the luciferin-luciferase technique was used to estimate the disappearance of ATP as when hydrolysis was measured by the pH method. Thus, the latter method was not subject to any pH artefact due to the presence of aurovertin.

The effects of ADP and P_i on the relationship between initial ATP concentration and initial velocity of ATP hydrolysis by the sonic particles are shown in the Lineweaver-Burk plots of fig. 2a. From these observations it follows that $(K_m)_{ATP}$ = 106 μM, there is no inhibition by P_i at a concentration ten times that of the ATP, and ADP is a strictly competitive inhibitor with $(K_i)_{ADP}$ = 8.6 μM. Inhibition of the ATPase of rat liver mitochondria by ADP was described previously [13], and inhibition of the ATPase of beef heart mitochondria by ADP has been described by several authors [14–17].

The data of fig. 2b show that when aurovertin was present, the V_{max} of the ATPase of the sonic particles was initially unchanged, but initially $(K_m)_{ATP}$ = 56 μM and $(K_i)_{ADP}$ = 180 μM.

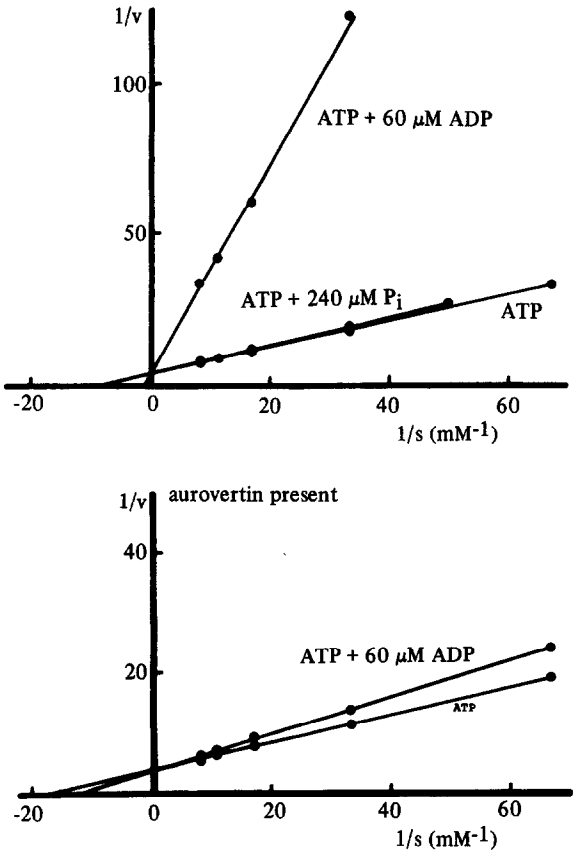

Fig. 2. Lineweaver-Burk plots of ATPase activity of sonic particles: a, in absence of aurovertin; b, in presence of 2.5 mg of aurovertin/g of particle protein. Conditions were as in the experiments of fig. 1. The initial rates of ATP hydrolysis represent the rates during the first 3 sec after addition of the pulses of ATP, (ATP + ADP) or (ATP + P_i). The value of V_{max} in these experiments corresponds to the hydrolysis of 2.8 μ-moles of ATP/sec g of particle protein.

The adenylate kinase activity of the sonic particle preparations was comparatively low, as can be seen from fig. 1, and this was confirmed by experiments in which the rate of hydrolysis of added ADP (120 μM) was shown to be less than 4% of the rate of hydrolysis of the same concentration of ATP. It was therefore possible to obtain from curves corresponding to fig. 1 the rate of ATP hydrolysis at various time-intervals after the initial addition of ATP, when the concentrations of ATP and ADP could be estimated. Analysis of such data by Lineweaver-Burk plots showed that

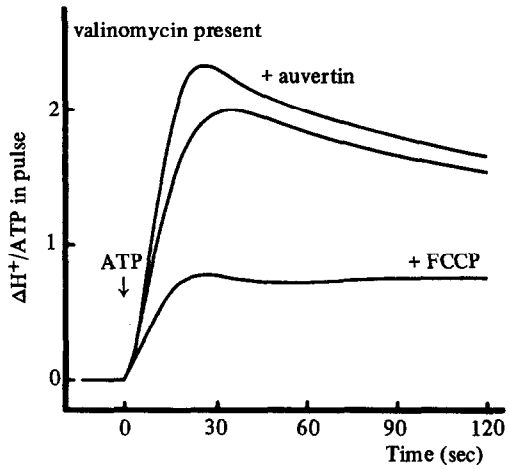

Fig. 3. Time-course of appearance of H^+ ions in the outer phase during proton translocation coupled to ATP hydrolysis by rat liver mitochondria. Mitochondria (6 mg of protein/ml) were suspended in an anaerobic medium containing 150 mM KCl, 25 mM sucrose, 3.3 mM glycylglycine, 1 mM EDTA and 10 μg of valinomycin/g of protein at pH 7.0–7.1 at 25°C. At the arrow, pulses of ATP (0.1 μmole) were added. Middle curve, no addition; upper curve, in presence of 2 mg of aurovertin/g of protein; lower curve, in presence of 1 μM FCCP.

in the presence of aurovertin (but not in its absence) the addition of ATP initiated a time-dependent fall in $(K_m)_{ATP}$, taking some 20 sec to complete. We have estimated that $(K_m)_{ATP}$ falls from the initial value of 56 μM to a steady-state value of 8.4 μM, whereas the value of V_{max} remains comparatively constant.

Experiments corresponding to those of figs. 1 and 2,done in presence of 6.8 mg of oligomycin/g of sonic particle protein, showed that the oligomycin-insensitive ATPase activity (7% of total) had the same $(K_m)_{ATP}$ and $(K_i)_{ADP}$ as the oligomycin-sensitive activity, and was similarly affected by aurovertin.

As illustrated in fig. 3, the presence of aurovertin did not decrease the stoichiometry of proton translocation accompanying ATP hydrolysis by intact mitochondria, but hastened completion of ATP hydrolysis. The extrapolated $\rightarrow H^+/P$ quotient was close to 2 in the presence of aurovertin as in its absence [10].

Our findings suggest that the stoichiometric of the mitochondrial ATPase system observed for isolated F_1 preparations by Lardy and co-workers (see 3, 18]) occurs also in the complete ATPase system and gives rise to the change of the affinity of the adenine nucleotide acceptor site for ATP and ADP. The differences between the effects of oligomycin and aurovertin that have been well discussed by Ernster, Lee and Janda [5] may be adequately explained by the fact that oligomycin reacts with the F_o component of the system [18], which appears to control V_{max}, whereas aurovertin reacts with the F_1 component, which appears to control the adenine nucleotide affinities. We suggest that the inhibitory effects of aurovertin and oligomycin on the reversal of the ATPase reaction are additive [1, 6] because aurovertin decreases the degree of saturation of the system with ADP while oligomycin decreases V_{max}.

References

[1] H.A.Lardy, J.L.Connelly and D.J.Johnson, Biochemistry 3 (1964) 1961.
[2] J.L.Connelly and H.A.Lardy, Biochemistry 3 (1964) 1969.
[3] H.A.Lardy and S.M.Ferguson, Ann. Rev. Biochem. 38 (1969) 991.
[4] G.Lenaz, Biochem. Biophys. Res. Commun. 21 (1965) 170.
[5] L.Ernster, C.P.Lee and S.Janda, in: Biochemistry of mitochondria, ed. E.C.Slater, Z.Kaniuga and L.Wojtczak (Academic Press, London, 1967) p. 29.
[6] C.P.Lee and L.Ernster, European J. Biochem. 3 (1968) 391.
[7] A.M.Robertson, C.T.Holloway, I.G.Knight and R.B. Beechey, Biochem. J. 108 (1968) 445.
[8] P.Mitchell and J.Moyle, Biochem. J. (in press).
[9] P.Mitchell and J.Moyle, Biochem. J. 104 (1967) 588.
[10] P.Mitchell and J.Moyle, European J. Biochem. 4 (1969) 530.
[11] M.Nishimura, T.Ito and B.Chance, Biochim. Biophys. Acta 59 (1962) 177.
[12] B.L.Strehler, in: Methods of enzymatic analysis, ed. H.U.Bergmeyer (Academic Press, New York, revised edition, 1965) p. 559.
[13] G.A.Vigers and F.D.Ziegler, Biochem. Biophys. Res. Commun. 30 (1968) 83.
[14] M.E.Pullman, H.Penefsky. A.Datta and E.Racker, J. Biol. Chem. 235 (1960) 3322.
[15] F.L.Bygrave and A.L.Lehninger, Proc. Natl. Acad. Sci. U.S. 57 (1967) 1409.
[16] M.J.Selwyn, Biochem. J. 105 (1967) 279.
[17] A.Tzagoloff, K.H.Byington and D.H.MacLennan, J. Biol. Chem. 243 (1968) 2405.
[18] M.E.Pullman and G.Schatz, Ann. Rev. Biochem. 36 (1967). 539.