# ON THE ROLE OF THE ADENOSINE DIPHOSPHATE-ADENOSINE TRIPHOSPHATE EXCHANGE REACTION IN OXIDATIVE PHOSPHORYLATION: EFFECT OF CALCIUM

F.L.BYGRAVE and K.C.REED

*Department of Biochemistry, The Australian National University, Faculty of Science, Canberra, A.C.T., Australia*

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## 1. Introduction

An important property of the ADP-ATP exchange reaction of oxidative phosphorylation is its susceptibility to loss of mitochondrial integrity as measured by the acceptor control ratio [1-3]. In the study of Bygrave and Lehninger [1] some indication was provided that the loss in exchange activity on loss of mitochondrial integrity was accompanied by an increase in ATPase activity. In our view this point is central in any evaluation of the mechanism of the exchange reaction in oxidative phosphorylation. We have therefore investigated this problem in greater detail by studying the response of these parameters to calcium. Our data provide substantial evidence for the view that the exchange reaction is directly related to the integrity of the isolated mitochondria and inversely related to mitochondrial ATPase. They further indicate that the likelihood of isolating this reaction from mitochondria as an ADP-ATP exchange enzyme is remote [2].

## 2. Methods and materials

Mitochondria were isolated from the livers of male albino rats essentially according to the procedure of Schneider [4]. The isolation medium consisted of 0.25 M sucrose. The mitochondria were washed twice in 0.25 M sucrose and suspended in this medium at a concentration of about 40 mg of protein per ml.

Acceptor control of respiration and oxidative phosphorylation were measured as described in the legends accompanying the appropriate figures; oxygen uptake was measured with a Gilson Oxygraph.

ADP-ATP exchange activity was measured as previously described [1] except that the nucleotides were separated by DEAE-cellulose paper chromatography [5]. In these experiments ATPase activity was determined by measuring the appearance of inorganic phosphate by a modification of the method of Allen [6]; in other experiments ATPase activity was measured by continuously monitoring the release of protons into the incubation medium. For this purpose a glass electrode was used in conjunction with a Townson Expansion pH meter (Townson & Mercer, Sydney) having a 100 mV recorder outlet (1.4 pH units) which was in turn connected to a 10 inch Rikadenki recorder (10 mV F.S.D.). Under the conditions used, changes in pH were a linear function of changes in proton concentration.

All reagents used were of A.R.grade. The nucleotides were obtained from C.F.Boehringer and Sonne, Mannheim, Germany or from Sigma Chemical Company, St. Louis, Missouri.

Radioactive ADP and radioactive orthophosphate were obtained from Amersham Radiochemical Centre.

## 3. Results

Fig.1 shows the results of an experiment in which ADP-ATP exchange and ATPase activities in intact rat liver mitochondria were measured as a function of increasing concentrations of added $Ca^{++}$. The data show that as the concentration of added $Ca^{++}$ in-

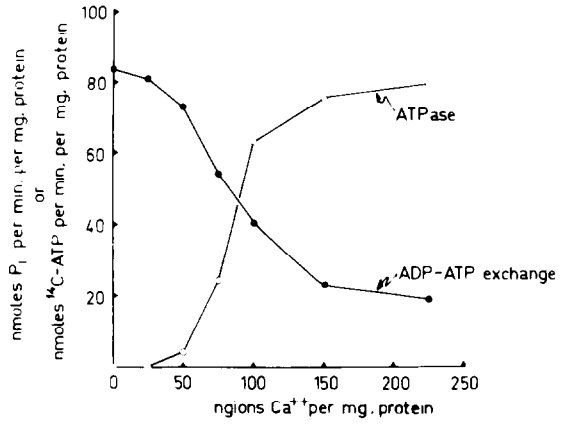


Fig.1. Influence of $Ca^{++}$ concentration on ATPase and ADP-ATP exchange activities in intact rat liver mitochondria. The reaction medium contained 80 mM sucrose, 16 mM tris chloride (pH 7.4), 2 mM ADP containing 0.1$\mu$Ci of $^{14}$C-labelled ADP, 0.2 mg mitochondrial protein and various concentrations of $Ca^{++}$ as indicated. After a 5 min preincubation, 6 mM ATP was added and the mixtures incubated for a further 10 min. The incubation temperature was 25° and the final volume 0.5 ml. Reactions were stopped with 0.2 ml of 2.5 M perchloric acid, neutralised with 2.5 M KOH and after centrifugation portions of the supernatant assayed for inorganic phosphate or used for measurement of exchange activity.

creases the ADP-ATP exchange activity decreases. A concomitant increase in ATPase activity is directly associated with this decrease in exchange activity. In this experiment the concentration of $Ca^{++}$ producing half-maximal effect is of the order of 85 ng ions/mg protein. Maximal effect is obtained at about 150 ng ions/mg protein. In other experiments (data not given) we have observed that oligomycin completely blocks the $Ca^{++}$-induced ATPase activity and inhibits the ADP-ATP exchange reaction to an extent comparable with the maximal inhibition produced by $Ca^{++}$.

When the experiment is carried out with aged preparations of rat liver mitochondria (fig.2), the effects of $Ca^{++}$ on the exchange and ATPase activity described above are not observed. It is seen that the rate of the exchange reaction in aged mitochondria measured in the absence of added $Ca^{++}$ is only about 20% of that observed in the original intact mitochondria, i.e. the same as the $Ca^{++}$-insensitive portion found with intact mitochondria. At the same time the activity of

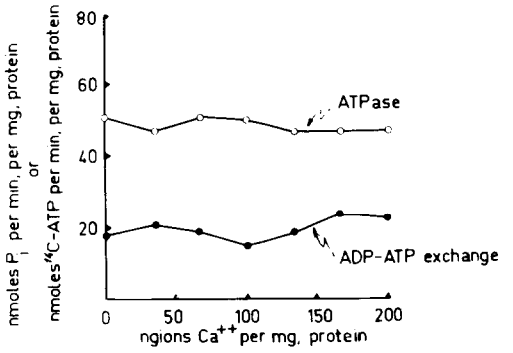


Fig.2. Influence of $Ca^{++}$ concentration on ATPase and ADP-ATP exchange activities in aged rat liver mitochondria. Experimental conditions were exactly as in fig.1 except that mitochondria were aged at 0° for 24 hr prior to incubation.

ATPase is approximately twice that of the exchange reaction. Furthermore, added $Ca^{++}$ has no effect on either the exchange or ATPase activities in the aged mitochondria. In other experiments [7] it was found that the rate of the ADP-ATP exchange in digitonin particles was similar to that observed in aged mitochondria and was also insensitive to $Ca^{++}$.

Although it has been recognised for some time that $Ca^{++}$ can uncouple oxidative phosphorylation in intact mitochondria [8], to our knowledge no data have appeared showing the effect of a wide range of concentrations of this ion on oxidative phosphorylation and related processes. It was important to examine this in our study as *both* the ADP-ATP exchange reaction and ATPase activity are considered to be intimately associated with oxidative phosphorylation [8,9].

Data in fig. 3 show the results of an experiment in which states III and IV of respiration, the acceptor control ratio, ATPase acitivity and the P/O ratio were measured in the presence of increasing concentrations of $Ca^{++}$. It is apparent that as the $Ca^{++}$ concentration is gradually increased, the rate of state III respiration progressively decreases. Since the rate of state IV respiration remains constant, the acceptor control ratio therefore also decreases. Little change in ATPase and in the P/O ratio is observed until the $Ca^{++}$ concentration approaches a value of about 150 ng ions per mg protein. At this point both states III and IV of respiration as well as the ATPase activity rise very sharply and the P/O ratio and acceptor control ratio decline very sharply to values of zero and one, respectively.

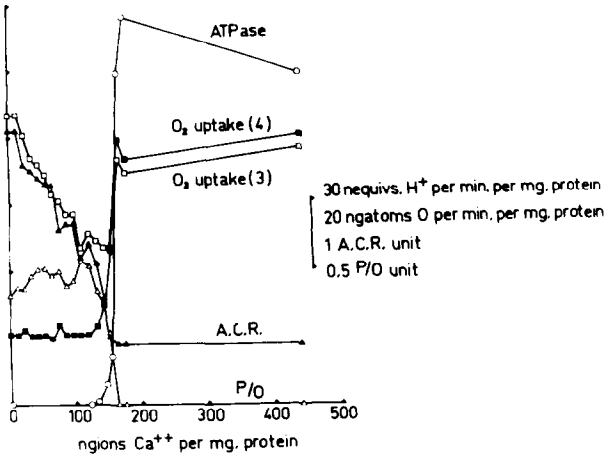


Fig. 3. Effect of increasing concentrations of $Ca^{++}$ on the P/O ratio, ATPase activity, acceptor control ratio and states III and IV of respiration in intact rat liver mitochondria. ATPase activity (○) was measured between 3 and 5 min after the addition of mitochondria by continuously monitoring $H^+$ release (see Methods) in a medium containing 80 mM sucrose, 16 mM tris-HCl (pH 7.4), 2 mM ADP, 6 mM ATP and 1.15 mg mitochondrial protein in a final volume of 1.5 ml. Respiratory parameters were measured in a medium containing 80 mM sucrose, 16 mM tris-HCl (pH 7.4), 1 mM phosphate, 7 mM succinate, $Ca^{++}$ as indicated and 2.3 mg mitochondrial protein in a final volume of 1.5 ml at 25°. Oxygen uptake in state IV (■) was measured for 3 min prior to the addition of 1 mM ADP and $0.5 \times 10^6$ cpm $^{32}Pi$; following this addition, oxygen uptake in state III (□) was measured for 2 min and an aliquot of the medium removed into perchloric acid for assay of esterified $^{32}Pi$ [19]. Acceptor control ratios (▲) are the ratios of the state III to state IV rates of oxygen uptake measured as described above. The P/O ratios (△) were calculated from the total oxygen uptake from the time of the combined addition of ADP and $^{32}Pi$ until the reaction was stopped in perchloric acid.

Thus at a critical concentration of $Ca^{++}$ a marked response is triggered in which the ADP-ATP exchange activity and the P/O ratio are almost totally abolished and the ATPase activity greatly stimulated, providing further evidence for the involvement of the ADP-ATP exchange reaction in oxidative phosphorylation.

## 4. Discussion

Several attempts have been made to isolate an exchange *enzyme* representative of the oligomycin-sensitive ADP-ATP exchange ***reaction*** of intact mitochondria [10-12]. However, despite intensive study there is little evidence to indicate that these isolated enzymes are intimately involved in the process of oxidative phosphorylation (see [13]).

It is evident from the data contained in figs. 1 and 3 that $Ca^{++}$ induces very gross changes in a variety of parameters closely associated with the overall mechanism of oxidative phosphorylation. Each of these changes occur at a similar concentration of $Ca^{++}$. The concomitant diminution of the ADP-ATP exchange activity with increase in ATPase activity (fig. 1) is significant since as mentioned above both of these activities are considered to reflect the terminal reactions of oxidative phosphorylation [8, 9]. This finding, together with (a) our observations that maximal rates of the oligomycin-sensitive ADP-ATP exchange activity are observed only in intact mitochondria possessing a high degree of functional integrity [1], i.e. minimal ATPase activity, and (b) with reports that purified preparations of ATPase as well as various submitochondrial particles (which can carry out a $^{32}Pi$-ATP exchange and phosphorylate ADP) do not possess ADP-ATP exchange activity [9, 14, 15], implies that the ATPase and ADP-ATP exchange are manifestations of the one and same activity, *viz* the terminal reaction(s) of oxidative phosphorylation. In intact mitochondria, when ATPase is 'latent' or 'masked', ADP-ATP exchange activity is maximal. Once mitochondrial integrity is lost, the ATPase becomes 'unmasked' and the exchange activity diminishes. Whether this transition is due to a loss in affinity of ATPase for ADP [1,15,16] or increased membrane permeability to $H^+$ [17] is not known.

These considerations further imply that the exchange is not an activity accountable for by an *enzyme per se,* but rather is an *activity* which is the resultant of an ordered system in the membranes of intact mitochondria and is functionally associated with a masked ATPase. This proposition would account for the experimental observations described above. Since this work was completed the suggestion was made [18] that the ADP-ATP exchange system is most likely a highly ordered membrane complex, containing, among other things, the mitochondrial ATPase.

The sigmoidal nature of the plots obtained in figs. 1 and 3 is the subject of a following communication.

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