THE REVERSIBLE ACTIVATION OF MITOCHONDRIAL ATPase by HIGH pH

A. Fonyo*, L. Szende and Ilona Mezei

Experimental Research Department, University Medical School, Budapest, Hungary.

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Two general theories of oxidative phosphorylation exist presently. The high-energy intermediate theory, formulated some 15 years ago (for review: Lehninger, 1964) and the recent "chemi-osmotic-coupling" hypothesis (Mitchell, 1961; Mitchell and Moyle, 1965). The two theories differ in the postulated role of ATPase. In the high-energy intermediate theory ATPase is supposed to function in one of the transfer reactions and is hydrolytic only under artificial conditions (as in the presence of uncouplers; (Penefsky et al. 1960). In the chemi-osmotic-coupling hypothesis, the "vectorial" ATPase has a postulated central role. Its function, driven by proton translocation through the mitochondrial membrane, would result in the synthesis of ATP. For both theories it is of importance to know, what conditions favor the ATP-hydrolysis and what may be the mechanism for the activation of the hydrolysis.

Activation of mitochondrial ATPase by increased pH was first reported by Swanson (1956) and later confirmed by Myers and Slater (1957). In the present paper, the similarity of this OH^- ion activation to the activation caused by the uncoupler 2,4-dinitrophenol (DNP) has been studied.

Experimental Procedure.

The experiments were performed on 2 types of preparations: intact rat heart mitochondria isolated in 0,25 M sucrose, and rat heart mitochondrial

* Present address: Pediatric Research Department, University of Maryland, Baltimore, Maryland, U.S.A.

fragments. The latter was a modification of the Keilin and Hartree heart muscle preparation as adapted for rat heart and performed in the cold: the heart was washed with distilled water and subsequently with 0,02 M phosphate buffer, homogenized in 0,02 M phosphate buffer pH 7,2 in an all glass homogenizer, centrifuged at 600 g for 20 min.; the supernatant was centrifuged at 8500 g for 15 min., the sediment washed in 0,25 M sucrose, recentrifuged at 20,000 g for 15 min. and finally suspended in 0,25 M sucrose. This preparation showed only a slight capacity to oxidatively phosphorylate. With succinate the P:O ratio was about 0,1. Nevertheless, its ATPase could be activated by DNP, although the specific activity of this DNP induced ATPase was about one third that of intact heart mitochondria. Both types of preparations yielded similar results.

ATPase activity was determined at 30° in a medium that contained 50 mM tris-Cl or tris-acetate buffer, 5 mM ATP, in the case of intact mitochondria 0,15 mg mitochondrial protein and in the case of fragments, 0,20 mg protein. The concentration of DNP employed was 0,2 mM. The incubation time was 10 min. and ATPase activity was calculated on the basis of inorganic phosphate liberated and expressed as µmole p/mg protein/10 min.

Results.

The ATPase of both intact mitochondria and fragments was activated by raising the pH; this activation took place in the absence of Mg^{2+} ions. In Fig. 1 the buffer used was tris-Cl. No measurement could be performed above pH 9.5 with tris, but the activation was similar with 2-amino-2-methyl-1-propanol-Cl and Ammonia-Cl buffers. The activity decreased above pH 9.5. The activation also occurred in the absence of added buffer when the pH of the reaction mixture was adjusted to pH 9.0 - 9.5 by the addition of NaOH.

This "high pH activated ATPase" was in many respects quite similar to the ATPase activated at pH 7.4 by DNP. They were similarly inhibited by high concentrations of sucrose (Fig.2), oligomycin (Fig.3) and by aging

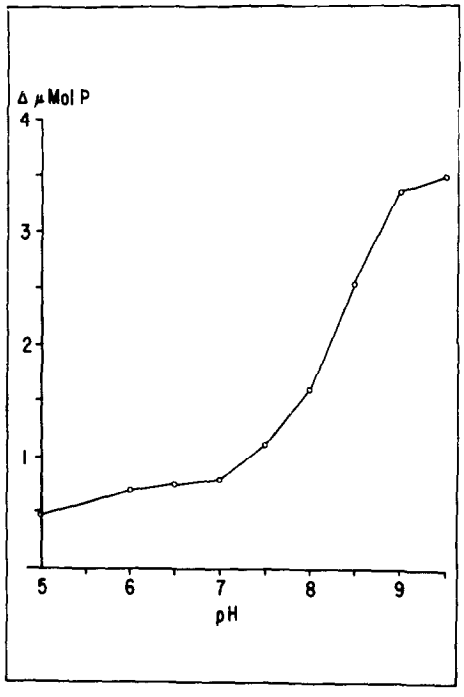

Fig.1. Effect of pH on ATPase activity of heart muscle mitochondrial fragments.

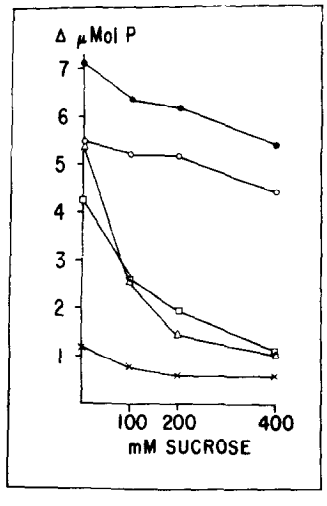

Fig. 2. Effect of sucrose on ATPase of heart muscle mitochondrial fragments. x———x pH 7,4 ; |¯|———|¯| pH 9,5 ; Δ———Δ pH 7,4, 0,2 mM DNP; o———o pH 7,4, 5 mM Mg^{2+};• ———• pH 9,5, 5 mM Mg^{2+}.

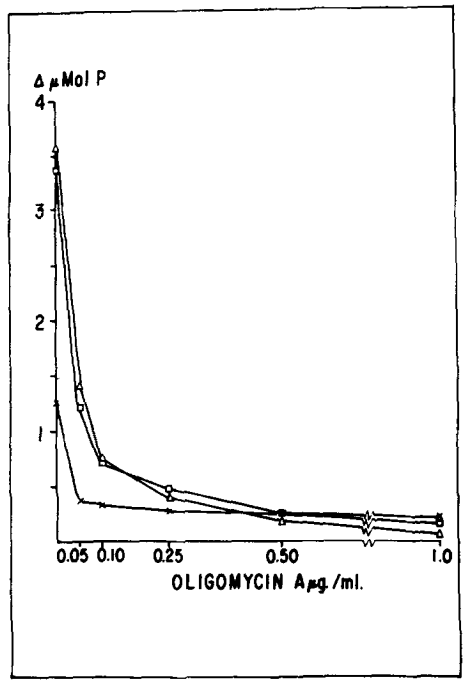

Fig. 3. Effect of oligomycin A on ATPase of heart muscle mitochondrial fragments.
x———x pH 7,4 ; |‾|——|‾| pH 9,5 ;
o———o pH 7,4, 5 mM Mg^{2+}; •———• pH 9,5, 5 mM Mg^{2+}

at 37° (Fig.4). The non-ionic detergent TRITON-X-100 and azide also inhibit. This is in sharp contrast with the behavior of the Mg^{2+} activated ATPase, which is inhibited very slightly if at all by sucrose, TRITON-X-100 or by aging. These data suggest that the high pH activated ATPase is different from the Mg^{2+} activated ATPase and may be closely related to or identical with the enzyme or system which responds to DNP.

The activating effect of high pH is to a large degree reversible (Fig.5). Part A is a control, shows the activity in the presence of 0.2 mM DNP at pH 9,5 and the effect of neutralization of a sample to pH 7,4 at 2,5 min. - no difference exists between the activities. In part B of this figure the lowest line represents the ATPase at pH 7,4, the upper line at pH 9,5. At

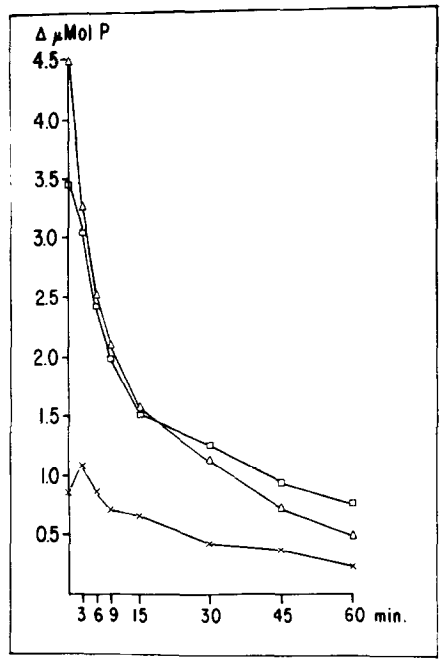

Fig. 4. Effect of aging on high pH activated and DNP activated ATPase heart muscle mitochondrial fragments preincubated in 0.25 M sucrose pH 7,4. ATPase measured at: x——x pH 7,4; |¯|——|¯| pH 9,5, Δ——Δ pH 7,4 0,2 mM DNP

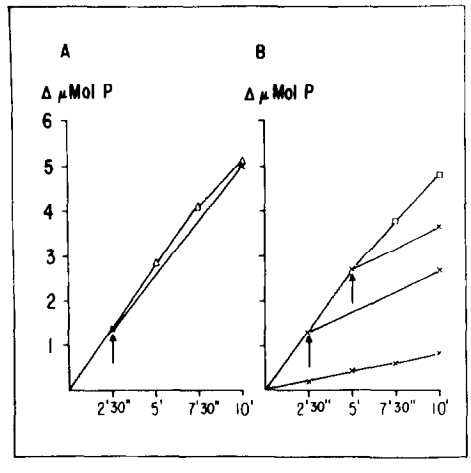

Fig. 5. Reversibility of high pH activated ATPase heart muscle mitochondrial fragments.
A: Δ——Δ pH 9,5, 0,2 mM DNP
At the arrow HCl was added to bring the pH to 7,4
B: x——x pH 7,4; |¯|——|¯| pH 9,5;
At the arrow HCl was added to bring the pH to 7,4

the arrows a titrated amount of HCl was added in a very small volume to the reaction medium at pH 9,5 to bring the pH to 7,4. After neutralization the rate of ATP-splitting decreased significantly. The fact that the rate after neutralization does not precisely parallel the control rate, suggests that the reversibility of the activation is about 60 per cent complete.

The apparent K_m values for ATP differed at 7,4 (in the presence of DNP) and at 9,5: at pH 7,4 the apparent K_m was of the order of 10^{-4} M and at pH 9,5 it was 6,4. 10^{-3} M. This suggests that the more highly ionized ATP molecule may be less tightly bound to the enzyme at the higher pH.

Discussion.

Those enzymes which play a role in ATP synthesis can catalyze hydrolysis under certain artificial conditions such as in the presence of the usual uncouplers of oxidative phosphorylation or by high pH. It remains to be demonstrated whether the high pH itself acts as a reversible uncoupler.

There are several different theoretical possibilities which might explain the activation of ATP hydrolysis at high pH. The degree of ionization of substrates and products at pH 7,4 and pH 9,5 may markedly influence the catalytic activity of the enzyme. ATP which is more highly ionized at pH 9,5 may be more susceptible to enzymic hydrolysis than ATP at pH 7,4. These possibilities are weakened but not ruled out by the K_m data which indicates that the less ionized species is the preferred substrate for hydrolysis. Another possibility is that the structure of the enzyme is altered reversibly at high pH. The ionization of a dissociable group might create an active center causing a change in the catalytic mechanism and favoring reaction with water. It has been suggested that some uncouplers may operate by a similar group-ionizing mechanism (Bovell et al. 1964). An alternative view, the chemi-osmotic theory, also gives a possible explanation. According to its newest formulation (Mitchell and Moyle, 1965; Jagendorf, pers. comm.) protons are translocated extramitochondrially during electron transport. The proton gradient from

outside to inside thus created, coupled with a reversible, spatially-oriented, membrane located ATPase is the cause of ATP synthesis. One might expect, that diminishing or reversing the proton gradient by increasing the external pH might direct ATPase in the direction of hydrolysis. This work is consistent with such a mechanism, but does not rule out a possibility of alterations in enzyme structure.

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