THE INSENSITIVITY OF MITOCHONDRIAL-CATALYZED ARSENATE-WATER OXYGEN EXCHANGE REACTION TO DINITROPHENOL AND TO OLIGOMYCIN

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Summary.

A comparison was made of the sensitivities of the mitochondrial-catalyzed arsenate-water oxygen exchange ($As_i \rightleftharpoons H_2O$) and the phosphate-water oxygen exchange ($P_i \rightleftharpoons H_2O$) to dinitrophenol and to oligomycin. The observed inhibition of the $P_i \rightleftharpoons H_2O$ exchange by oligomycin and by dinitrophenol was in accord with previously reported findings. The mitochondrial-catalyzed $As_i \rightleftharpoons H_2O$ exchange was not inhibited by either compound. These results suggest that the prominent $As_i \rightleftharpoons H_2O$ exchange is not directly related to the effect of As_i on oxidative phosphorylation. These findings provide no support for the idea that arsenate serves as an alternative substrate in place of phosphate in a partial reaction of oxidative phosphorylation.

ATP supports a mitochondrial-catalyzed $P_i \rightleftharpoons H_2O$ exchange by a process unrelated to electron transfer reactions (1). Although the detailed relationship between mitochondrial $P_i \rightleftharpoons H_2O$ exchange and oxidative phosphorylation and its reversal is not clearly understood, the sensitivity of this exchange to DNP (2,3) and to oligomycin (4) suggests that this exchange represents a partial reaction of oxidative phosphorylation. Inhibition of $P_i \rightleftharpoons H_2O$ exchange by As_i has been reported by Chan et. al. (5). Further evidence for similarity between the behavior of As_i and P_i in mitochondrial systems has been presented by Itada and Cohn (6). These authors found that mitochondria catalyze a pronounced $As_i \rightleftharpoons H_2O$ exchange. More recently Chan et. al. (7) have used radioactive As_i and have provided interesting evidence that As_i may be activated by mitochondrial processes similar to those involved in P_i activation.

The purpose of this paper is to report significant differences in the responses of the $As_i \rightleftharpoons H_2O$ and the $P_i \rightleftharpoons H_2O$ exchange reactions to oligomycin and to DNP.

Methods

Rat liver mitochondria were isolated in 0.25 M sucrose containing 0.1 mM EDTA, pH 7.5 and were washed and resuspended in 0.25 M sucrose. The $P_i \rightleftharpoons H_2O$ oxygen exchanges were started by the addition of mitochondria at zero time and reactions were stopped at 5 minutes by addition of $HClO_4$ to a final concentration of 0.3 N. The extent of ^{18}O incorporation into inorganic phosphate from water (0.84 atom% excess of ^{18}O) was determined as described by Boyer and Bryan (8). Highly enriched $KH_2As^{18}O_4$ was prepared by equilibration of the unlabelled salt with $H_2{}^{18}O$. Nonenzymatic exchange was minimized by dissolving $KH_2As^{18}O_4$ (approximately 60 atom% excess ^{18}O) in cold KOH before use. Sufficient H_2SO_4 was added to the incubation mixture to bring the final pH to 7.5 upon arsenate addition. Arsenate was added 30 seconds before the mitochondria. The $As_i \rightleftharpoons H_2O$ oxygen exchange was measured by removal of 0.1 ml aliquots 30 seconds and 5 minutes 30 seconds after the addition of mitochondria. Further exchange was prevented by rapidly freezing the samples in liquid nitrogen. The water was subsequently distilled off at -15°C. under reduced pressure. The amount of ^{18}O incorporated into H_2O from arsenate was determined as previously described (8).

Results and Discussion

Table 1 shows an experiment in which ATP was used as the energy source to drive a $P_i \rightleftharpoons H_2O$ exchange reaction. Respiration was blocked with 1 mM KCN. The $P_i \rightleftharpoons H_2O$ exchange was markedly inhibited by oligomycin, in agreement with the findings of Lardy, et. al. (4). The exchange was considerably reduced but not abolished in the presence of 1mM DNP. Maintenance of a low level $P_i \rightleftharpoons H_2O$ exchange in the presence of levels of DNP sufficient to

Table 1: The Failure of Oligomycin and DNP to Inhibit the $As_i \rightleftharpoons H_2O$ Oxygen Exchange in Contrast to Their Inhibitory Effect on the $P_i \rightleftharpoons H_2O$ Oxygen Exchange Catalyzed by Intact Rat Liver Mitochondria.

Reaction mixture	μatoms O exchanged/min./mg. protein
$As_i - {}^{18}O \rightleftharpoons H_2O$	
1. Complete	10.5
2. Complete + oligo. (2.5 μg/ml)	10.5
3. Complete + DNP (1 mM)	9.4
4. Mitochondria omitted	0.0
$P_i \rightleftharpoons H_2{}^{18}O$	
1. Complete	1.83
2. Complete +oligo. (1 μg/ml)	0.195
3. Complete + DNP (1 mM)	0.69

Mitochondria were incubated in a medium containing 0.25 M sucrose, 10 mM Tris sulfate, 1 mM KCN, 5 mM $MgSO_4$, 4 mM ATP, at pH 7.5, 38°. Protein concentration was 0.84 mg/ml. $As_i \rightleftharpoons H_2O$ exchange incubation mixtures contained in addition 20 mM As_i (final volume 1 ml). P_i, where added, was 10 mM, in final volume of 2.5 ml. Values for $As_i \rightleftharpoons H_2O$ exchange are calculated on the exchange which occurred between 30 seconds and 5 minutes 30 seconds following addition of mitochondria.

uncouple oxidative phosphorylation is in agreement with previous reports (9).

The mitochondrial-catalyzed $As_i \rightleftharpoons H_2O$ exchange, unlike the corresponding $P_i \rightleftharpoons H_2O$ exchange reaction, is quite insensitive to either DNP or to oligomycin as shown in Table 1. The observation that mitochondria catalyze an $As_i \rightleftharpoons H_2O$ exchange 50 to 100 times faster than the rate of ATP hydrolysis (6) has prompted Ernster, et. al. (10) to attempt to reconcile these findings with those of the effect of As_i on respiration and on ATPase. However,

the insensitivity of $As_i \rightleftharpoons H_2O$ exchange to an inhibitor (oligomycin) and to an uncoupler (DNP) of oxidative phosphorylation suggests that this prominent exchange is not directly related to the effect of As_i on oxidative phosphorylation. The inability of oligomycin, an inhibitor of As_i-induced respiration (11), to inhibit the $As_i \rightleftharpoons H_2O$ exchange reaction is of special interest.

No detectable nonenzymatic exchange occurred during the incubation period in the absence of mitochondria, in agreement with the stability of ^{18}O-labelled arsenate in $H_2{}^{16}O$ at pH 7.5 (12).

Results in Table 1 do not exclude the possibility that As_i activation may proceed with formation of a stable intermediate which undergoes exchange at a rate comparable to a corresponding phosphorus compound. A small $As_i \rightleftharpoons H_2O$ exchange proceeding at 1/10 the rate of the overall observed $As_i \rightleftharpoons H_2O$ exchange would not have been detected in these experiments.

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