THE UBIQUITY OF AN ENZYME-LIKE ACTIVITY FOR PROMOTING AN ARSENATE-WATER OXYGEN EXCHANGE REACTION IN RAT LIVER CELL FRACTIONS

Robert A. Mitchell

Department of Biochemistry, Wayne State University, Detroit, Michigan 48201

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Summary

The capacity of different rat liver cell fractions to catalyze an arsenate-water oxygen exchange reaction was tested. High rates of exchange (2 to 3 μgatoms per min per mg of protein, at 25°, pH 7.5) were found for all fractions (nuclear, mitochondrial, microsomal, and soluble). This activity was lost on boiling the fractions, and was absent from bovine serum albumin, thereby pointing to a ubiquitous enzyme activity as the cause of exchange. These results further point to a need for caution in invoking mitochondrial As_i=H_2O exchange data to support proposed mechanisms for oxidative phosphorylation.

Rat liver mitochondria are known to catalyze an exchange of oxygen between inorganic arsenate and water (As_i=H_2O exchange[1]) in a reaction which is rapid in comparison with the apparent As_i-stimulated ATPase of mitochondria (Itada and Cohn, 1). These workers logically assumed that the exchange was analogous to the mitochondrial P_i=H_2O exchange and that it was therefore in some manner indicative of an As_i activation step. DeMaster and Mitchell (2) and Mitchell et al. (3) have pointed out that attempts by subsequent workers e.g. Ernster et al. (4) and Cross and Wang (5) to use the As_i=H_2O exchange to establish details of a scheme for oxidative phosphorylation may be invalid, since this exchange does

[1]Abbreviations: As_i, inorganic arsenate; P_i, inorganic phosphate

not possess properties similar to the mitochondrial P_i=H_2O exchange, and indeed lends little support for an uncoupling mode of action by As_i based on arsenolysis of a hypothetical As-containing intermediate. Recently, two articles have appeared where the mitochondrial catalyzed As_i=H_2O exchange has been incorporated into yet further novel proposals for a mechanism for oxidative phosphorylation (Green, 6; Young et al., 7). It is the purpose of this paper to show that the activity for catalysis of As_i=H_2O exchange is quite widely-distributed in the cell, thereby reemphasizing earlier work from this laboratory suggesting the need for caution in interpreting this exchange in terms of specific mechanisms for oxidative phosphorylation.

METHODS

Liver obtained from a fasted 200g female rat (fasted overnight and sacrificed by decapitation) was homogenized in 0.25 M sucrose containing 1 mM EDTA, pH 7.5) and fractions designated nuclear, mitochondrial, and microsomal were obtained by sedimentation at 750 x g (12 min), 10,000 x g (12 min) and 78,000 x g (60 min). The mitochondrial and nuclear fractions were resuspended and recentrifuged twice. The 78,000 x g cell supernate was designated "soluble". The fractions were stored in sucrose-EDTA at -15° and aliquots were thawed just prior to use. As_i=H_2O exchange was measured as described previously (3) using a CEC model 21-401 mass spectrometer to measure isotope ratios of unknown CO_2 samples and of standards prepared from H_2O of known ^{18}O enrichment. Protein was determined by the Lowry method using bovine serum albumin as the standard.

TABLE 1. Distribution of As_i=H_2O exchange activity in various cell fractions.

Fraction	Exchange rate
Mitochondrial	2.59
Soluble	2.18
Microsomal	2.85
Nuclear	1.80

Incubations were carried out at 25°, pH 7.0 in buffer containing 0.2 M sucrose, 40 mM Tris sulfate, 4 mM $MgSO_4$, 28 mM K_2SO_4, 33 mM potassium arsenate, and from 0.6 to 1.2 mg of protein. Final volume was 0.45 ml and rates of exchange were calculated from the ^{18}O content of water samples taken immediately after the start of incubation, 10 min after the initial sample, and 24 hours after the start of incubation (equilibrium value). Rates of exchange are expressed in µgatoms of oxygen exchanged per min per mg of protein.

RESULTS

Table 1 shows that all fractions tested possessed the ability to catalyze an As_i=H_2O exchange. The rate of exchange of the mitochondrial fraction is somewhat lower than that reported by DeMaster and Mitchell for intact freshly-prepared rat liver mitochondria (10.5 µgatoms oxygen exchanged per min per mg of protein) but this decrease could reflect some loss of activity as a result of the freeze-thaw process used in the later experiment, or differences in incubation conditions. It is of interest that the soluble fraction also possessed appreciable capacity for exchange.

This observation would appear to rule out the possibility that the exchange is merely some type of surface-catalyzed phenomenon dependent on a particulate system. In another experiment to test the possibility of a glass-surface catalyzed exchange, labelled As_i was incubated with glass beads (250 mg per ml) of 5, 25, and 500 micron diameter, in the presence of a small amount of Triton X-100 as a wetting agent. After 5 min of incubation, the extent of exchange was the same for all three samples and did not differ significantly from a control with the beads omitted, (about 0.2 µgatoms per min).

The results of experiments to determine the heat sensitivity of the exchange reaction catalyst are shown in Table 2. Boiling the fraction for 1 min before addition of As_i abolished exchange capacity. Thus, after correcting for the background exchange, there was no detectable exchange capacity after boiling the soluble or the mitochondrial fraction. On the other hand, the unboiled fractions catalyzed exchanges of 2.2 and 1.30 µgatoms per min per mg of protein for the soluble and mitochondrial fraction respectively. These results are strongly suggestive of an enzymic activity as the basis for the exchange. To test if the exchange might result from a nonspecific protein catalysis, the capacity for exchange by bovine serum albumin was tested. As shown in Table 2, no capacity for $As=H_2O$ exchange by albumin was detected.

DISCUSSION

Although various authors have cited the observations of Itada and Cohn (1) on the presence of a mitochondrial-catalyzed $As_i=H_2O$ exchange to support the concept of a covalent activation of As_i to form a high-energy compound, presumably readily susceptible to

TABLE 2. Heat inactivation of the capacity for As_i=H_2O oxygen exchange in various rat liver cell fractions.

Expt.	Fraction	Exchange rate
1.	Mitochondrial	1.92
	Mitochondrial (boiled)	0.116
	Control (no protein)	0.308
2.	Soluble	2.13
	Soluble (boiled)	0.39
	Bovine serum albumin	0.267
	Control (no protein)	0.51

Incubation conditions were: 0.25M sucrose, 50 mM Tris sulfate, 50 mM $MgSO_4$, 20 mM K_2SO_4, 30 mM potassium arsenate, final volume 0.5 ml, pH 7.5, at 25°. The rates are expressed in µgatoms per min. Experiment #1 contained 1.24 mg of mitochondrial protein and #2 contained 0.73 mg of soluble protein or 1 mg of bovine serum albumin.

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hydrolysis, it is frequently overlooked that in the mitochondrial system experiments with ^{18}O labelled As_i have not provided the same clear-cut definitive results that have been obtained by Slocum and Varner (8) or Itada and Cohn (1) in the soluble enzyme systems which they studied. Thus, the recent suggestion by Green (6) for a common origin of the As_i=H_2O and the P_i-H_2O exchanges takes no account of the considerable differences in the properties of the two types of exchange (DeMaster and Mitchell, 2; Mitchell et al., 3). On the other hand, Young et al. (17) have recognized the difficulty of reconciling a dinitrophenol-resistant, nonenergy-requiring

mitochondrial As_i=H_2O exchange with the oxidative phosphorylation scheme by suggesting that the longer length of the As-O bond (as compared to the P-O bond in P_i) may permit exchange to occur at an open configuration of a catalytic site, rather than at the energized closed site postulated for the P_i=H_2O exchange. This would suggest that energizing the mitochondrial membrane should if anything, inhibit As_i=H_2O exchange. However, no evidence for this type of inhibition was reported by Mitchell et al. (3). The rather widespread distribution of an apparent enzymic capacity for As_i=H_2O exchange in rat liver cell fractions thus further serves to emphasize the need for caution in invoking this reaction in schemes for oxidative phosphorylation.

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