A MANGANESE STIMULATED, NUCLEOTIDE DEPENDENT ^{18}O-INORGANIC PHOSPHATE EXCHANGE REACTION CATALYZED BY HEAVY MEROMYOSIN[1]

J. Robert Swanson[2] and Ralph G. Yount

Departments of Agricultural Chemistry and Chemistry
Washington State University, Pullman, Washington

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Myosin is now known to catalyze two apparently different types of ^{18}O-phosphate exchange reactions (see discussions by Koshland and Levy, 1964, and Boyer, 1964). The first of these exchange reactions (Levy and Koshland, 1959) has been shown by these workers to occur during the hydrolysis of ATP at some intermediate level between tightly bound ATP and products and for this reason is called intermediate exchange. It is detected by measuring the incorporation of ^{18}O from $H_2{}^{18}O$ into the phosphate cleaved from ATP. The significance of this reaction lies in the fact that it is presently the only method which has detected any intermediate(s) during myosin catalyzed hydrolysis of nucleoside triphosphates.

The second type of exchange reaction catalyzed by myosin was discovered by Boyer and co-workers (Dempsey and Boyer, 1961; Dempsey *et al.*, 1963) who showed that myosin in the presence of ATP would catalyze an exchange between $Pi^{18}O$ and $H_2{}^{16}O$. This type of exchange, "medium" exchange, has been found by Boyer and co-workers to predominate in their reactions while Levy *et al.* (1962) have shown that under their reaction conditions medium exchange is small when compared to

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intermediate exchange. While Dempsey and Boyer (1961) originally claimed that all the exchange observed could be explained by medium exchange they now also find a preferential exchange with the phosphate originating from ATP (Boyer, 1964).

Of importance to this problem is the observation that heavy meromyosin, the enzymically active tryptic fragment from myosin, has been shown, at least under one set of conditions (Mg^{++}, pH 7.4), to give essentially complete intermediate exchange and no medium exchange (Yount and Koshland, 1963). This finding has led to the suggestion (Kielley, 1964) that perhaps medium exchange is a property of a contaminant in myosin and actomyosin preparations.

To answer this possibility and others we have begun a systematic investigation of the properties of medium exchange and the relationship of these properties to those of intermediate exchange previously investigated (Levy et al., 1960; Yount and Koshland, 1963). In this paper we wish to report that heavy meromyosin does catalyze medium exchange but only in the presence of certain divalent metal ions and only in the presence of ADP or ATP.

Table I shows the results of two sets of experiments designed to detect medium exchange at different divalent metal ion concentrations. As can be seen on line 2, Mn^{++} is by far the most effective metal ion co-factor for this exchange reaction with essentially complete exchange being observed in 20 hours at the higher metal ion and enzyme concentrations. Co^{++} and Ca^{++} (lines 4,8) also function as co-factors but less efficiently. The other metal ions tested, Mg^{++}, Ni^{++}, Sr^{++}, Zn^{++}, (lines 1,5,6,7) were all much less effective and were, in general, within experimental error of zero ($\sim$ 0.20 atoms exchanged or less). Little or no exchange was observed when the divalent metal ions were omitted (lines 11, 12).

Of particular interest is the observation that either ADP or ATP

TABLE I.) Metal Ion Dependence of HMM[1] Catalyzed "Medium" Exchange

Conditions: 0.1M Tris·Cl (pH 7.4), 0.1M KCl, 5mM $KH_2P^{18}O_4$ (1.38 atom % ^{18}O), 0.33 mg HMM/ml, 20 Hr. Phosphate was isolated as KH_2PO_4 (Dempsey et al., 1963), converted to CO_2 by the guanidine·HCl method (Boyer et al., 1961) and analyzed on a Nuclides Analysis Associates Mass Spectrometer, Model 6-60-100 GU2[2]. HMM prepared as described previously (Yount and Koshland, 1963).

	Divalent Metal Ion	Nucleotide Added	0.5 mM Divalent Metal Ion Added: Atoms ^{18}O exchanged per Pi Molecule[d]	0.5 mM Divalent Metal Ion Added: % ATP Cleaved	5 mM Divalent[a] Metal Ion Added: Atoms ^{18}O exchanged per Pi Molecule[d]	5 mM Divalent[a] Metal Ion Added: % ATP Cleaved
		-5mM-				
1.)	Mg^{++}	ATP	0.19	74	0.36	99
2.)	Mn^{++}	ATP	2.14	98	3.89	92
3.)	Mn^{++}	none	0.01[b]	--	0.00[b]	--
4.)	Co^{++}	ATP	0.36	97	1.34	99
5.)	Ni^{++}	ATP	0.14	90	0.00	92
6.)	Sr^{++}	ATP	0.23	86	0.21	85
7.)	Zn^{++}	ATP	0.29	85	0.06[c]	26
8.)	Ca^{++}	ATP	0.35	88	0.61	92
9.)	Ca^{++}	ADP	---	--	0.95	--
10.)	Ca^{++}	none	---	--	-0.05[b]	--
11.)	none	ATP	0.30	87	0.25	81
12.)	none	none	0.14	--	---	--

[1] HMM = Heavy Meromyosin.

[2] We are indebted to Prof. J. P. Hunt for his helpful advice and for the use of this instrument.

[a] 0.75 mg HMM/ml.

[b] A metal ion phosphate precipitate was visible from zero time. Approx. 30% of the Pi and divalent metal stay in solution at 5 mM Ca^{++} and Mn^{++} under the conditions used here.

[c] A Zn^{++} HMM precipitate forms.

[d] Calculated by the method of Levy et al. (1962).

have to be present for exchange to occur (lines 3, 10). It has previously been shown with myosin (Dempsey et al., 1963) that ATP was required for detectable medium exchange to occur in the presence of Mg^{++}. The possibility that the true co-factor is ADP rather than ATP is difficult to check since most myosin preparations are contaminated with myokinase, a situation leading to ATP production. The finding here that Ca^{++} is a co-factor of myosin for medium exchange while it is not for myokinase (Noda, 1958) allowed us to test this possibility. The experiment shown on line 9 demonstrates that ADP[1] is actually a more effective co-factor than ATP[2]. This finding makes the possibility of medium exchange occuring at the active site more plausible since it is difficult to see how both ATP and Pi can be binding to the same site simultaneously.

Finally it should be emphasized that while heavy meromyosin does catalyze medium exchange over long time periods, other experiments (Swanson and Yount, unpublished) have shown that over short time periods (1-2 hours) with Mn^{+2} or Mg^{+2} as co-factors, medium exchange is undetectable while intermediate exchange shows 3 to 4 atoms of ^{18}O incorporated per Pi cleaved from ATP. Thus these results plus those reported here confirm the earlier findings made with heavy meromyosin (Yount and Koshland, 1963) and myosin (Levy *et al.*, 1962) but also lend considerable support to the proposal that medium exchange is a true catalytic property of myosin[3] and myosin systems.

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[1] No conversion of ADP to ATP was detected either by paper chromatography or by Pi analysis.

[2] H. M. Levy and D. E. Koshland, Jr., have also found that ADP will serve as well or better than ATP for Mg-myosin catalyzed medium exchange (personal communication).

[3] Experiments with myosin (Swanson and Yount, unpublished) have shown a divalent metal dependence for medium exchange essentially identical to that given here for heavy meromyosin.

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