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SOME PROPERTIES OF THE ADP–ATP EXCHANGE REACTION IN TURTLE BLADDER MICROSOMES

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SUMMARY

The catalyzed exchange reaction, [^{14}C]ADP + ATP $\rightleftharpoons$ [^{14}C]ATP + ADP, found in the microsomal pellet from isolated mucosal epithelial cells of the bladder of fresh water turtles has an absolute requirement for Mg^{2+} in the presence or absence of Na^+ and/or of $Na^+ + K^+$. The rate of exchange is reduced by Na^+ and is increased by ouabain over a wide range of Mg^{2+} levels. The Na^+-induced decrement of exchange is elicited in the presence and in the absence of ouabain. In fact, Na^+ can entirely abolish the exchange reaction in the native microsomal proteins. The rate of exchange in the presence or absence of Na^+ is independent of the pH over a wide pH range. Whereas oligomycin inhibits hydrolysis without affecting the exchange rate, *N*-ethylmaleimide inhibits both processes. The nucleotide preference of the exchange rate with Mg^{2+} alone was ATP > GTP > ITP > UTP > CTP; and with $Mg^{2+} + Na^+$ was ATP > CTP > ITP > UTP > GTP.

INTRODUCTION

The microsomal pellet extracted from the isolated mucosal epithelial cells of the turtle bladder possesses an $Na^+ + K^+$-stimulated ouabain-inhibited ATPase activity. This activity has an absolute dependence on the presence of Mg^{2+}; and the activity in the presence of $Mg^{2+} + Na^+ + K^+$ is *ca.* twice that in the presence of Mg^{2+} alone[1,2]. Subsequent findings on microsomal binding showed that the addition of Na^+ caused an increase in ^{32}P-binding to microsomes incubated with [γ-^{32}P]ATP, and a decrease in ^{14}C-binding to microsomes incubated with [^{14}C]ATP (ref. 2).

These observations led to the present experiments on the catalyzed exchange reaction between ADP and ATP. Present experiments involved a chromatographic measurement of the rate of conversion of [^{14}C]ADP to [^{14}C]ATP in the presence of the microsomal proteins via the reaction,

$$[^{14}C]ADP + ATP \underset{}{\overset{Mg^{2+}}{\rightleftharpoons}} [^{14}C]ATP + ADP,$$

where the γ-phosphate of ATP is transferred to [^{14}C]ADP in a transphosphorylation step. The major findings in the present report show that the rate of exchange in the

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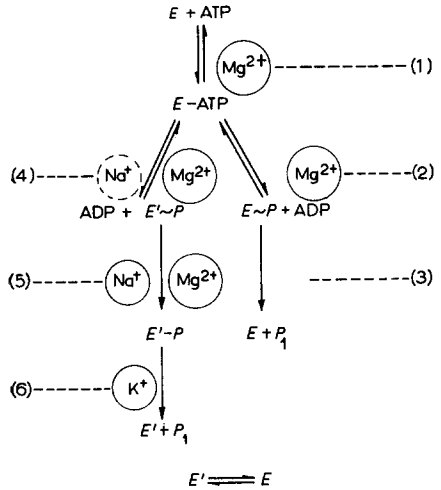
above reaction is dependent upon Mg^{2+} and is inhibited by Na^+. The present findings together with those previously reported on microsomal binding[2] and hydrolysis[1,2], could be fitted into a single reaction system integrating all three processes, exchange, intermediate complex formation, and hydrolysis.



In Diagram 1, E denotes the enzyme. The Mg^{2+}-dependent hydrolysis is assumed to proceed through Reactions 1–3 inclusive, and the $Na^+ + K^+$-dependent hydrolysis is assumed to proceed through Reactions 1, 4, 5 and 6. E–ATP is assumed to be the common precursor for both paths. E' denotes a sodium modified enzyme of the same protein. In turtle bladder microsomes, we postulated that the major site of action for Na^+ is Reaction 5, whereas the minor site of action for Na^+ is Reaction 4. To explain data from microsomes of certain other tissues, it was necessary to infer that Na^+ acts on Reaction 4. A detailed discussion of the results will be presented later.

The purpose of the present work, implicit to the reaction model, was to determine whether the exchange reaction between ADP and ATP was an integral part of the system involving intermediate complex formation and hydrolysis.

METHODS

Materials

Microsomes of turtle bladders were prepared as described previously[1]. The preparation was stored at −30° where it retained its activity for several weeks. [8-^{14}C]ADP was obtained from International Chemical and Nuclear Corp., Calif. as the ammonium salt. ATP, GTP, UTP, CTP, ITP and ADP as Tris salts, ouabain, *N*-ethylmaleimide and oligomycin were obtained from Sigma.

Transphosphorylation assay (exchange rate)

The standard incubation mixture, in terms of the final concentrations, consisted of the following: 5 mM Tris–ATP, 0–3 mM $MgCl_2$, 0–125 mM NaCl, 1.6 mM [^{14}C]ADP (specific activity $1 \cdot 10^6$ counts/min per μmole), 40 mM Tris–HCl (pH 7.3) as buffer, 0.1 mM EDTA–Tris, and about 1–5 μg of microsomal protein in a total volume of 30 μl.

The exchange assay can be quantitated only when ATP and ADP remain reasonably constant[3]. This was achieved by omitting K^+, and checked experimentally by determining the percentage of the Mg^{2+}-dependent hydrolysis. Results showed that 12 % or less of ATP was hydrolyzed under the conditions used in the routine assay.

The samples, in tubes (5 mm × 50 mm), were incubated at 38° for 10 min. The reaction was stopped by adding 5 μl 10 % (w/v) formic acid in methanol. Preliminary tests showed that this treatment gave less Mg^{2+}-dependent hydrolysis than did the treatment of placing the rack of tubes in a boiling water bath for 2 min prior to placing it in an ice bath[4]. $HClO_4$ was not used to terminate the reaction because of its interference with the thin-layer chromatography. Each sample, along with similar volumes of standard known nucleotides, 3–5 μl, was applied to the chromatographic plates. After separation on the plates, counting was performed according to the procedure used by FAHN *et al.*[4]. Protein was determined according to the method of LOWRY *et al.*[5]. Under the aforementioned assay conditions for measuring the exchange rate, no [^{14}C]AMP formation was detected, thus eliminating any contribution of adenylate kinase activity to the exchange rate. The exchange rate was calculated from the specific activity of the [^{14}C]ADP; and expressed in terms of the [^{14}C]ATP formed as a percentage of the total [^{14}]ADP present initially.

The procedures for pre-incubation and for assay of ATP hydrolysis were as has been described previously[2].

Assay of the exchange rates of ITP, CTP, GTP and UTP, with [^{14}C]ADP

The composition of the incubation mixture, in millimolar concentrations, was as follows: 5 mM Tris–ITP; or (of the Tris salts of CTP, GTP, or UTP) when indicated; 3 mM $MgCl_2$; 0 or 85 mM NaCl as indicated; 1.6 mM [^{14}C]ADP; 40 mM Tris–HCl (pH 7.3) as the buffer; 0.1 mM EDTA–Tris; and approx. 8 μg of microsomal protein in a total volume of 30 μl. Aliquots of the reaction mixture were run on thin layer chromotography and assayed for the [^{14}C]ATP formed. The rest of the assay procedure was as described under the section on transphosphorylation assay.

RESULTS

(*A*) *Factors affecting the exchange rate*

Amount of enzyme protein

Fig. 1 presents two composite plots of values on the rate of conversion of [^{14}C]-ADP to [^{14}C]ATP as a function of the amount of microsomal proteins. The rate of the exchange was expressed in terms of the amount of [^{14}C]ATP formed as a percentage of the initial amount of [^{14}C]ADP. Each point on each of the curves is the mean of four determinations from four separate experiments on the same batch of microsomes.

The lower curve shows the experimentally determined values for the exchange rate. The upper curve shows the values after correcting for the hydrolytic loss of the [^{14}C]ATP formed during the exchange reaction. This hydrolytic loss, presumably due to the action of the Mg^{2+}-dependent ATPase, was 10 μmoles/mg of microsomal protein per h, on the average, as determined in a separate set of experiments on the same microsomes.

Even without correcting for hydrolytic losses of [^{14}C]ATP, as much as 17 % of the ^{14}C label of ADP was incorporated into ATP during a 10-min period of incubation

at 38°. The corrected and uncorrected values do not differ appreciably in the presence of 4 μg or less of microsomal protein. However, an appreciable correction to the exchange rate is required in the presence of 5 μg or more of microsomal protein. The Mg^{2+}-dependent ATPase activity accounted for the hydrolysis of 10% of the total ATP in the 10 min of incubation with maximal amounts of microsomal proteins (10 μg) used for the experiments on the exchange reaction.

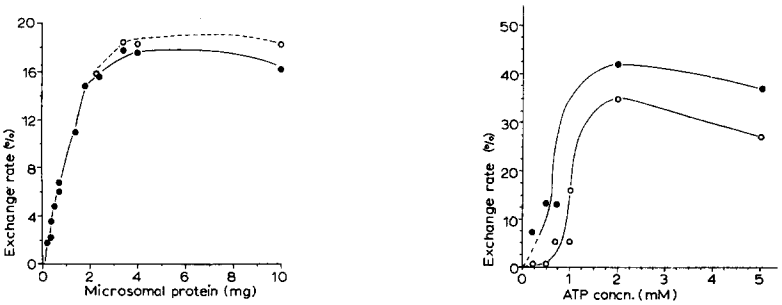


Fig. 1. Exchange rate as a function of the amount of microsomal protein. Exchange rate is defined as the amount of [^{14}C]ATP formed in 10 min at 38° as percentage of the initial amount of [^{14}C]ADP. The standard errors are represented by the vertical bars. Whenever the radius of the circle was equal to or bigger than the S.E., the vertical bars are not shown in the graph. (This definition of the exchange rate and of its S.E. will be the same throughout the remainder of this report.) Appropriate dilutions of the enzyme were added to each reaction mixture, and the subsequent incubation and assay were as described in METHODS. Each tube contained 3 mM $MgCl_2$, 5 mM ATP, 1.6 mM [^{14}C]ADP, 0.1 mM Tris–EDTA, 40 mM Tris–HCl (pH 7.3) as buffer, and varying amounts of microsomal protein as indicated along the abscissa in a total volume of 30 μl. ●—●, experimentally observed results; ○------○, the corresponding corrected values of experimentally observed results for the hydrolytic loss of the [^{14}C]ATP formed during the exchange reaction.

Fig. 2. Exchange rate *versus* ATP concentration. The ATP/ADP ratio, 4:1, was kept constant. ●—●, in the presence of 3 mM $MgCl_2$; ○—○, in the presence of 3 mM $MgCl_2$ + 85 mM NaCl. The amount of microsomal protein in each vessel was 5 μg. Other conditions and ion concentrations were the same as those described for Fig. 1 and as those in METHODS.

The pattern of corrected values *versus* amounts of enzyme resembles that expected of an exchange reaction[3], whereas the pattern of raw values *versus* amount of enzyme (particularly in the presence of high concentrations of microsomal protein) deviates somewhat from that expected of a pure exchange reaction.

Concentration of ATP and ADP

In the next set of experiments, the amount of microsomal protein (*ca.* 2 μg) and the nucleotide concentration ratio, [ATP]/[ADP] = 4.0 were fixed, thereby permitting observations on the exchange rate as a function of the concentration of ATP.

Fig. 2 is a composite plot of values of the catalyzed rate of the ADP–ATP exchange reaction as a function of the concentration of ATP in the presence of Mg^{2+} (upper curve), and in the presence of Mg^{2+} + Na^+ (lower curve). Each point on each of the curves is the mean of four determinations from four separate experiments on the same batch of microsomes.

The most obvious finding is that the catalyzed rate of exchange in the presence of Mg^{2+} was significantly greater than that in the presence of Mg^{2+} + Na^+ over the entire range of ATP concentrations (0.2–5.0 mM) used. The Na^+-induced decrement in

the exchange rate, reminiscent of the previously reported Na^+-induced decrement in [^{14}C]ATP binding on the microsomes, is consistent with the reaction model postulated in INTRODUCTION. Thus, with no Na^+ present, the exchange reaction proceeds *via* Reactions 1 through 2 and 1 through 4, where transphosphorylation can be accomplished through the two reversible reactions. When Na^+ is added, the irreversible Reaction 5 is accelerated, which provides a sink for $E' \sim P$ thereby reducing the reversal of transphosphorylation *via* Reactions 1 and 4.

In the previous report showing a Na^+-induced decrement of ^{14}C-binding to microsomes, the postulated site of Na^+ action was Reaction 4, which would predict a Na^+-induced increase of transphosphorylation. However, Fig. 2 shows a clear-cut Na^+-induced decrease in transphosphorylation, which excluded Reaction 4 as the sole site of Na^+ action, and which forced the invoking of a new postulate holding that Na^+ acts mainly on reaction 5. This new postulate is fully consistent with the Na^+-induced decrease in the exchange rate (Fig. 2) as well as with the Na^+-induced decrease in binding of [^{14}C]ATP to the microsomes.

The functional form of the plot shown in Fig. 2 is somewhat complex. At low concentrations of ATP, 0.2–1.0 mM, the rate function resembled that expected of cooperative homotropic kinetics[6]; and at high concentrations of ATP, 2.5–5.0 mM, the rate function resembled that expected of substrate inhibition at or near the saturation range of the reaction.

Effect of pH

Fig. 3 presents two composite plots of values on the rate of exchange in the presence of Mg^{2+}, and in the presence of Mg^{2+} + Na^+ *versus* pH. Each point is the mean value of four determinations from four individual experiments on a single pool of microsomes.

Neither the Mg^{2+}- nor the Mg^{2+} + Na^+-dependent exchange rate showed any

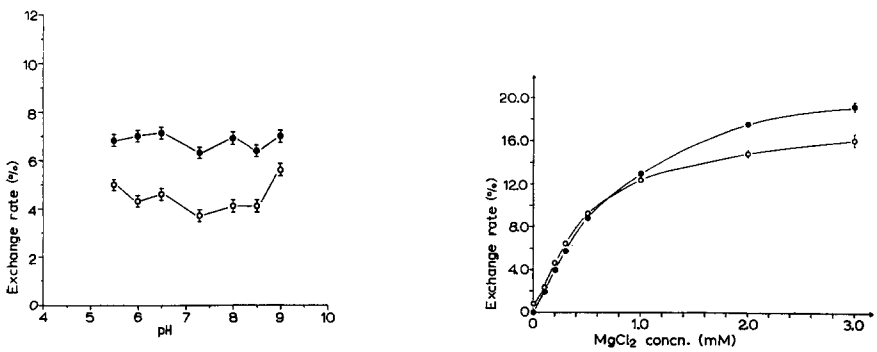


Fig. 3. Exchange rate *versus* pH. ●—●, in the presence of Mg^{2+}; ○—○, in the presence of Mg^{2+} + Na^+. Each tube contained 3 mM $MgCl_2$, 5 mM ATP, 1.6 mM [^{14}C]ADP, 0.1 mM Tris–EDTA, 40 mM Tris–HCl (pH 7.3) as buffer, 5 μg of microsomal protein in a total volume of 30 μl. When present, the concentration of NaCl was 85 mM .Other conditions were as described in METHODS.

Fig. 4. Exchange rate *versus* Mg^{2+} concentration. ●—●, in the presence of Mg^{2+}; ○—○, in the presence of Mg^{2+} + Na^+. In addition to Mg^{2+}, each tube contained 5 mM ATP, 1.6 mM [^{14}C]ADP, 0.1 mM Tris–EDTA, 40 mM Tris–HCl (pH 7.3), 5 μg of microsomal protein in a total volume of 30 μl. When present, NaCl concentration was 85 mM. Incubation and assay procedures were as described in METHODS.

marked change due to variation of the pH from 5.5 to 9.0. The Na^+-induced decrement of the exchange rate was readily detectable over the entire pH range.

The pH independence of the Mg^{2+}-dependent exchange rate in the turtle bladder was similar to that found in rat brain[7]; but the pH independence of the Na^+-modified exchange rate differed from that reported for other tissues[4,7].

Concentration of Mg^{2+}

Fig. 4 presents two composite plots of values on the rate of the exchange reaction as a function of Mg^{2+} concentrations in the presence of Na^+ (lower curve), and in the absence of Na^+ (upper curve). In both cases (with and without Na^+), the kinetic pattern of the exchange rate was that of a Michaelis-like function. At low concentrations of Mg^{2+} (0.0–0.6 mM) there is no marked Na^+-induced stimulation, as has been observed for the electric eel microsomes[4]. At high concentrations of Mg^{2+} (0.6–3.0 mM), the presence of Na^+ resulted in a significant decrease in the exchange rate, as has been observed by others[4,8] in different tissues.

The aforementioned kinetic patterns are consistent with Reactions 1, 2, 4 and 5 in Diagram 1. With Mg^{2+} alone, the exchange is mediated through Reactions 1 and 2 of the Mg^{2+}-dependent ATPase sequence, and simultaneously through Reactions 1 and 4 of the $(Na^+ + K^+)$-ATPase sequence. With $Mg^{2+} + Na^+$, the exchange is mediated through the same reactions, but the Na^+-induced sink for $E' \sim P$ reduces effective transphosphorylation of ADP by the $E' \sim P$.

The Mg^{2+}-dependent reaction sequence terminates in Reaction 3, which may or may not require Mg^{2+}.

Concentration of Na^+

Fig. 5 presents a composite plot of values on the exchange rate as a function of Na^+ concentrations in the presence of a low Mg^{2+} concentration. The increase in Na^+ concentration resulted in a marked decrease in the Mg^{2+}-dependent exchange rate with a possible secondary peak in activity at 90 mM. The exchange rate approached zero when the Na^+ concentration was increased to 120 mM.

Such a Na^+-induced decrease in the exchange rate is consistent with the data of Fig. 4 on exchange activity *versus* Mg^{2+} in the presence of Na^+; and is also consistent with the postulate that Na^+ acts mainly on Reaction 5 in the model reaction system, and supports the contention that Reaction 5 is a nearly irreversible step.

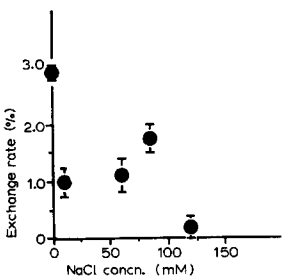


Fig. 5. Exchange rate *versus* Na^+ concentration. All reaction mixtures contained 0.1 mM $MgCl_2$. Apart from $MgCl_2$ and NaCl, the rest of ionic concentrations and conditions were as described for Fig. 4.

K^+

The effect of 15 mM K^+ on the exchange rate was examined in 2 experiments on a single batch of microsomal proteins. The conditions for incubation and the composition of the reaction mixture were the same (except for K^+ additions) as has been described herein. The mean values of each exchange rate, expressed as percentages of that obtained with Mg^{2+} alone (raw value for Mg^{2+}: 15 %), were as follows: Mg^{2+}, 100; K^+ alone, 3; $Mg^{2+} + K^+$, 100; $Mg^{2+} + Na^+$, 68; $Mg^{2+} + Na^+ + K^+$, 65. In other words, the addition of K^+ in the presence of Mg^{2+} and $Mg^{2+} + Na^+$ had no detectable effect on the measured exchange rate.

The data on $(Na^+ + K^+)$-ATPase and K^+-induced stripping of ^{32}P from the phosphoprotein[2] are consistent with placing the site of the K^+ effects at Reaction 6. This, in turn, is particularly consistent with the lack of any K^+-induced effect on the exchange reaction, suggesting that the rate constant for Reaction 6 is less than that of Reaction 5.

(B) Effect of inhibitors on the exchange rate

Table I, a summary of results on a single batch of microsomes, presents values of relative activity of the exchange rate and of the overall hydrolysis in the presence of three inhibitors under the specified ionic conditions. Values of the exchange rate are expressed as percentages of the native Mg^{2+}-dependent exchange activity; and those of hydrolysis (ATPase activity), as percentages of the native Mg^{2+}-dependent or of the native $(Na^+ + K^+)$-ATPase activity. Apart from 14 separate determinations on the native microsomes, the mean values and standard errors were estimated from the data of four separate experiments on a single pool of microsomal pellets, one aliquot of which was treated with ouabain, one with *N*-ethylmaleimide and one with oligomycin.

In the native microsomal pellet (first row), the exchange rate in the presence of $Mg^{2+} + Na^+$ was 83 % of that in the presence of Mg^{2+} alone ($P < 0.001$). The native Mg^{2+}-dependent exchange rate was 12 μmoles/mg protein per h. Concomitantly, the

TABLE I

EXCHANGE RATE AND ATPASE ACTIVITY OF THE NATIVE AND OF THE INHIBITOR-TREATED MICROSOMAL PROTEIN

Each reaction tube contained: 3 mM $MgCl_2$, and when indicated, 85 mM NaCl and 15 mM KCl. Other ion concentrations and conditions were as described for Fig. 4. $(Na^+ + K^+)$-ATPase activity and exchange rate were determined as described in METHODS. The 100 % value for the Mg^{2+}-dependent exchange was 12 μmoles/mg protein per h; that for Mg^{2+}-dependent ATPase, 10; and that for $(Na^+ + K^+)$-ATPase, 11.

Conditions	*Exchange rate (% of Mg^{2+}-dependent)*		*ATPase activity (% of control)*	
	Mg^{2+}	*$Mg^{2+} + Na^+$*	*Mg^{2+}*	*$Na^+ + K^+$*
Native	100	83 ± 2	100	100
Ouabain (1 mM) pre-treated	145 ± 2	132 ± 2	105 ± 3	4 ± 2
N-Ethylmaleimide (1 mM) pre-treated	16 ± 9	31 ± 5	92 ± 3	55 ± 3
Oligomycin (6.8 μg/ml)	111 ± 4	96 ± 12	90 ± 2	50 ± 5

hydrolytic activity of the same pellet (rate of P_i released), was 10 μmoles/mg protein per h with Mg^{2+}, and 21 μmoles/mg protein per h with $Mg^{2+} + Na^+ + K^+$.

In the inhibitor-treated aliquots of the same microsomal pellet, the exchange rates were: (a) increased in the presence of ouabain ($P < 0.001$) which completely inhibited the ($Na^+ + K^+$)-ATPase activity; (b) decreased in the presence of *N*-ethylmaleimide ($P < 0.01$) which inhibited 45 % of the ($Na^+ + K^+$)-ATPase activity; and (c) hardly changed in the presence of oligomycin ($0.05 > P < 0.1$) which inhibited 50 % of the ($Na^+ + K^+$)-ATPase activity.

Ouabain

Fig. 6 presents two composite plots on values of the rate of the exchange reaction in ouabain-treated (1 mM) microsomes as a function of the Mg^{2+} concentration without Na^+ (upper curve), and with Na^+ (lower curve).

The maximal rates of exchange observed in the ouabain-treated microsomes, 25 % with Mg^{2+} alone and 22 % with $Mg^{2+} + Na^+$, were greater than the corresponding rates (19 and 15 %, respectively) in the native microsomes. The ouabain-induced acceleration of the Mg^{2+}-dependent ADP–ATP exchange rate in turtle bladder microsomes contrasted with the lack of change reported for the Mg^{2+}-dependent exchange rate in microsomes of the electric eel[4].

Fig. 6 also shows that Na^+ retarded the exchange rate in the ouabain-treated microsomes over the entire range of Mg^{2+} concentration used (0.1–3.0 mM). In the native microsomes, Na^+ failed to retard the exchange rate in the presence of low Mg^{2+} concentrations (0.1–0.6 mM), but did retard the exchange rate at high Mg^{2+} concentrations (1.0–3.0 mM).

Several investigators[9,10], have suggested that ouabain inhibits the ($Na^+ + K^+$)-ATPase by interacting at the K^+-site of the enzyme (Reaction 6). The new finding of the present report that ouabain induces acceleration of the exchange rate in turtle bladder microsomes cannot be explained by the contention that ouabain acts solely on the K^+-site (Reaction 6) of the enzyme. Such a finding is consistent with the postulate that ouabain interacts at site of Reaction 5 either solely or in addition to its interaction at the site of Reaction 6. Inhibition at the site of Reaction 5 would slow down the rate of conversion of $E' \sim P$ to E'–P, thus allowing a greater exchange rate.

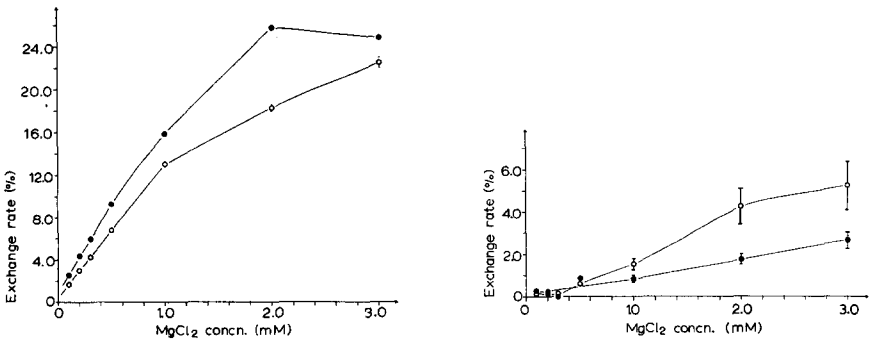


Fig. 6. Exchange rate in ouabain-treated microsomes. Microsomes were treated with 1 mM ouabain. Ionic concentrations and conditions were as described for Fig. 4.

Fig. 7. Exchange rate in *N*- ethylmaleimide-treated microsomes. Microsomes were treated with 1 mM *N*-ethylmaleimide. Other ionic concentrations and conditions were as described for Fig. 4.

N-Ethylmaleimide

Fig. 7 presents two composite plots of values on the rate of the exchange reaction of *N*-ethylmaleimide-treated (1 mM) microsomes as a function of Mg^{2+} concentration, without Na^+ (lower curve) and with Na^+ (upper curve).

Apparently *N*-ethylmaleimide, at 1 mM, caused a large decrease in both exchange rates, that in presence of Mg^{2+} and that in presence of $Mg^{2+} + Na^+$ (see also Table I). Despite this inhibition, the addition of Na^+ was associated with a small increase in the exchange rate of the *N*-ethylmaleimide-poisoned enzyme system. The results are consistent with the model reaction scheme, if one postulates that *N*-ethylmaleimide blocks the Reaction at sites 4 and 5.

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF OLIGOMYCIN ON THE EXCHANGE RATE

Each tube contained 0.3 mM $MgCl_2$ and when used, 85 mM NaCl. Other ion concentrations and conditions were as described for Fig. 4.

Oligomycin (*μg/ml*)	*Exchange rate* (%)	
	Mg^{2+}	$Mg^{2+} + Na^+$
0 (native)	2.4 ± 0.6	1.8 ± 0.2
1.7	2.9 ± 0.3	2.5 ± 0.8
4.0	2.7 ± 0.5	1.6 ± 0.4
6.8	2.8 ± 0.2	2.6 ± 0.1

Oligomycin

Table II presents mean values ± S.E. of the catalyzed exchange rates in microsomes treated with progressively increasing concentrations of oligomycin. The range of concentration used was similar to that used by others in similar studies on other tissues[4,7]. The first column denotes the concentration of oligomycin; the second and third columns, the values of Mg^{2+}- and $Mg^{2+} + Na^+$-dependent exchange rates, respectively.

Increases in the concentration of oligomycin resulted in little or no increase in the exchange rate. Addition of Na^+ caused a slight decrease in the exchange rate in the native and in the oligomycin-treated microsomal preparations.

No conclusions were made with respect to the effect of oligomycin on the model scheme presented in INTRODUCTION, because the oligomycin effects were little or nothing under the present conditions.

(*C*) *Substrate specificity*

Fig. 8 presents, in columnar form, the mean values ± S.E. from four determinations on the same batch of microsomes which had been incubated with 5 mM concentrations of the designated nucleotides in the presence of [^{14}C]ADP.

The data on nucleotide preference of the Mg^{2+}-dependent exchange rate were similar to those reported in electric eel microsomes[4] and rat brain microsomes[7]. However, the Na^+-induced decreases of the nucleotide exchange rates in turtle bladder microsomes was in sharp contrast to that of a Na^+-induced increase on the nucleotide

exchange rate for electric eel microsomes at low Mg^{2+} concentrations[4] and for rat brain microsomes treated with NaI (ref. 7).

In the presence of Mg^{2+} alone, the order of nucleotide preference in terms of the exchange rate was: ATP, 17; GTP, 3.8; ITP, 3.5; UTP, 2.7; and CTP, 1.8; which in terms of percentage becomes ATP, 100; GTP, 22; ITP, 21; UTP, 16; and CTP, 11.

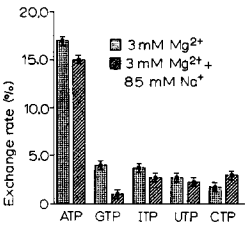


Fig. 8. Nucleotide specificity of the exchange rate. Each reaction tube contained 5 mM of the appropriate nucleotide (ITP, CTP, GTP, UTP or ATP); and 3 mM $MgCl_2$. The remaining constituents of the reaction mixture were as described for Fig. 4.

In the presence of $Mg^{2+} + Na^+$, the above order was changed only slightly in that the preference for GTP and CTP was reversed. This effect on nucleotide preference was accounted for by the fact that Na^+ increased slightly the exchange rate between CTP and ADP. This small effect of sodium on the ADP:CTP exchange was the exchange between ADP and all of the other nucleotides.

DISCUSSION

What can be said on the basis of the present data on the catalyzed exchange rate is that ADP is phosphorylated by a high energy phosphoprotein, and that this reaction, as well as those of enzyme substrate complexing and overall hydrolysis, is highly dependent upon the concentration of Mg^{2+}, Na^+ and K^+ in the ionic environment. Whereas the microsomal enzyme mixture may have ionic requirements identical to those of the intact transport system, it lacks the spatial orientation which provides directionality for the active transport process.

The reaction model, like any enzyme–substrate reaction model, requires that the enzyme be regenerated after formation of the final reaction product P_i, which means that the Na^+ form, E' is converted to the original form, E, as expressed by the reaction, $E' \rightleftharpoons E$. In this connection, the separation of a Na^+-independent, Mg^{2+}-dependent, moiety of the exchange reaction in rat brain microsomes[11,12] may be related to a low value for the equilibrium ratio, E'/E in the case of turtle bladder microsomes.

Problems remaining to be solved include: (a) the further separation of Mg^{2+}-dependent from $Na^+ + K^+$ dependent activity; (b) the nature of the intermediary complexes formed; and (c) the exact role of membrane ATPase in active Na^+ transport.

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