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Effects of Oligomycin on the $(Na^+ + K^+)$-Dependent Adenosine Triphosphatase

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SUMMARY

Oligomycin inhibited a rat brain $(Na^+ + K^+)$-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) preparation, but the inhibition was incomplete even at high oligomycin concentrations, and Dixon plots were concave downward. Inhibition increased as the incubation temperature was lowered. Elevated $MgCl_2$ concentrations antagonized the inhibition, and relative inhibition was less at low (1 μM) than at high (1–3 mM) ATP concentrations. In the presence of ouabain, which also inhibited the enzyme, the relative efficacy of oligomycin decreased. Oligomycin influenced the kinetics of cation activation, diminishing V_{max} and n (the slope of the Hill plot, an index of cooperativity) for both NaCl and KCl, and also reducing $K_{0.5}$ (the concentration for half-maximal activation) for NaCl. Inhibition of the associated K^+-dependent phosphatase activity could be demonstrated in the presence of NaCl, and oligomycin reduced V_{max} and n for KCl activation in this case also. The data suggest that oligomycin decreases ATPase activity by affecting the equilibria between alternative allosteric forms of the enzyme, oligomycin favoring a form with a lower V_{max} and a higher Q_{10}, and with n for cation activation near 1.0; this shift in equilibria would be opposed by $MgCl_2$ and temperature. It is proposed that both forms, reminiscent of E_1 and E_2, catalyze $(Na^+ + K^+)$-dependent hydrolysis and cation transport, since inhibition of transport by oligomycin is also reported to be incomplete.

INTRODUCTION

Inhibition of the $(Na^+ + K^+)$-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) by oligomycin has been documented extensively (1–13), yet a number of peculiar aspects of this inhibition remain ill-defined and unexplained. Thus, although oligomycin inhibits the enzyme, this inhibition does not appear to reach completion (3, 4, 7–11), and stimulation occurs under certain circumstances (7, 14, 15). This study was undertaken to explore some of these issues in terms both of the reaction schemes for the ATPase and of the models for cation transport base on these schemes.

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METHODS

The enzyme was obtained from a rat brain microsomal preparation by treatment with deoxycholate and then NaI, as described previously (16, 17).

$(Na^+ + K^+)$-dependent ATPase activity was measured routinely in terms of the production of P_i, as described previously (17). The standard medium contained 50 mM Tris-HCl (pH 7.8), 3 mM $MgCl_2$, 3 mM ATP (as the Tris salt), 90 mM NaCl, 10 mM KCl, 2 μl/ml of ethanol (as a solvent for the oligomycin when it was present), and the enzyme

preparation (0.1 mg of protein per milliliter). The reaction was started by adding the enzyme, and incubation was carried out for 4–8 min at 37°; P_i production was linear with time during this period. Activity in the absence of added Na^+ and K^+ ("MgATPase") was measured concurrently; such activity averaged only a small percentage of the $(Na^+ + K^+)$-dependent activity under standard conditions (17), and was subtracted from the total activity in the presence of Na^+ and K^+ to give the $(Na^+ + K^+)$-dependent activity.

In some experiments ATPase activity was measured in terms of the liberation of $^{32}P_i$ from [γ-^{32}P]-ATP. Experiments were performed similarly, but with tracer amounts of ^{32}P-ATP added; the liberated $^{32}P_i$ was extracted by a slight modification of the method of Neufeld and Levy (18), and the radioactivity was measured with a liquid scintillation counter.

K^+-dependent phosphatase activity was measured in terms of the production of *p*-nitrophenol after incubation with *p*-nitrophenyl phosphate, as described previously (19). The standard medium contained 50 mM Tris-HCl (pH 7.8), 3 mM $MgCl_2$, 3 mM *p*-nitrophenyl phosphate (as the Tris salt), 10 mM KCl, 2 μl/ml of ethanol, and the enzyme preparation (0.1 mg of protein per milliliter). Incubation was carried out for 8–15 min at 37°; *p*-nitrophenol production was linear with time during this period. Activity in the absence of added K^+ was measured concurrently; such activity averaged only a small percentage of the K^+-dependent activity under standard conditions (19), and was subtracted from the total activity in the presence of K^+ to give the K^+-dependent activity.

To assess the reversibility of the oligomycin inhibition of the ATPase, incubations were performed in the presence and absence of oligomycin (5 μg/ml) in the standard medium scaled to 5.0 ml. After incubation for 5 min at 37°, an aliquot was removed to measure the production of P_i, and 30 ml of ice-cold 0.1 M Tris-HCl (pH 7.8) containing 0.2% ethanol (v/v) were added to the reaction mixture, which then was centrifuged at 2° for 25 min at 32,000 × *g*. The pellets obtained were mixed with 35 ml of Tris–ethanol and again centrifuged. Finally, the second pellets were resuspended in small volumes of 0.01 M Tris-HCl (pH 7.8) and assayed for ATPase activity, in terms of the protein content. The reversibility of the inhibition of the ATPase by ouabain was assessed similarly. Incubations in the standard medium scaled to 5 ml, in the presence and absence of 0.1 mM ouabain, were carried out for 5 min at 37°, and aliquots were removed to measure P_i production. The mixture was then diluted with 30 ml of 0.1 M Tris-HCl (pH 7.8) at room temperature (22–25°) and centrifuged at room temperature as above. The pellets obtained were mixed with 35 ml of the Tris buffer, centrifuged again, and finally suspended in small volumes of ice-cold 0.01 M Tris-HCl (pH 7.8) for subsequent assay.

ATP and *p*-nitrophenyl phosphate were purchased from Sigma Chemical Company as the sodium salts and were converted to the Tris salts; oligomycin was also purchased from Sigma. [γ-^{32}P]-ATP, 22.8 Ci/mmole, was purchased from New England Nuclear Corporation. All solutions were made in water redistilled in an all-glass still. Protein was measured by the biuret method, using bovine serum albumin as a standard.

Experimental points are the averages of five or more experiments performed in duplicate (for ^{32}P experiments in triplicate), and the data are presented with standard errors of the mean where appropriate.

RESULTS

Inhibition by oligomycin. Oligomycin, at a concentration of 2.5 μg/ml, inhibited the $(Na^+ + K^+)$-dependent ATPase 64%; prior incubation of the enzyme with oligomycin under various conditions had little additional effect (Table 1). Moreover, the percentage inhibition was not detectably different after 2, 4, and 8 min of incubation. To assess the reversibility of the inhibition by oligomycin, the enzyme was incubated with 5 μg/ml of oligomycin and then washed (see METHODS); this procedure restored activity from 28% of control (unwashed) to 71% (washed). From these observations it was concluded that conventional kinetic analyses of the inhibi-

TABLE 1

Effects of prior incubation with oligomycin on inhibition of (Na^+ + K^+)-dependent ATPase

The enzyme preparation was first incubated for the time specified at 37° in the standard medium (see METHODS), minus the substances listed, in the presence and absence of 2.5 μg/ml of oligomycin; the reaction was then initiated by adding the omitted substance. Activity in the presence of oligomycin is presented as a percentage of the concurrent control without oligomycin.

Prior incubation conditions		Activity
Omission	Time	
	min	*% control*
ATP	5	31 ± 4
	10	31 ± 3
	20	29 ± 5
KCl	5	30 ± 5
NaCl	5	32 ± 2
KCl and NaCl	5	32 ± 4
No prior incubation		36 ± 3

tion occurring during brief incubations were appropriate.

Although inhibition increased with the concentration of oligomycin, it appeared to approach a maximal level before total inhibition was achieved (Fig. 1). Furthermore, inhibition was greater at lower incubation temperatures (Fig. 1), and thus the Q_{10} for the ATPase activity in the presence of oligomycin was greater than in its absence (Table 2); similar data have been reported by Gruenor and Avi-Dor (7). This diminishing effect of added increments of oligomycin is particularly apparent in Dixon plots (Fig. 1, inset), where, instead of a linear relationship between the inhibitor concentration and $1/v$, the curves are concave downward for all temperatures.

Oligomycin and cation activation. Activation by monovalent cations was affected by oligomycin, and for NaCl the concentration for half-maximal activation, $K_{0.5}$, was halved under conditions in which V_{max} was reduced markedly (Fig. 2). Reductions in both $K_{0.5}$ and V_{max} were also found by Inturrisi and Titus (10). In addition, the degree of sigmoidicity of the affector-velocity plot, as indicated by the slope of the Hill plot, n, was reduced (Fig. 2). This reduction in

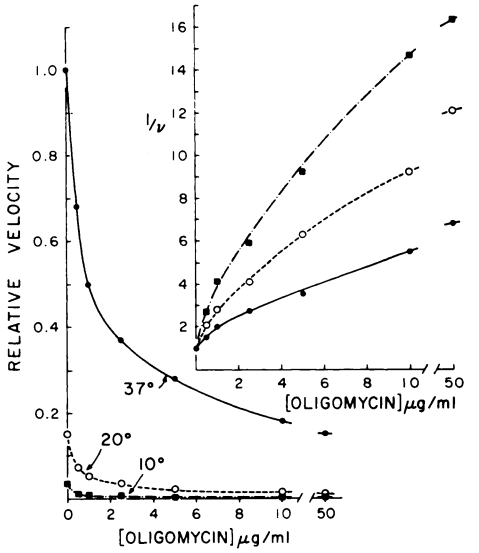

FIG. 1. *Inhibition of ATPase by oligomycin*

(Na^+ + K^+)-dependent ATPase activity was measured in the presence and absence of oligomycin in the standard medium (see METHODS) at 37° (●——●), 20° (○- - -○), and 10° (■-·-·■). Reaction velocities are presented relative to that of control incubations at 37°, defined as 1.0. In the inset, data are replotted in the form of a Dixon plot, with velocities expressed, for each temperature, relative to that in the absence of oligomycin, defined as 1.0.

TABLE 2

Effects of oligomycin and ouabain on Q_{10} of (Na^+ + K^+)-dependent ATPase

The Q_{10} was calculated from incubations at 30° and 20° in the standard medium, in the presence and absence of oligomycin and ouabain, as indicated.

Additions	Q_{10}
None	3.1
Oligomycin, 5 μg/ml	3.7
Ouabain, 0.01 mM	2.8

sigmoidicity in the presence of oligomycin reflects an alteration in the response of the ATPase to low concentrations of NaCl, such that oligomycin could stimulate at low NaCl concentrations (Fig. 2).

For KCl (Fig. 3) oligomycin had little effect on $K_{0.5}$, although the value of n was decreased as it was for NaCl. Inturrisi and

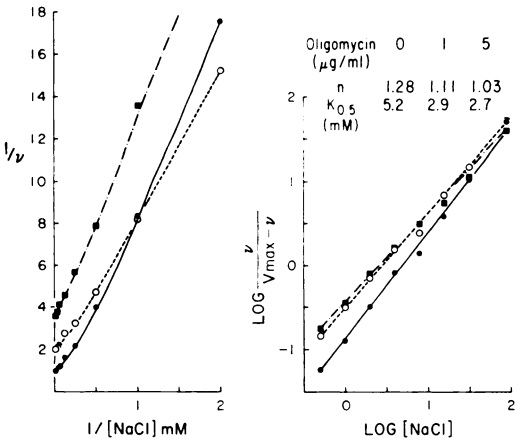

FIG. 2. *Effects of oligomycin on activation of ATPase by NaCl*

$(Na^+ + K^+)$-dependent ATPase activity was measured in incubations at 37° in the standard medium modified to contain the concentrations of NaCl indicated, and containing either no oligomycin (●——●), 1 μg/ml of oligomycin (○- - -○), or 5 μg/ml of oligomycin (■-·-·■). Data are presented in the left-hand panel in the form of a Lineweaver-Burk plot, with velocities expressed relative to that in the standard medium, defined as 1.0. In the right-hand panel data are presented in the form of a Hill plot, with values for n and $K_{0.5}$ derived from the equations of the lines fitted by the method of least squares.

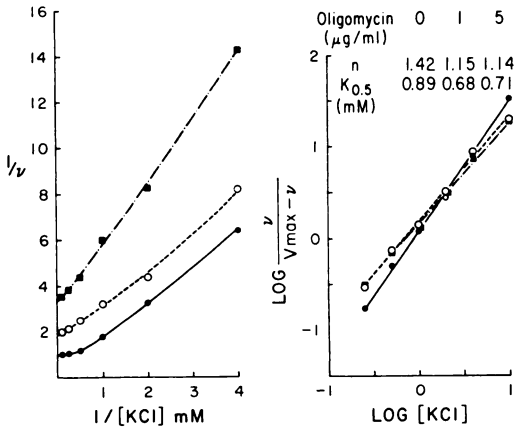

FIG. 3. *Effects of oligomycin on activation of ATPase by KCl*

Experiments were performed and the data are expressed as in Fig. 2, except that KCl was varied in the absence (●——●) or presence of oligomycin: ○- - -○, 1 μg/ml; ■-·-·■, 5 μg/ml.

Titus (10) also found a reduction in V_{max} with a negligible change in $K_{0.5}$.

Inhibition by oligomycin was far greater at low than at high concentrations of $MgCl_2$ (Table 3).

Inhibition at low ATP concentration. The relative inhibition by 10 μg/ml of oligomycin was less with 1 μM ATP than with 1 mM ATP at equimolar $MgCl_2$ concentrations (Table 4); moreover, the inhibition in the presence of 1 μM ATP could be prevented by 1 mM $MgCl_2$.

TABLE 3

Effect of $MgCl_2$ on inhibition of ATPase by oligomycin

Incubation was carried out in the standard medium, but with the $MgCl_2$ concentrations indicated, in the presence and absence of oligomycin. Activity in the presence of oligomycin is presented as a percentage of the concurrent control without oligomycin.

$MgCl_2$ concentration	Activity	
	With 0.5 μg/ml of oligomycin	With 2.5 μg/ml of oligomycin
mM	*% control*	
0.1	40 ± 4	19 ± 3
0.3	46 ± 2	19 ± 4
1.0	70 ± 2	34 ± 2
3.0	68 ± 3	37 ± 3
6.0	83 ± 5	53 ± 5

TABLE 4

Variation in oligomycin inhibition of ATPase with concentration of ATP

ATPase activity was measured in terms of the liberation of $^{32}P_i$ from [γ-^{32}P-]ATP (see METHODS) during incubations in the standard medium, but with the concentrations of ATP and $MgCl_2$ listed, in the presence and absence of 10 μg/ml of oligomycin. Activity in the presence of oligomycin is presented as a percentage of the activity of the concurrent control without oligomycin. In the presence of 1 mM $MgCl_2$ the control activity with 1 μM ATP was only 44% of that with 1 μM $MgCl_2$.

ATP concentration	$MgCl_2$ concentration	Activity
μM	*μM*	*% control*
1	1	65 ± 5
1	1000	110 ± 8
1000	1000	20 ± 4

Inhibition by ouabain. Under certain circumstances ouabain inhibition has been shown to be time-dependent (20–22), and an essentially irreversible inhibition of the ATPase can occur (20, 21, 23; but see ref. 24). However, in the experiments reported here, in which the reaction was started by adding the enzyme to the incubation mixture, the percentage inhibition was not measurably different at 2, 4, and 8 min. In addition, such inhibition was reversible to the extent that the 86% inhibition in the presence of 0.1 mM ouabain could be diminished by washing (see METHODS) to 22%.

Under these conditions the concentration ($K_{-0.5}$) of ouabain required to inhibit the ($Na^+ + K^+$)-dependent ATPase by 50%, was 3 μM. However, in the presence of oligomycin (2.5 μg/ml) the $K_{-0.5}$ for ouabain was increased to 18 μM. Similarly, the $K_{-0.5}$ for oligomycin was successively increased as the concentration of ouabain was raised (Fig. 4).

In contrast to the inhibition with oligomycin, inhibition with ouabain was, if anything, diminished as the temperature for incubation was lowered (Table 2).

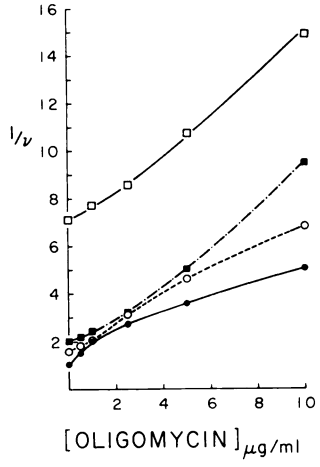

FIG. 4. *Effects of ouabain on inhibition of ATPase by oligomycin*

Experiments were performed as in Fig. 1, but in the absence (●——●) or presence of ouabain: ○- - -○, 1 μM; ■·-·-■, 3 μM; □——□, 100 μM. Under these conditions the $K_{-0.5}$ for oligomycin rose from 1 μg/ml in the absence of ouabain to 2.6, 4.1, and 9.2 μg/ml, respectively.

Effects of oligomycin on K^+-dependent phosphatase activity. Phosphatase activity measured in the standard medium was only slightly affected by high concentrations of oligomycin, even at lowered temperatures (Fig. 5), but in the presence of NaCl (which itself can inhibit the K^+-dependent phosphatase activity) oligomycin inhibited appreciably (Fig. 5). Most earlier reports describe little or no inhibition of the phosphatase by oligomycin (8–11, 14, 15); the experiments by Gruenor and Avi-Dor (7) showing inhibition appear to have been performed in the presence of Na^+ (from the sodium salt of *p*-nitrophenyl phosphate), and are thus in accord with these data.

The kinetic response of the phosphatase activity to KCl is quite sensitive to NaCl

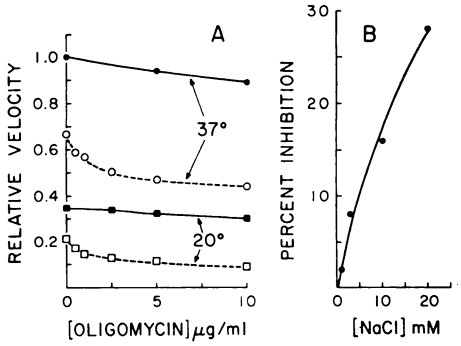

FIG. 5. *Effects of oligomycin on K^+-dependent p-nitrophenyl phosphatase activity*

A. Results of experiments in which K^+-dependent phosphatase activity was measured at 37° and at 20° in the standard medium (see METHODS) (●, ■) and in media containing in addition 20 mM NaCl (○, □), in the presence and absence of oligomycin. Reaction velocities are expressed relative to that of control incubations at 37°, defined as 1.0. B. Effects of NaCl on inhibition due to oligomycin. In these experiments phosphatase activity was measured at 37° in the standard medium in the presence and absence of 10 μg/ml of oligomycin and in the presence and absence of NaCl. The percentage inhibition on the ordinate refers to the increment in inhibition due to oligomycin as a function of the NaCl concentration, calculated as follows:

$$100 \times \left[1 - \frac{\left(\frac{\text{activity with NaCl + oligomycin}}{\text{activity with NaCl}}\right)}{\left(\frac{\text{activity with oligomycin}}{\text{control activity}}\right)} \right]$$

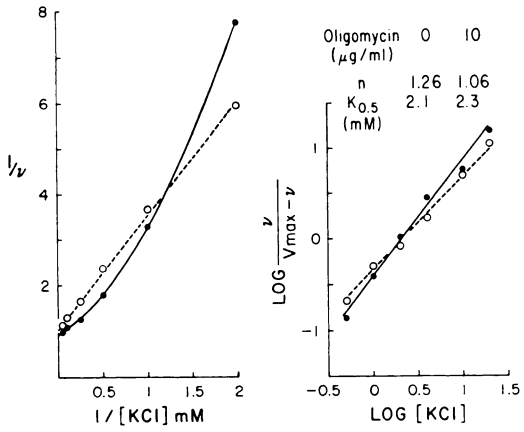

FIG. 6. *Effects of oligomycin on activation of p-nitrophenyl phosphatase by KCl*

K^+-dependent phosphatase activity was measured in incubations at 37° in media containing 50 mM Tris-HCl (pH 7.8), 3 mM *p*-nitrophenyl phosphate, 3 mM $MgCl_2$, 3 mM NaCl, the concentration of KCl indicated, and in the absence (●——●) and presence (○- - -○) of oligomycin, 10 μg/ml. Data are presented as in Fig. 3.

(15, 19, 25), and the effects of oligomycin on K^+ kinetics are therefore difficult to decipher. However, in the presence of a low concentration (3 mM) of NaCl, which itself has only a small effect on the response to KCl, oligomycin can be shown to affect the phosphatase as it did the ATPase: $K_{0.5}$ was affected only slightly, but n was diminished (Fig. 6).

DISCUSSION

A striking feature of the influence of oligomycin on the $(Na^+ + K^+)$-dependent ATPase is its diminishing efficacy as the concentration is raised, coupled with its seeming inability to effect complete inhibition (Fig. 1). Although residual $(Na^+ + K^+)$-dependent ATPase activity in the presence of very high concentrations of oligomycin has been noted repeatedly (3, 4, 7–11), the consequences of this inhibitory response have been largely ignored. Incomplete inhibition with a Dixon plot concave downward can occur from three types of interactions. (a) The enzyme-inhibitor complex retains some catalytic activity even though totally saturated with the inhibitor; however, such "partial inhibition" (26) is rare. (b) If two or more enzyme forms exist with differing affinity for the inhibitor, such a kinetic response can occur, but only if there are no equilibria between the forms, for, if the forms are interconvertible, addition of an inhibitor shifts the equilibrium to the form with greater affinity, and a straight-line Dixon plot results. (c) With alternative enzyme forms in equilibrium, a compound may inhibit by shifting the equilibrium toward a form inherently less active, and, without altering the catalytic activity of that form, decrease the total velocity. In view of the considerable evidence indicating the allosteric nature of the ATPase (16, 17, 19, 20, 25, 27–31) and the occurrence of multiple enzyme conformations that such a formulation implies, the consequences of this last manifestation of incomplete inhibition, case (c), may be considered.

The conventional formulation (6, 32) of the reaction sequence of the ATPase, i.e.,

$$E_1 + \text{ATP} \xrightleftharpoons{Na^+,\ Mg^{2+}} E_1\text{-P} \xrightleftharpoons{Mg^{2+}} E_2\text{-P} \xrightleftharpoons{K^+} E_1 + P_i$$

proposes a conformational change of the phosphorylated enzyme (E_1-P to E_2-P) that is sensitive to the Mg^{2+} concentration and that appears to be the point of action of oligomycin [reactions to the left are stimulated by oligomycin (6, 12) while the over-all reaction is inhibited]. With this scheme it is improbable that oligomycin inhibits by forming an inactive or poorly active enzyme-oligomycin complex: under case (b), incomplete inhibition occurs by preferential binding to one of several alternative forms, but the forms must not be interconvertible or a straight-line Dixon plot results; under case (a), in which binding of the inhibitor forms a partially inhibited complex, there should be at high oligomycin concentrations essentially no E_1 form remaining if E_1 and E_2 are interconvertible; yet in the presence of high concentrations of oligomycin an "E_1 activity," the ADP-ATP exchange, is stimulated. The remaining possibility, case (c), that oligomycin inhibits by shifting the equilibrium toward an inherently less active form (with-

out influencing the catalytic activity of that form), is supported by the apparent antagonism between the Mg^{2+} concentration and oligomycin inhibition (Table 3) and between ouabain and oligomycin (Fig. 4). Considerable evidence has accumulated that is consistent with ouabain inhibiting the ATPase by binding to the E_2 form of the enzyme (24, 32). In such a scheme ouabain would be expected to diminish the inhibitory efficacy of oligomycin (as manifested in $K_{-0.5}$) if oligomycin were bound to a different enzyme form in equilibrium with that to which ouabain binds (E_1·oligomycin $\rightleftarrows E_1 \rightleftarrows E_2 \rightleftarrows E_2$· ouabain).

Although a reaction pathway through E_1 to E_2 has been shown to occur (32), the data do not require that this indeed be the normal route in the presence of nonlimiting Mg^{2+} concentrations and/or in the absence of inhibitors. The persistence of hydrolytic activity in the presence of high oligomycin concentrations suggests that the E_1-P of the conventional scheme is hydrolyzed at a slow but significant rate, and K^+-dependent hydrolysis under E_1 conditions (low Mg^{2+} concentrations) has been demonstrated (32). Furthermore, no data indicate that E_2-P (i.e., a form with poor or absent ADP-ATP exchange activity) cannot be formed directly from the enzyme and ATP. Hence an alternative reaction scheme, with coexisting interconvertible forms, would be compatible with these data:

$$E_I + ATP \xrightleftharpoons{Na^+,\ Mg^{2+}} E_I\text{-}P \xrightleftharpoons{K^+} E_I + P_i$$

$$\Updownarrow$$

$$E_{II} + ATP \xrightleftharpoons{Na^+,\ Mg^{2+}} E_{II}\text{-}P \xrightleftharpoons{K^+} E_{II} + P_i$$

(Roman numerals are used to suggest forms analogous to those of the conventional scheme; the possibility of additional intermediate forms of E should be recognized.) For this scheme interconversion between E_I and E_{II} should be possible at all steps; oligomycin would act by shifting the equlibrium from E_{II}-P to E_I-P (and perhaps between all states of E_{II} and E_I : $E_{II} \rightleftarrows E_I + E_I$·oligomycin). Such a formulation is consistent with allosteric mechanisms and the actions of allosteric effectors to modify enzymatic activity, and permits a characterization of the extreme forms, E_I and E_{II} (Table 5); n, the slope of the Hill plot for cation activation, is a function of the number of sites for the cation and the interaction between the sites (17).

In contrast to the effects of oligomycin, the equilibria would be shifted toward E_{II} by increased Mg^{2+} concentrations (6, 32) (Tables 3 and 4) and higher temperatures [oligomycin sensitivity decreases (Fig. 1), the Q_{10} decreases (7, 33), the K_m for ATP increases (17), and $K_{0.5}$ and n for Na^+ and K^+ increase (17) as the temperature is raised]. Moreover, substrate-velocity plots for the ATPase indicate two distinct K_m values for ATP (18, 34), and such data fit a model in which the low-K_m form (E_I) contributes a significant fraction of the hydrolytic activity at low ATP concentrations (approximately 1 μM) whereas the high-K_m form (E_{II}), with a much higher V_{max}, overwhelmingly predominates at high ATP concentrations (approximately 1 mM). In such circumstances oligomycin would have less effect in the presence of 1 μM ATP than with 1 mM ATP; addition of 1 mM $MgCl_2$ with 1 μM ATP would itself inhibit somewhat (shifting

TABLE 5

Comparison of enzymatic properties of the two forms of ($Na^+ + K^+$)-dependent ATPase

The characteristics of the two extreme forms of the ATPase, E_I and E_{II}, are listed in terms of (a) properties described for E_1 and E_2 and/or (b) properties with low ATP, Na^+, or K^+ concentrations for the ATPase, and with low nucleotide or oligomycin concentrations for the phosphatase, taken to represent E_I conditions.

Property	E_I	E_{II}
ATP hydrolysis:		
V_{max}	Low	High
Q_{10}	High	Low
K_m for ATP	Low	High
$K_{0.5}$ for Na^+, K^+	Low	High
n for Na^+, K^+	1	>1
ADP-ATP exchange:		
V_{max}	High	Low ?
Phosphatase activity:		
V_{max}	Moderate	High
$K_{0.5}$ for K^+	Low	High
n for K^+	1	>1

the equilibrium to the E_{II} form with high K_m and V_{max}) but would also compete against the effects of oligomycin (Table 4).

For the K^+-dependent phosphatase activity, which reflects some aspects of the terminal hydrolytic steps (11, 19), the formulations are less clear, and alternative reaction pathways may be available (15) in addition to the occurrence of multiple enzyme forms. "Activation" of the phosphatase, in terms of a decreased $K_{0.5}$ for K^+, occurs in the presence of Na^+ together with low concentrations of either certain nucleotides (19, 35, 36) or oligomycin (14, 15), although the V_{max} may be somewhat diminished in these circumstances. However, higher concentrations of either nucleotides or oligomycin, or both together, abolish this "activation" (14, 15, 19).

Thus the phosphatase activity may proceed fairly well with either form of the enzyme, whereas the reduction in $K_{0.5}$ and n (19) may reflect the shift toward an E_I form; the biphasic response would thus represent the opposing influences on V_{max} and $K_{0.5}$. The role of Na^+ in oligomycin interactions is unclear, and with the ATPase such a requirement may go unnoticed; for the phosphatase, however, Na^+ may be necessary for oligomycin binding or for allosteric changes (cf. ref. 10), or merely to permit operation of the Na^+-K^+ pathway of the phosphatase (15), so that the oligomycin effect is made manifest.

Finally, the implications for the transport models of the incomplete inhibition by oligomycin should be considered, since oligomycin also does not block active cation transport completely (37). Thus, in contrast to some models for the ATPase (20, 24), the E_1-P to E_2-P conversion may not be an essential part of the translocation scheme; moreover, neither may the previously proposed (17) allosteric changes of the E_I to E_{II} form play such a role, although such transformations should play a vital role in regulating rates of cation transport (38). Indeed, oligomycin has been reported to reduce the number of potassium ions transported per ATP molecule hydrolyzed (5), consistent with this scheme.

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