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SENSITIVITY OF THE ($Na^+ + K^+$)-ATPase TO STATE-DEPENDENT INHIBITORS

EFFECTS OF DIGITONIN AND TRITON X-100

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Summary

Treatment of a purified ($Na^+ + K^+$)-ATPase preparation from dog kidney with digitonin reduced enzymatic activity, with the ($Na^+ + K^+$)-ATPase reaction inhibited more than the K^+-phosphatase reaction that is also catalyzed by this enzyme. Under the usual assay conditions oligomycin inhibits the ($Na^+ + K^+$)-ATPase reaction but not the K^+-phosphatase reaction; however, treatment with digitonin made the K^+-phosphatase reaction almost as sensitive to oligomycin as the ($Na^+ + K^+$)-ATPase reaction. The non-ionic detergents, Triton X-100, Lubrol WX and Tween 20, also conferred sensitivity to oligomycin on the K^+-phosphatase reaction (in the absence of oligomycin all these detergents, unlike digitonin, inhibited the K^+-phosphatase reaction more than the ($Na^+ + K^+$)-ATPase reaction). Both digitonin and Triton markedly increased the $K_{0.5}$ for K^+ as activator of the K^+-phosphatase reaction, with little effect on the $K_{0.5}$ for K^+ as activator of the ($Na^+ + K^+$)-ATPase reaction. In contrast, increasing the $K_{0.5}$ for K^+ in the K^+-phosphatase reaction by treatment of the enzyme with acetic anhydride did not confer sensitivity to oligomycin. Both digitonin and Triton also increased the inhibition of the K^+-phosphatase reaction by ATP and decreased the inhibition by inorganic phosphate and vanadate. These observations are interpreted as digitonin and Triton favoring the E_1 conformational state of the enzyme (manifested by sensitivity to oligomycin and a greater affinity for ATP at the low-affinity substrate sites), as opposed to the E_2 state (manifested by insensitivity to oligomycin, greater sensitivity to phosphate and vanadate, and a lower $K_{0.5}$ for K^+ in the K^+-phosphatase reaction). In addition, digitonin blocked activation of the phosphatase reaction by Na^+ plus CTP. This effect is consistent with digitonin dissociating the catalytic subunits of the

enzyme, the interaction of which may be essential for activation by Na^+ plus nucleotide.

Introduction

Early studies [1—3] on the $(Na^+ + K^+)$-ATPase (ATP phosphohydrolase, EC 3.6.1.3) demonstrated two different conformational states of the phosphorylated enzyme, an ADP-sensitive form termed E_1-P and a K^+-sensitive form termed E_2-P, suggesting the following reaction sequence [1—5]:

$$E_1 \overset{ATP,\,Na}{\rightleftharpoons} E_1ATP(Na) \underset{-ADP}{\rightleftharpoons} E_1\text{-}P(Na) \underset{-Na}{\rightleftharpoons} E_2\text{-}P \overset{K}{\underset{-P_i}{\rightleftharpoons}} E_2(K) \underset{-K}{\rightleftharpoons} E_1$$

Oligomycin, which inhibits the K^+-dependent dephosphorylation of the enzyme but not the ADP ↔ ATP exchange reaction [1,2], thus appeared to be a determinant of the major enzyme conformations through blocking the conversion of E_1-P to E_2-P.

This ATPase also catalyzes a K^+-dependent phosphatase reaction with various substrates (e.g., nitrophenyl phosphate, acetyl phosphate), but this reaction, under the usual assay conditions, is not inhibited by oligomycin [6—9] although it seems to reflect the terminal K^+-dependent hydrolytic steps of the $(Na^+ + K^+)$-ATPase reaction sequence that are sensitive to oligomycin [10—12]. Since oligomycin appeared to be an uncompetitive inhibitor toward ATP in the $(Na^+ + K^+)$-ATPase reaction [6], an explanation for such selectivity would be that oligomycin binds to the E_1-P form of the enzyme with subsequent blockade of the conversion to the E_2-P form [13]. By this argument, oligomycin would not inhibit the K^+-phosphatase reaction since the E_1-P form was thought not to participate in that reaction sequence.

During studies on the interaction of digitonin with this enzyme a surprising sensitization of the K^+-phosphatase reaction to oligomycin was noticed: with concentrations of digitonin that themselves had relatively little effect on the K^+-phosphatase reaction, oligomycin inhibited almost as much as it did in the $(Na^+ + K^+)$-ATPase reaction in either the absence or presence of digitonin. This report describes these experiments and further studies exploring the ability of digitonin and certain non-ionic detergents to influence interactions of the enzyme with ligands that select specific conformational states.

Methods and Materials

The enzyme preparation was obtained from the medulla of frozen canine kidneys by a modification [14] of the procedure of Jorgensen [15]. $(Na^+ + K^+)$-ATPase activity was measured in terms of the production of inorganic phosphate, as previously described [16]: the standard medium contained 30 mM histidine · HCl-Tris (pH 7.8), 3 mM ATP, 3 mM $MgCl_2$, 90 mM NaCl and 10 mM KCl. Potassium nitrophenyl phosphate activity was measured in terms of the production of *p*-nitrophenol, as previously described [17]: the standard medium contained 30 mM histidine · HCl-Tris (pH 7.8), 3 mM *p*-nitrophenyl phosphate, 3 mM $MgCl_2$ and 10 mM KCl. Potassium acetyl phosphatase

activity was measured in terms of the loss of acetyl phosphate measured by the hydroxamate reaction, as previously described [8]: the standard incubation mixture was the same as that above except for the substitution of 3 mM acetyl phosphate for *p*-nitrophenyl phosphate.

In experiments to measure the effects of digitonin on enzymatic activity, a solution of digitonin in ethanol was evaporated in the reaction tubes under N_2, leaving a film of digitonin on the walls [18]: the concentration of digitonin listed is that in the final incubation mixture. All the reactants except substrate (ATP, nitrophenyl phosphate, or acetyl phosphate) were then added and the mixture incubated at 37°C, routinely for 8 min. The enzymatic reaction was initiated by adding the missing substrate and incubation was continued at 37°C for 6—12 min. In experiments to measure the effects of the detergents, Triton X-100, Lubrol WX and Tween 20, solutions of the detergents were added directly to the standard incubation medium: routinely, the interaction between enzyme and detergent and the assay of enzymatic activity were initiated simultaneously by adding the enzyme preparation to the medium without prior equilibration with detergent. For modification by acetic anhydride, the enzyme preparation was reacted for 10 min at 0°C with 0.16 μl acetic anhydride per ml of medium, in 30 mM imidazole · HCl (pH 7.8), then diluted and collected by centrifugation as previously described [19].

Data presented are averages of four or more determinations, each performed in duplicate. Where appropriate, values are presented ± S.E. For the figures, curves were fitted by eye. To evaluate the kinetic parameters, least-squares regression lines were calculated for linear Lineweaver-Burk and Hill plots [16].

Frozen dog kidneys were obtained from Pel-Freeze; ATP, CTP, nitrophenyl phosphate, acetyl phosphate, oligomycin, Triton X-100, Lubrol WX and Tween 20 from Sigma; and digitonin and ammonium orthovanadate from Fisher.

Results

As previously shown by Winter and associates [14,18,20], digitonin inhibited enzyme activity in a dose-dependent fashion, with the $(Na^+ + K^+)$-ATPase reaction far more sensitive than the K^+-phosphatase reaction (Fig. 1). For both reactions the inhibition was reversible (i.e., activity returned to control levels after dilution, centrifugation to collect the particulate enzyme, and resuspension): the onset of inhibition was essentially instantaneous (i.e., no difference could be determined between incubation begun 5 s or 10 min after mixing enzyme and digitonin); and the extent of inhibition did not change during the incubation times used here.

Oligomycin, although a potent inhibitor of the $(Na^+ + K^+)$-ATPase reaction, did not inhibit the K^+-phosphatase reaction in the standard incubation medium (Fig. 2) using nitrophenyl phosphate as substrate. However, treatment of the enzyme with digitonin conferred sensitivity to oligomycin (Fig. 2) comparable to that of the ATPase reaction. The lack of sensitivity of the phosphatase reaction in the absence of digitonin occurred whether the enzyme was first equilibrated with oligomycin for 10 min at 37°C or added at the beginning of the incubation: similarly, the increased sensitivity in the presence of digitonin occurred whether the enzyme was equilibrated with oligomycin for 10 min or

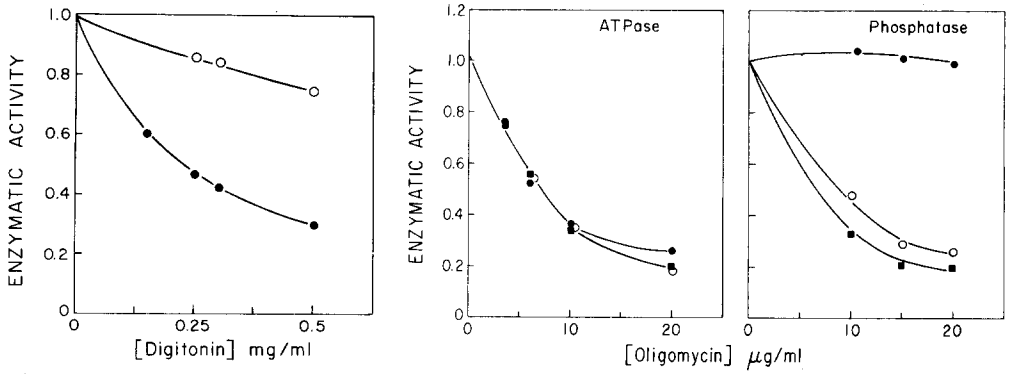


Fig. 1. Inhibition of enzymatic activity by digitonin. The enzyme preparation was first incubated in the standard incubation medium for the $(Na^+ + K^+)$-ATPase or K^+-phosphatase reaction without substrate but with the concentrations of digitonin indicated, for 8 min at 37°C, as described in Methods and Materials. The enzymatic reaction was then initiated by addition of either ATP or nitrophenyl phosphate and incubation continued for a further 6—12 min. $(Na^+ + K^+)$-ATPase (●) and K^+-phosphatase (○) activities are presented relative to control values measured concurrently in the absence of digitonin, defined as 1.0.

Fig. 2. Inhibition of enzymatic activity by oligomycin. In the left-hand panel are shown experiments measuring $(Na^+ + K^+)$-ATPase activity in the standard medium containing, in addition, the concentrations of oligomycin indicated, for untreated enzyme preaprations (●) or those treated with 0.5 mg digitonin/ml incubation medium (○) or 0.08 μl Triton X-100/ml incubation medium (■), as described in Methods and Materials. In each case the change in enzymatic activity with oligomycin is presented relative to concurrent incubations in the absence of oligomycin defined as 1.0. In the right-hand panel are shown corresponding experiments measuring the K^+-phosphatase activity in the standard medium plus the concentration of oligomycin indicated for untreated (●), digitonin-treated (○) or Triton-treated (■) enzyme preparations, as above. Again, the change in activity with oligomycin concentration is plotted relative to control incubations for treated and untreated enzyme in the absence of oligomycin, each defined as 1.0.

added at the beginning of the incubation.

The K^+-phosphatase reaction also hydrolyzes acetyl phosphate. With this substrate hydrolysis was again insensitive to oligomycin in the absence of digitonin, but became sensitive in the same range of digitonin concentrations (data not presented).

To determine whether this striking alteration in sensitivity to oligomycin was unique to digitonin, the effects of several non-ionic detergents on these processes were examined. Triton X-100 also conferred on the K^+-phosphatase reaction sensitivity to oligomycin (Fig. 2). In contrast to digitonin, however, Triton inhibited the K^+-phosphatase reaction more than the $(Na^+ + K^+)$-ATPase reaction (Fig. 3). Inhibition of both reactions was dose-dependent, reversible, instantaneous in onset and constant throughout the incubation period. Tween 20 (final concentration 1%, v/v) and Lubrol-WX (final concentration 0.1%, w/v) produced similar results.

To explore the implications of this induced sensitivity to oligomycin, the effects of digitonin and Triton on other enzyme-ligand interactions were examined. Both digitonin and Triton increased the $K_{0.5}$ for K^+ at the moderate-affinity α-sites of the enzyme [21] through which the K^+-phosphatase reaction is activated (Fig. 4, Table I), but had little effect on the $K_{0.5}$ for K^+ at the high-affinity β-sites [21] through which the $(Na^+ + K^+)$-ATPase reaction is activated (Fig. 5, Table II). The reagents increased the $K_{0.5}$ for Na^+ in the $(Na^+ + K^+)$-ATPase reaction slightly (Table II). Although the K_m for nitrophenyl phos-

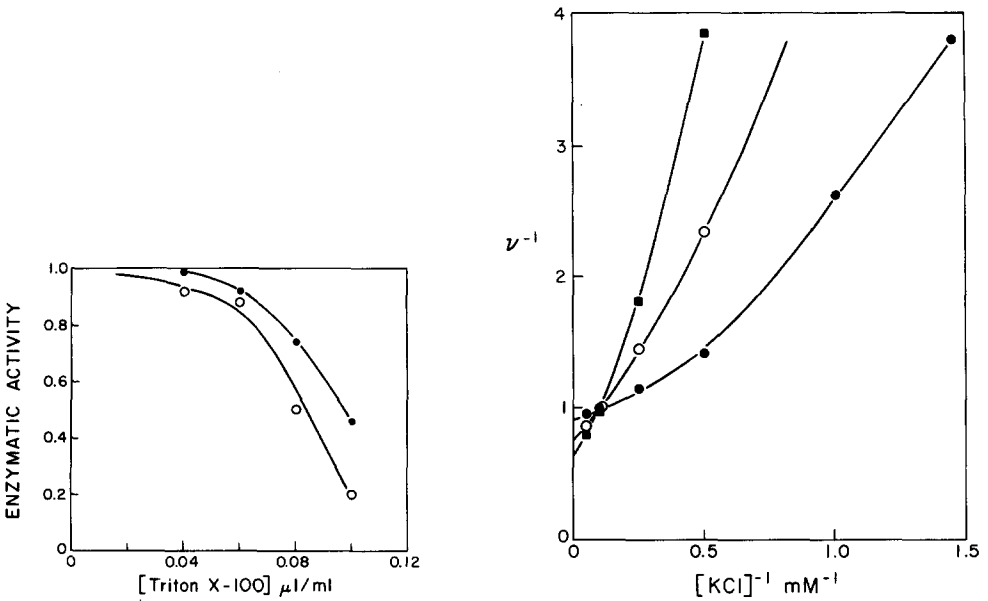


Fig. 3. Inhibition of enzymatic activity by Triton X-100. The enzyme preparation was incubated in the standard incubation media containing, in addition, the concentrations of Triton indicated, as described in Methods and Materials. ($Na^+ + K^+$)-ATPase (•) and K^+-phosphatase (○) activities are presented relative to control values, measured concurrently in the absence of Triton, defined as 1.0.

Fig. 4. Effects of digitonin and Triton on K^+-activation of the K^+-phosphatase reaction. Incubations were performed in the standard medium but with the concentrations of KCl indicated, with the enzyme untreated (•), treated with 0.5 mg digitonin/ml incubation medium (○) or treated with 0.08 μl Triton X-100/ml incubation medium (■). Data are presented in double-reciprocal form: for ease of comparison the activity in the presence of 10 mM KCl is defined as 1.0 in each case.

phate in the K^+-phosphatase reaction was unaffected, both reagents produced small decreases in the K_m for ATP at the low-affinity substrate sites [22] in the ($Na^+ + K^+$)-ATPase reaction (Table II).

To determine if an increase in $K_{0.5}$ for K^+ at the α-sites alone could confer

TABLE I

EFFECTS OF DIGITONIN AND TRITON X-100 ON THE KINETIC PARAMETERS OF THE K^+-PHOSPHATASE REACTION

Experiments were performed as in Fig. 4, in the absence of Na^+ plus CTP, or in media containing, in addition, 10 mM NaCl plus 0.3 mM CTP. The kinetic parameters were evaluated by least-squares linear regression of Hill plots of the data [16].

Enzyme treatment	Kinetic parameter			
	Without (Na^+ + CTP)		With (Na^+ + CTP)	
	V	$K_{0.5}$ for K^+ (mM)	V	$K_{0.5}$ for K^+ (mM)
Untreated (control)	1.10 ± 0.03	1.4 ± 0.2	0.83 ± 0.05	0.08 ± 0.02
Digitonin-treated	1.01 ± 0.07	3.7 ± 0.4	0.77 ± 0.06	0.32 ± 0.07
Triton-treated	0.81 ± 0.06	6.2 ± 0.9	—	—

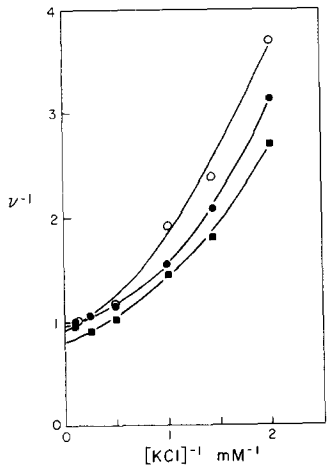


Fig. 5. Effects of digitonin and Triton on K^+-activation of the $(Na^+ + K^+)$-ATPase reaction. Experiments were performed and the data are presented as in Fig. 4, except that the enzymatic activity measured is the $(Na^+ + K^+)$-ATPase reaction: incubations were in the standard medium for the reaction but with the concentrations of KCl indicated for untreated (●), digitonin-treated (○) and Triton-treated (■) enzyme preparations.

oligomycin sensitivity on the K^+-phosphatase reaction, the enzyme preparation was reacted with acetic anhydride [19]. This treatment increased the $K_{0.5}$ for K^+ in the K^+-phosphatase reaction to 3.4 mM [19]. Nevertheless, the K^+-phosphatase reaction catalyzed by the treated enzyme preparation was still insensitive to oligomycin, although subsequent treatment with digitonin could still produce such sensitivity to oligomycin.

Alternatively, the effects of digitonin and Triton may be interpreted as a shift in conformational states away from those binding K^+ at the α-sites to those binding ATP at the low-affinity substrate sites and those conferring sensitivity to oligomycin. Consequently, the effects of digitonin and Triton were pursued in terms of three other interactions that also appear to reflect

TABLE II

EFFECTS OF DIGITONIN AND TRITON X-100 ON THE KINETIC PARAMETERS OF THE $(Na^+ + K^+)$-ATPase REACTION

Experiments were performed as in Fig. 5, in which KCl, NaCl and ATP were successively varied in the presence of fixed concentrations of the other reactant. The kinetic parameters were evaluated [16] by least-squares linear regression of the data plotted in the form of Hill plots (for Na^+ and K^+) or Lineweaver-Burk plots (for ATP).

Enzyme treatment	Kinetic parameter			
	V	$K_{0.5}$ for ATP (mM)	$V_{0.5}$ for K^+ (mM)	$K_{0.5}$ for Na^+ (mM)
Untreated (control)	1.05 ± 0.02	0.45 ± 0.04	0.8 ± 0.1	7.9 ± 1.2
Digitonin-treated	0.34 ± 0.03	0.36 ± 0.03	0.9 ± 0.1	11.8 ± 2.4
Triton-treated	0.93 ± 0.08	0.33 ± 0.05	0.8 ± 0.2	9.8 ± 1.9

TABLE III

EFFECTS OF DIGITONIN AND TRITON X-100 ON INHIBITORS OF THE K^+-PHOSPHATASE REACTION

K^+-phosphatase activity was measured, as in Fig. 2, in the presence of 10 or 20 mM KCl and the inhibitors, ATP, phosphate and vanadate. Inhibition is calculated relative to the corresponding incubation without that inhibitor.

Enzyme treatment	KCl concentration (mM)	Percent inhibition with		
		ATP (0.3 mM)	Phosphate (2 mM)	Vanadate (0.1 μM)
Untreated (control)	10	57 ± 2	42 ± 3	59 ± 1
	20	44 ± 3	44 ± 2	69 ± 1
Digitonin-treated	10	79 ± 3	22 ± 4	38 ± 3
	20	71 ± 2	22 ± 3	47 ± 3
Triton-treated	10	70 ± 2	29 ± 3	48 ± 2
	20	69 ± 4	34 ± 3	58 ± 4

specific conformational states of the enzyme: inhibition of the K^+-phosphatase reaction by ATP, by inorganic phosphate and by vanadate.

ATP inhibits the K^+-phosphatase reaction, competitively toward nitrophenyl phosphate, with a K_i consistent with its occupying the low-affinity substrate sites [22]. Higher concentrations of K^+ decrease this inhibition (Table III) and increase the K_m for ATP at the low-affinity substrate sites in the ($Na^+ + K^+$)-ATPase reaction [16,23]. Both digitonin and Triton increased inhibition of the K^+-phosphatase reaction by ATP (Table III).

Inorganic phosphate is also a competitive inhibitor of the K^+-phosphatase reaction [24], but in this case sensitivity to the inhibitor is not affected by increasing the K^+ concentration (Table III). Both digitonin and Triton decreased inhibition by phosphate (Table III).

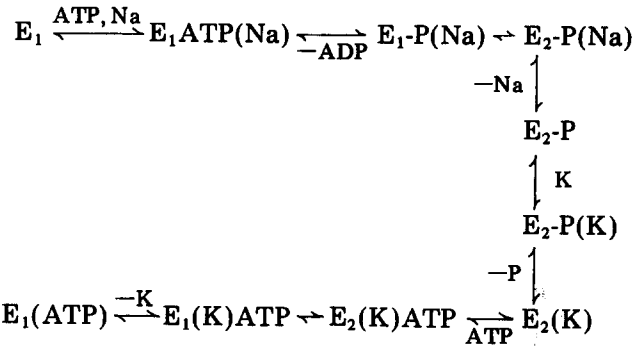
Vanadate is a potent inhibitor of both the K^+-phosphatase and ($Na^+ + K^+$)-ATPase reactions, with higher concentrations of K^+ augmenting the inhibition [25]. Both digitonin and Triton decreased inhibition by vanadate in the K^+-phosphatase reaction (Table III) and in the ($Na^+ + K^+$)-ATPase reaction (data not presented).

At low K^+ concentrations the K^+-phosphatase reaction is stimulated by certain nucleotides, including ATP and CTP, when Na^+ is also present [17]. One hypothesis [12,26] to account for the decrease in the $K_{0.5}$ for K^+ that underlies the stimulation proposes that, in an enzyme composed of two catalytic subunits and with one high- and one low-affinity substrate site, the nucleotide in the presence of Na^+ can phosphorylate the enzyme through its high-affinity substrate site, thereby making available the high-affinity β-sites for K^+ not otherwise available to the K^+-phosphatase reaction. The phosphatase substrate would be hydrolyzed at the low-affinity substrate site on the alternate catalytic subunit. Liang and Winter [20] have provided strong evidence that digitonin dissociates the two catalytic subunits of the enzyme, consistent with marked inhibition of the ($Na^+ + K^+$)-ATPase reaction (which requires both substrate sites) and relative sparing of the K^+-phosphatase reaction (which may

require only one). If the above hypothesis and interpretation are correct, then digitonin would preferentially inhibit the stimulation of the K^+-phosphatase reaction due to nucleotide plus Na^+. This was the case (Table I): digitonin increased the $K_{0.5}$ for K^+ from the range of the β-sites, available in the presence of CTP plus Na^+, toward the range of the α-sites, normally available in the K^+-phosphatase reaction in the absence of nucleotide plus Na^+, with little effect on V. The interpretation is supported by the relatively small effect of digitonin and Triton on the $K_{0.5}$ for K^+ at the β-sites measured in the ($Na^+ + K^+$)-ATPase reaction (Table II), and the identification of the activating sites for K^+ in the presence of nucleotide plus Na^+ as β-sites: the latter is supported not only by the apparent affinity for K^+, but also in terms of the differential sensitivity to pH and to modifers [21,26] (in these experiments CTP was used as the activating nucleotide because in the absence of Na^+ inhibition by 0.3 mM CTP was negligible in the absence or presence of digitonin).

Discussion

Recent formulations [22,27,28] of the reaction sequence for the ($Na^+ + K^+$)-ATPase incorporate not only the progression of E_1, E_1-P, E_2-P and E_2 states of the enzyme, but also the sequential filling of high- and low-affinity substrates sites (K_D values for ATP approx. 0.1 μM and 0.1 mM, respectively) with their different functional roles. Whereas ATP at the high-affinity substrate site can phosphorylate the enzyme, ATP at the low-affinity substrate site can decrease inhibition by K^+ at moderate-affinity inhibitory sites (perhaps identical with the α-sites): conversely, K^+ at such sites increases the K_m for ATP at the low-affinity sites [16,23]. In addition, studies [29] on tryptic digestion of the enzyme reveal one pattern of hydrolysis in the presence of K^+ (reflecting the K-form of the enzyme, termed 'E(K)'), and another in the absence of K^+ or the presence of Na^+ (reflecting the Na-form of the enzyme, termed 'E(Na)'). Moreover, addition of ATP can convert the tryptic hydrolytic pattern in the presence of K^+ to that corresponding to the Na-form. Studies on the effects of Na^+ and K^+ on intrinsic protein fluorescence [30] support this classification. These experiments thus confirm the antagonism between K^+ and ATP, and emphasize the relationship of E_1 to the E(Na) form and of E_2 to the E(K) form. An expanded reaction sequence to incorporate these interactions was proposed by Karlish et al. [28].



How the K^+-phosphatase fits into this reaction scheme is not certain, but the requirement for K^+ and the inhibition by ATP would indicate that the reaction proceeds with the E_2 or E(K) forms of the enzyme. The insensitivity to oligomycin could then be explained by this reagent interacting only with the E_1 or E(Na) form of the enzyme (recent experiments [28,31] demonstrating effects of oligomycin in the absence of enzyme phosphorylation make a requirement for E_1-P as the sole reacting enzyme species untenable). In accord with this formulation is the sensitization of the K^+-phosphatase reaction to oligomycin in the presence of Na^+ and of Na^+ plus nucleotide [7—9].

The experiments described here, with digitonin and the non-ionic detergents, Triton, Tween and Lubrol, suggest that these agents favor the E_1 or E(Na) enzyme state over the E_2 or E(K) enzyme state. (i) Sensitivity to oligomycin is induced in the presence of K^+ but in the absence of Na^+ and of nucleotide. (ii) The $K_{0.5}$ for K^+ is increased at the α-sites, equated [21,26] with the inhibitory and state-determining K^+ sites. (iii) The K_m for ATP at the low-affinity substrate sites is decreased and inhibition of the K^+-phosphatase reaction by ATP at the low-affinity substrate sites is increased. (iv) Inhibition by inorganic phosphate is decreased, consistent with inorganic phosphate, in the reaction scheme above, favoring the E_2-P form (indeed, the enzyme can be phosphorylated by inorganic phosphate [5]). (v) Inhibition by vanadate is also decreased: Cantley et al. [25] proposed that vanadate is a potent inhibitor of the enzyme by its mimicking the transition state of phosphate during hydrolysis of the phosphoenzyme complex (inhibition by vanadate is stimulated by K^+ [25] at sites with properties characteristic of the α-sites (Robinson, J.D., unpublished observations)).

Although these characteristics strongly imply that digitonin and Triton favor the E_1 or E(Na) conformational states of the enzyme over the E_2 or E(K) states, the mechanism by which they act is not clear. An increase in the $K_{0.5}$ for K^+ at the α-sites could account for the transformations, but this need not be the sole mode of action. Indeed, acetic anhydride, which increases the $K_{0.5}$ for K^+ at the α- but not the β-sites [19], failed to confer sensitivity to oligomycin on the K^+-phosphatase reaction. Nevertheless, two other agents that reduce the $K_{0.5}$ for K^+ at the α-sites, dimethylsulfoxide and phlorizin [21], appear to favor the E_2 or E(K) form over the E_1 or E(Na) form, as indicated by decreased sensitivity of the K^+-phosphatase reaction to ATP and increased sensitivity to inorganic phosphate (Robinson, J.D., unpublished observations).

A drawback to this overall formulation is the lesser sensitivity of the K^+-phosphatase reaction than that of the (Na + K)-ATPase to digitonin: the argument presented above, that digitonin favors the E_1 or E(Na) form, should imply that the K^+-phosphatase reaction is at least as sensitive as the (Na^+ + K^+)-ATPase reaction. That this is not the case may reflect an additional action, proposed by Liang and Winter [20]: digitonin alters subunit interactions, as manifested in chemical crosslinking experiments, and these subunit interactions may be more crucial for the (Na^+ + K^+)-ATPase than for the K^+-phosphatase reaction. The further sensitivity of the K^+-phosphatase reaction in the presence of Na^+ and a nucleotide, a reaction process that may require subunit interactions [12,26], is consistent with this interpretation. Moreover, digitonin would not be expected to abolish selectively the stimulation of the K^+-phosphatase

reaction by nucleotide plus Na^+ if the effect of digitonin were confined to favoring the E_1 or E(Na) conformational states: such stimulation requires that Na^+ and nucleotide bind to the high-affinity substrate site in order to phosphorylate the enzyme, and this binding is favored in the E_1 or E(Na) conformational states.

An alternative explanation for the lesser sensitivity of the K^+-phosphatase reaction to digitonin might lie in the differential slowing of the two transitions from E_1 to E_2: transition (i) from E_1-P to E_2-P being slowed more than transition (ii) from $E_1 + K$ to $E_2(K)$. Since the first of these transitions is part of the $(Na^+ + K^+)$-ATPase reaction cycle (and also of the stimulation of the phosphatase reaction by nucleotide plus Na^+), whereas the second is involved in activation of the K^+-phosphatase reaction, digitonin would then inhibit the K^+-phosphatase reaction less than the $(Na^+ + K^+)$-ATPase reaction (or the phosphatase reaction in the presence of nucleotide plus Na^+). Conversely, Triton, which inhibited the K^+-phosphatase reaction more than the $(Na^+ + K^+)$-ATPase reaction, might act by slowing the second transition from E_1 to E_2 more than the first. With both Triton and digitonin, however, sensitivity of the K^+-phosphatase reaction to oligomycin would result from slowing the transition from E_1 (oligomycin-reactive) to E_2K (oligomycin-resistant) forms.

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