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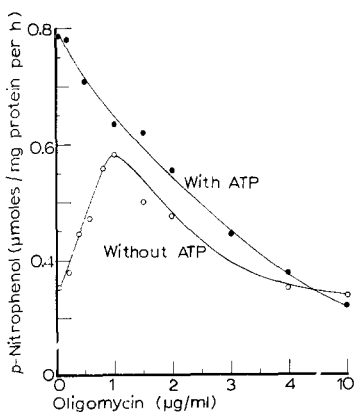
Similar modifying effects of oligomycin and ATP on the K^+-dependent *p*-nitrophenylphosphatase activity of the (Na^+ + K^+)-activated ATPase

A modifying effect of ATP on the (Na^+ + K^+)-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) was first proposed by SKOU[1] and subsequently by others[2-4]. Evidence in support of such a role of ATP was provided by the demonstration of an activating effect of ATP on the K^+-dependent *p*-nitrophenylphosphatase activity which is considered to be a component of the (Na^+ + K^+)-ATPase complex[5-6]. It has been shown that oligomycin, an inhibitor of the (Na^+ + K^+)-ATPase, has no effect on the K^+-dependent *p*-nitrophenylphosphatase but inhibits the ATP activation of the *p*-nitrophenylphosphatase[6]. Based on these results, we suggested that oligomycin interferes either with the binding of ATP to the modifying site of the enzyme complex, or with the activating process which follows ATP binding[6]. The purpose of this communication is to present our recent data which indicate that oligomycin not only blocks the activating effect of ATP but that under proper conditions it can, like ATP, also activate the *p*-nitrophenylphosphatase.

Preparation of the enzyme and assays were described before[6]. Substrates, oligomycin and ouabain were obtained from Sigma Chemical Co. (St. Louis, Mo.). Fig. 1 shows the effects of varying concentrations of oligomycin on the *p*-nitrophenylphosphatase activity in the presence and absence of a fixed concentration of ATP. Fig. 2 contains the data on the effects of different concentrations of ATP on the enzyme activity in the presence and absence of oligomycin. From the results the following points are evident. (1) Either oligomycin or ATP alone can stimulate the activity. The effects of the two compounds are qualitatively similar. Both activation curves have peaks, and at higher concentration of each compound the activating effect is



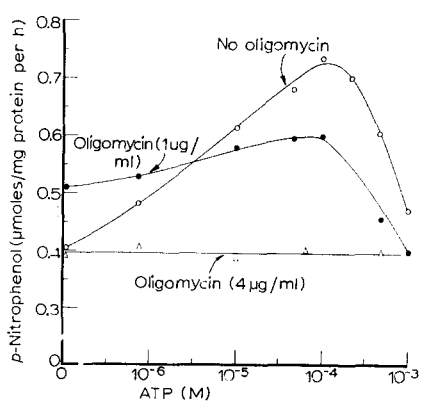


Fig. 1. Effects of varying concentrations of oligomycin on the *p*-nitrophenylphosphatase activity in the presence and absence of ATP (0.1 mM). Each reaction mixture contained 40 mM Tris–HCl (pH 7.4), 4 mM *p*-nitrophenyl phosphate, 4 mM $MgCl_2$, 20 mM NaCl, 0.4 mM KCl, 0.3 mg of enzyme protein, and the indicated amounts of oligomycin and ATP in a final volume of 2.5 ml.

Fig. 2. Effects of varying concentrations of ATP on the *p*-nitrophenylphosphatase activity in the presence and absence of oligomycin. The reaction conditions were the same as described for Fig. 1.

lost. (2) Maximum activation by oligomycin never approaches that obtained with ATP. (3) The activating effect of ATP, over a wide range of ATP concentrations, is blocked either partially or completely by increasing concentrations of oligomycin. These results suggest the existence of a common modifying site on the enzyme complex for oligomycin and ATP. Assuming this, and considering the estimated molecular weight of oligomycin[7,8], it would seem that the apparent affinity of oligomycin for the modifying site is much greater than that of ATP. The higher maximum activating effect obtained with ATP suggests, however, that ATP is more efficient in producing the modification which leads to the activation of the K^+-dependent hydrolytic step.

The data presented in Table I show the necessity of the presence of both Na^+

TABLE I

DEPENDENCE OF ACTIVATING EFFECTS OF OLIGOMYCIN AND ATP ON THE PRESENCE OF Na^+ AND K^+

Reaction conditions were the same as those described for Fig. 1.

Alkali cation	*p-Nitrophenylphosphatase activity (μmoles p-nitrophenol per mg protein per h)*		
	No activators	*Oligomycin (1 μg/ml)*	*ATP (0.1 mM)*
None	0.35	0.35	0.31
Na^+ (20 mM)	0.39	0.38	0.33
K^+ (0.4 mM)	0.42	0.40	0.37
Na^+ (20 mM) + K^+ (0.4 mM)	0.46	0.85	0.98
Na^+ (20 mM) + K^+ (0.4 mM) + ouabain (1 mM)	0.34	0.36	0.32

and K^+ for the activating effect of oligomycin and demonstrate the sensitivity of the oligomycin activation to ouabain. Similar cation requirements were observed before for the effects of ATP on the enzyme[6] and are also included in Table I for comparison. YOSHIDA *et al.*[9] have shown that the Na^+-dependent activating effect of ATP on the *p*-nitrophenylphosphatase is accompanied by a decrease in the apparent K_m for K^+. Our studies, not presented here, indicate that the same is true about the effect of oligomycin. It is appropriate to point out that due to this fact the activating effect of oligomycin can only be shown in the presence of low concentrations of K^+ such as those used in the above experiments. From the data presented here and in the context of the working hypothesis suggested before[6], it may be concluded that the binding of either ATP or oligomycin to the modifying site requires Na^+ and that the consequence of this event is an increase in the activating effect of K^+ on the hydrolytic step.

The demonstration of similar modifying effects of oligomycin and ATP on the ($Na^+ + K^+$)-ATPase complex gives further support to the proposal[1] that Na^+-dependent ATP binding to the enzyme complex, rather than phosphorylation of the enzyme by ATP, may be the primary event in producing certain conformational changes in the membrane, and perhaps in the process of Na^+ translocation through the membrane.

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Purification and mode of action of a lytic enzyme from *Bacillus cereus*

We have described earlier a lytic enzyme which is formed in ultraviolet-irradiated cultures of *Bacillus cereus*[1]. The enzyme is able to hydrolyze the isolated *B. cereus* cell wall[2]. After induction by ultraviolet irradiation, the enzyme activity greatly increases in the culture media, and we have attempted the isolation of the lytic enzyme.

The lytic activity is expressed in arbitrary units, 1 unit of the enzyme is defined as the amount which leads to a decrease of 0.001 unit of absorbance in 20 min at 20° when present in 4 ml of a cell-wall suspension having an initial absorbance of 0.5. Details of the test were described earlier[2].

B. cereus was grown on a casamine medium[1], induced by ultraviolet irradiation and harvested 90–100 min after induction. The cells were resuspended in one-tenth of the original volume and sonicated[1]. The disintegrated cells were recombined with the supernatant culture fluid and thus a crude extract was obtained. 2.5 l of the crude extract were mixed with 2.8 l of acetone (−5°) and centrifuged at 0°. The precipitate was discarded, and the enzyme precipitated from the supernatant by further addition of 2.15 l of cold acetone. The precipitate was dissolved in 250 ml of 0.01 M Tris–HCl buffer (pH 7.8). This was Fraction 1.

Further purification was achieved by adding 150 ml of acetone to the solution obtained in the previous step. The precipitate of the inactive proteins was removed by centrifugation, and the enzyme was precipitated from the supernatant by the addition of a new portion of 150 ml of acetone. The precipitate was dissolved in 25 ml of 0.01 M Tris–HCl buffer (pH 7.6) and dialyzed against the same buffer solution at 3–5° for 24 h (Fraction 2).

DEAE-cellulose chromatography was performed (Fig. 1) applying 45 mg protein obtained in the previous step to the column. Elution was performed by Tris–HCl