

BBA Report

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The binding of ouabain to $\text{Na}^+ - \text{K}^+$ -dependent ATPase treated with phospholipase

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SUMMARY

1. An ($\text{Na}^+ - \text{K}^+$)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) preparation treated with phospholipase showed nearly the same kinetics of [^3H] ouabain binding as those of the control.
2. Phospholipase-treated ATPase preparation which bound ouabain showed no activation of ($\text{Na}^+ - \text{K}^+$)-ATPase activity in the presence of phospholipids.
3. The experiments suggested that the binding of ouabain to ($\text{Na}^+ - \text{K}^+$)-ATPase did not require phospholipids directly.

When an $\text{Na}^+ - \text{K}^+$ -dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) preparation from ox brain microsomes was treated with phospholipase A (phosphatide acylhydrolase, EC 3.1.1.4), its ATPase activity was reduced to 20–25% of the control value and approximately 70% of phospholipids in the preparation were changed to lyso-compounds. When the preparation was treated with phospholipase C (phosphatidylcholine choline-phosphohydrolase, EC 3.1.4.3), both the ATPase activity and the content of phospholipids of the preparation were reduced to 40% of the control value, respectively. After the preparation had been treated with phospholipase A or C, its ATPase activity was reactivated to nearly the control value by addition of acidic phospholipids. Treatment with phospholipase A reduced the rate of phosphorylated enzyme formation by about 60% and reduced the rate of phosphorylated enzyme decomposition by more than 90%. The rates of formation and decomposition of the phosphorylated enzyme were increased to the control level and to 40% of the control level, respectively, by addition of acidic phospholipids. From these results, Taniguchi and Tonomura¹ have proposed that the role of phospholipids in ($\text{Na}^+ - \text{K}^+$)-ATPase is to give a negative charge and hydrophobicity in the vicinity of the active site of ATPase, rather than as components of the active site itself.

Abbreviation: GEDTA, glycoletherdiaminetetraacetic acid.

In this report, the binding of ouabain to $(\text{Na}^+-\text{K}^+)\text{-ATPase}$ treated with phospholipase will be described.

The method of partial purification of $(\text{Na}^+-\text{K}^+)\text{-ATPase}$ was reported previously¹. The ATPase preparation was treated with phospholipase A prepared from *Naja naja* venom according to Imai and Sato² or phospholipase C (*Clostridium wellchii*) in incubation mixture containing 1 mg/ml ATPase protein, 0.1 mg/ml phospholipase A or 0.4 mg/ml phospholipase C, 2.95 M sodium acetate or 1.1 M sucrose, 29.5 mM Tris, 67.5 mM 2-mercaptoethanol, 0.07 mM EDTA and 11.2 mM CaCl_2 (pH 7.4) at 37°. The treatment was terminated by addition of GEDTA (glycoletherdiaminetetraacetic acid)-Tris-HCl buffer (pH 7.4) at a final concentration of 28.6 mM GEDTA and 190 mM Tris.

Binding of [³H] ouabain to the ATPase was measured in 1 ml of reaction medium by the method of Barnett³ with slight modifications. The ATPase preparation (0.067 mg/ml) was preincubated with 5 mM MgCl_2 , 140 mM NaCl, 196 mM sodium acetate, 14 mM KCl, 3 mM ATP, 32 mM sucrose, 24.6 mM Tris, 4.5 mM 2-mercaptoethanol, 1.91 mM GEDTA and 0.745 mM CaCl_2 (pH 7.4) at 37° for 5 min. The reaction was started by addition of [³H] ouabain ($4 \cdot 10^8$ counts/min per μmole) at the final concentration of 1.75 μM . At various time intervals, cold unlabeled ouabain (pH 7.4 with 40 mM Tris-HCl) was added to the reaction medium at the final concentration of 5 mM to stop the reaction. The enzyme suspension was immediately filtered through a membrane filter (pore size 0.45 μm) and washed with 100 ml of cold 100 μM ouabain (pH 7.4) and 75 ml of cold water. The ³H present on the filter was counted after dissolving in 12 ml of Bray's⁴ solution.

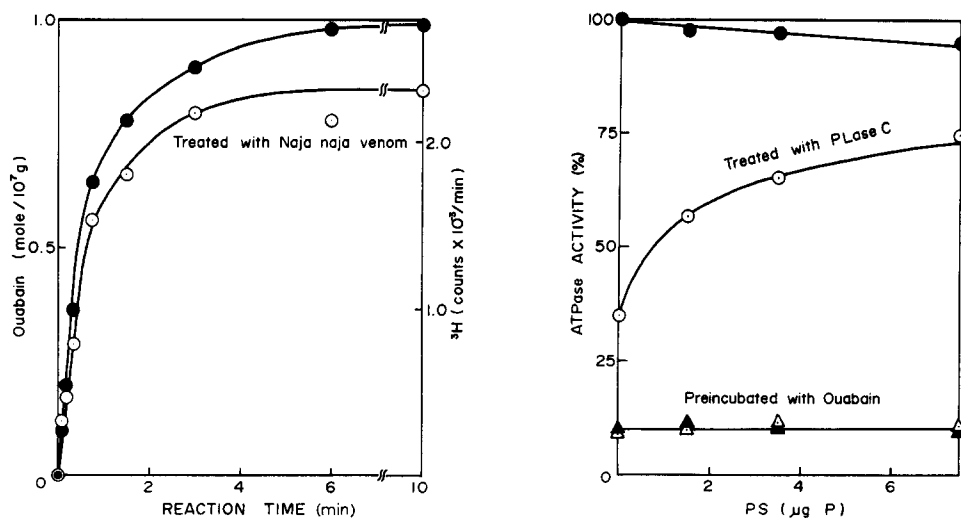


Fig. 1. Ouabain binding to $(\text{Na}^+-\text{K}^+)\text{-ATPase}$. Amount of ouabain bound to 10^7 g protein; ○-○, phospholipase A-treated for 15 min; ●-●, control preparation.

Fig. 2. Reactivation of $(\text{Na}^+-\text{K}^+)\text{-ATPase}$ activity by phosphatidylserine (PS) in the presence or absence of ouabain. ATPase preparation (0.1 mg/ml) treated with phospholipase C for 2 h was incubated with 5 mM MgCl_2 , 140 mM NaCl, 3 mM ATP, 32 mM sucrose and 0.5 μM ouabain (pH 7.4) at 37° for 10 min. ATPase activity was measured 10 μg /ml ATPase protein, 5 mM MgCl_2 , 140 mM NaCl, 14 mM KCl and various concentrations of phosphatidylserine in the absence or presence of 0.05 μM ouabain. ○-○, △-△, phospholipase C-treated; ●-●, ▲-▲, control; △-△, ▲-▲, preincubated with ouabain.

The measurement of ATPase activity, the estimation of protein concentration and other details were similar to those reported¹.

The time course of [³H] ouabain binding to (Na⁺-K⁺)-ATPase preparation was shown in Fig. 1. The final amount bound was approximately 1 mole/10⁷ g protein for the control and 0.85 mole/10⁷ g protein for phospholipase A-treated preparation and 0.80 mole/10⁷ g protein for phospholipase C-treated preparation (data not cited here). The time required for half the maximum binding was approximately 30 sec for those preparations, while the ATPase preparation treated with phospholipase A (data not cited here) or C (Fig. 2) showed a marked decrease in (Na⁺-K⁺)-ATPase activity as reported previously¹.

ATPase preparations treated with ouabain showed no activation of the ATPase activity in the presence of phosphatidylserine as shown in Fig. 2, while phospholipase C-treated ATPase preparation showed a marked activation of the (Na⁺-K⁺)-ATPase activity in the presence of phosphatidylserine.

Approximately 70% of phospholipids in the preparation were changed to lyso-compounds by treatment with phospholipase A, while the content of phospholipids was reduced to approximately 40% by treatment with phospholipase C¹. It was interesting that ouabain did bind (Na⁺-K⁺)-ATPase in spite of phospholipase A or C treatment.

These facts strongly supported that the binding of ouabain to (Na⁺-K⁺)-ATPase does not require phospholipids directly and that the role of phospholipids in (Na⁺-K⁺)-ATPase is to give a negative charge and hydrophobicity in the vicinity of the active site of ATPase, rather than as components of the active site itself.

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