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DETERGENT EFFECTS OF KINETIC PROPERTIES OF $(Na^+ + K^+)$-ATPase FROM KIDNEY MEMBRANES

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Studies on $(Na^+ + K^+)$-ATPase generally employ detergents such as SDS and deoxycholate. Under such conditions, the purified enzyme possesses high specific activity. The $(Na^+ + K^+)$-ATPase from kidney membranes was unmasked by deoxycholate and SDS as described by Jørgensen and its kinetic properties were studied. The results suggest that these detergents induce some irreversible alterations in the kinetic properties of the native enzyme. Another detergent, saponin, unmasked the $(Na^+ + K^+)$-ATPase as effectively as did SDS, but it seems to affect the kinetic properties of the native enzyme to a lesser extent.

Introductions

$(Na^+ + K^+)$-ATPase plays a role in the active transport of Na^+ and K^+ across plasma membranes of eucaryotic cells. This membrane-embedded enzyme is composed of two subunits, a catalytic subunit (α) and a smaller subunit (β) [1]. The role of the smaller subunit, a glycoprotein, has not been completely elucidated.

In the native state, the specific activity of $(Na^+ + K^+)$-ATPase in certain membrane preparations is low. Only a small fraction of the total ATPase activity is dependent on Na^+ and K^+, and is inhibited by ouabain [2]. A higher specific activity can be obtained following unmasking and purification of the enzyme by detergents such as deoxycholate and SDS [3,5]. The normal function of $(Na^+ + K^+)$-ATPase requires a hydrophobic environment created by membrane lipids [6], and detergents can modify membrane structure [7,12]. It is thus possible that unmasking and purification of $(Na^+ + K^+)$-ATPase by such detergents is responsible for modification in the regulatory role of the membrane.

Enzyme-membrane interactions of sheep-kidney $(Na^+ + K^+)$-ATPase were studied by analysing the kinetic response of $(Na^+ + K^+)$-ATPase and potassium-dependent phosphatase in the native state and after unmasking by three detergents: deoxycholate, SDS and saponin.

Materials and Methods

Preparation of plasma membrane from sheep kidney

The preparation of microsomal membranes was obtained according to Jørgensen [4] with minor modifications. After homogenization of outer medullary sheep-kidney in a buffer comprising 30 mM histidine/0.25 M sucrose (pH 7.2), the preparation was centrifuged at $1000 \times g$ for 15 min (Beckman JB21). The pellets were washed and the supernatants centrifuged at $4300 \times g$ for 15 min. The new supernatants were recentrifuged at 40000 $\times g$ for 60 min. The pellets was suspended by gentle homogenization in the same buffer as above. The protein concentration was 4.5–5 mg/ml. The preparation was stored at $-30°C$.

Membrane treatment by deoxycholate or SDS

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followed the method of Jørgensen [4]. Saponin treatment was as follows: membranes (0.5 mg/ml protein) were incubated 15 min at 20°C in a buffer comprising 30 mM imidazole (pH 7.5)/0.2 mg/ml saponin.

Detergents were eliminated by high velocity centrifugation [5,13] at 60000 rpm (Spinco L2 65B). In these conditions, 15–18% of SDS remained bound to the proteins.

Kinetic properties of $(Na^+ + K^+)$-ATPase

$(Na^+ + K^+)$-ATPase activities. The activity of $(Na^+ + K^+)$-ATPase was measured by determining the rate of liberation of inorganic phosphate in a medium containing 200 μM free magnesium (magnesium concentration was held constant so that its effect on the enzyme did not vary), 140 mM NaCl, 20 mM KCl, 40 mM histidine (pH 7.5) and various concentrations of ATP (50, 100, 200, 500, 1000 μM). The activity of $(Na^+ + K^+)$-ATPase as measured as the difference in activity with and without addition of ouabain $5 \cdot 10^{-4}$ M. After sample equilibration at 37°C for 5 min, 12.5–25 μg enzymatic protein were added to 2 ml solution. The quantity of phosphate liberated was measured after definite invervals for each concentration of ATP [4].

Na^+-dependent ATPase activities. The stimulation by Na^+ alone of the ATPase activity of membrane $(Na^+ + K^+)$-ATPase was determined in a medium containing 200 μM free Mg^{2+}, 160 mM NaCl 30 mM dimidazole (pH 7.5) and 50 μM [$\gamma - ^{32}$P]ATP. Incubations were performed at 37°C. ATPase activity was estimated from the amount of [^{32}P]P_i released from [γ-^{32}P]ATP following a procedure already described [15]. Na^+-ATPase was determined as the difference between the activity in media with Na^+ and in media with Na^+ and $5 \cdot 10^{-4}$ M ouabain.

Cation affinities.

K^+. The affinity for K^+ was obtained from enzyme activity determination carried out at six different concentrations of K^+ (0.3, 0.5, 1, 2, 10 and 20 mM). The reaction medium contained 160–140 mM NaCl, 200 μM free Mg^{2+} and 1 mM ATP. The same procedure as above was employed.

Na^+. A similar procedure was used with 5 mM K^+ and five concentrations of Na^+ (2, 3, 4, 8 and 20 mM). Choline chloride was used to adjust the ionic strength to 0.16.

Mg^{2+}. Determination of kinetic parameters (V_m and K_m) was carried out at five concentrations of free Mg^{2+}. Potassium and sodium concentrations were kept constant at 20 and 120 mM, respectively. The effect of magnesium on $(Na^+ + K^+)$-ATPase activity was analysed using the curves $1/V_m = f(1/[Mg^{2+}_{free}])$. The affinity constant between ATP and Mg, as determined by a potentiometric technique [16], was found to be 60 μM.

Kinetic properties of K^+ phosphatases

K^+-dependent activity. K^+-dependent phophatase activity was measured as the rate of liberation of fluorescent methyl 7-hydroxycoumarin produced from methyl umbelliferone phosphate [8]. The reaction solution contained 5 mM $MgCl_2$, 8 mM K^+, 20 mM imidazole, 160 mM choline (pH 7.5) and methyl-UMP at various concentrations (0.5, 1, 2, 3, 4 mM). After 5 min equilibration at 37°C, 25 μg membrane suspension were added to the raction solution. Kinetic measurements were determined as a continuous recording at $\lambda_{em} =$ 455 nm. The potassium phosphatase activity was obtained from the difference between activities in the presence and absence of K^+.

K^+ affinities. Potassium affinity was studied in solutions containing 5 mM Mg^{2+}, 1 mM UMP, 20 mM imidazole, 160 mM choline (pH 7.5) and for six K^+ concentrations (0.5, 1, 3, 5, 8 mM) according to the procedure described above.

Phosphorylation by [γ-^{32}P]ATP

Incorporation of ^{32}P was performed at 37°C according to a method similar to that described by Jørgensen [18]. 40–50 μg of protein were incubated for 15 s in the reaction medium 30 mM imidazole (pH 7.5)/200 μM free Mg^{2+}/160 mM Na^+. The final volume was 2 ml. The reaction was started by adding 50 μM [γ-^{32}P]ATP. The reaction was allowed to proceed for 9 s then stopped by adding 2 ml 10% cold trichloroacetic acid. Pellets obtained after centrifugation were washed three times with a solution containing 1% trichloroacetic acid, 0.5 mM ATP and 10 mM P_i and then dissolved in 250 μl 1 M NaOH. Proteins were assayed according to the method of Lowry et al. (as described in Ref. 3) in the sediment. Radioactivity was measured in 10 ml water (Cerenkov effect). The amount of the

phosphorylated intermediate produced in the presence of 160 mM K^+ was substracted from the preceding results.

[^3H]Ouabain binding induced by P_i

This study was performed according to the method described by Hansen [19] with 3 mM Mg^{2+}, 3 mM P_i and 20 mM Tris (pH 7.5; 37°C). [^{3}H] Ouabain had a specific activity of 49 Ci/mol and its purity was 90–95% according to the method of Hansen and Skou [20]. 40–50 μg protein were incubated with $1 \cdot 10^{-7}$ M ouabain in 2 ml reaction medium. After 30 min, the samples were placed in a ice-bath, then centrifuged at 100000 × g for 1 h. The pellets were dissolved in 250 μl 1 M NaOH for 15 min at 60°C. Radioactivity was determined in 10 ml of the scintillant medium. The nonspecific fixation of ouabain was determined in the same medium as above but in the presence of $5 \cdot 10^{-4}$ M ouabain.

Each of the experimental procedures described above were repeated several times on different membrane preparations in order to determine an average representative measurement of the different activities of the enzyme bound to native or unmasked membranes.

Results

Numerous studies performed with deoxycholate or SDS [3,4,21] show that ($Na^+ + K^+$)-ATPase is activated over a narrow range of concentrations and that the enzyme is rapidly inactivated when the concentration of detergent increases. The analysis of the effects of saponin on the unmasking and inactivation of the enzyme indicates the existence of a plateau followed by a progressive phase of inhibition. The optimal concentration under our experimental conditions is close to that reported for SDS (Fig. 1).

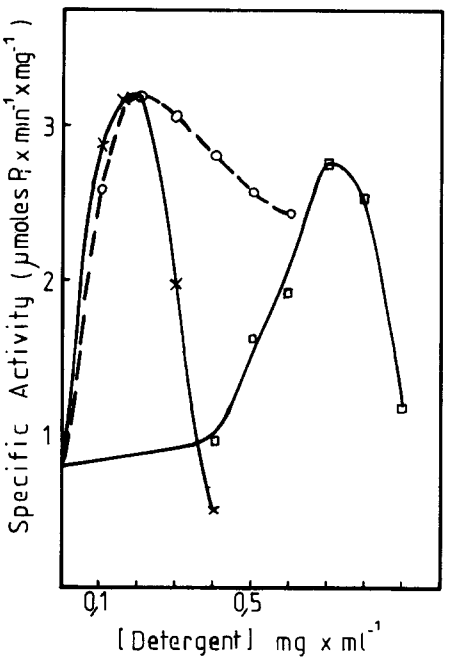

Fig. 1. Activation of ($Na^+ + K^+$)-ATPase as a function of detergent concentration. O——O, Saponin: incubation solution (pH 7.5); 25 mM imidazole; reaction time 15 min at 20°C; protein concentration, 0.5 mg/ml; ×——×, SDS: identical conditions for 30 min; □——□, deoxycholate: incubation medium (pH 7) for 30 min. After the given intervals, 50 μl membrane suspension were added to a 2 ml test-tube containing 140 mM Na^+, 20 mM K^+, 30 mM histidine (pH 7.5, 37°C), 0.2 mM free Mg^{2+} and 1 mM ATP. After 5 min, 80 μl were placed in a coloration tube. The enzymatic activity was measured as the difference between the activity with and without $5 \cdot 10^{-4}$ M ouabain.

TABLE I

DETERMINATION OF KINETIC PARAMETERS FOR NATIVE ($Na^+ + K^+$)-ATPase AND AFTER UNMASKING BY DETERGENTS

	Native ($Na^+ + K^+$)-ATPase	($Na^+ + K^+$)-ATPase + saponin	($Na^+ + K^+$)-ATPase + SDS	($Na^+ + K^+$)-ATPase + deoxycholate
K_m before (μM)	220 ± 15 (7)	216 ± 12 (14)	111 ± 6 (13)	173 ± 14 (7)
K_m after (μM)		223 ± 11 (5)	211 ± 14 (5)	203 ± 10 (3)
V_m [a] (μM $P_i \cdot mg^{-1} \cdot h^{-1}$)	61	180	175	150

[a] In the native state, the specific activity varied from 35 to 80 μM $P_i \cdot mg^{-1} \cdot h^{-1}$. The activation of ($Na^+ + K^+$)-ATPase by detergents varied from 2.5 to 5 according to the preparations.

Kinetic properties of $(Na^+ + K^+)$-ATPase

Enzyme ATP-Mg affinity. Each of the three detergents increases the specific of $(Na^+ + K^+)$-ATPase. However, deoxycholate and SDS induce an increase in the affinity of the enzyme for its substrate (Table I). This process is reversible after elimination of deoxycholate and SDS (Table I). Saponin does not modify the affinity of the enzyme for ATP-Mg at the low-affinity site.

Cation affinity. K^+. Ouabain-sensitive enzymatic activity is measured as a function of potassium concentration. The results shown in Fig. 2 indicate the existence of several K^+ sites. Hill diagrams (Fig. 3) were used to study the degree of cooperativity and the affinity constant for K^+ ($K_{0.5}$). It can be seen that the same degree of cooperativity between the K^+ sites was obtained with and without the detergents. However, deoxycholate and SDS induced an irreversible alteration of the affinity of the enzyme for potassium (Table II). An identical response to K^+ was obtained with native membranes and membranes treated by saponin. Moreover, addition in the assay medium of small amounts of detergents (equal to those remaining bound to the protein) did not change this kinetic parameter.

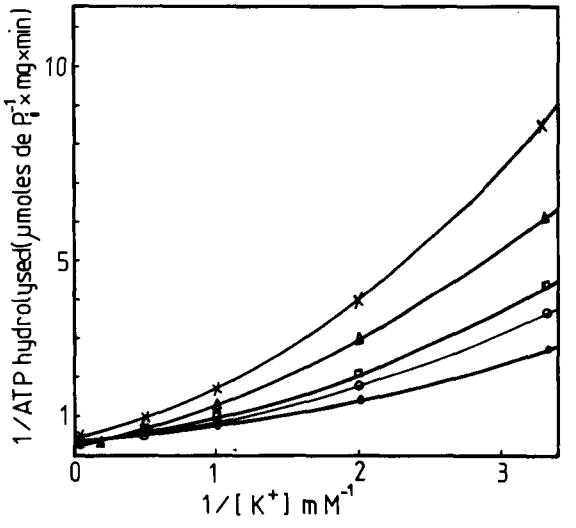

Fig. 2. Lineweaver-Burk plot: $1/v = f(1/[K^+])$. 50 μl of 0.5 mg/ml membrane suspension were added to 2 ml reaction medium containing 1 mM ATP, 0.2 mM free Mg^{2+} (pH 7.5), 30 mM histidine. Na^+ and K^+ were variable. Ionic strength was 0.16, temperature 37°C. After 5 min, 80 μl of reaction medium were added to the coloration tube. Symbols: $(Na^+ + K^+)$-ATPase: □——□, native (200 μl of membrane suspension); ○——○, treated by saponin; ×——×, treated by SDS followed by elimination of SDS; △——△, treated by SDS; ●——●, treated by deoxycholate.

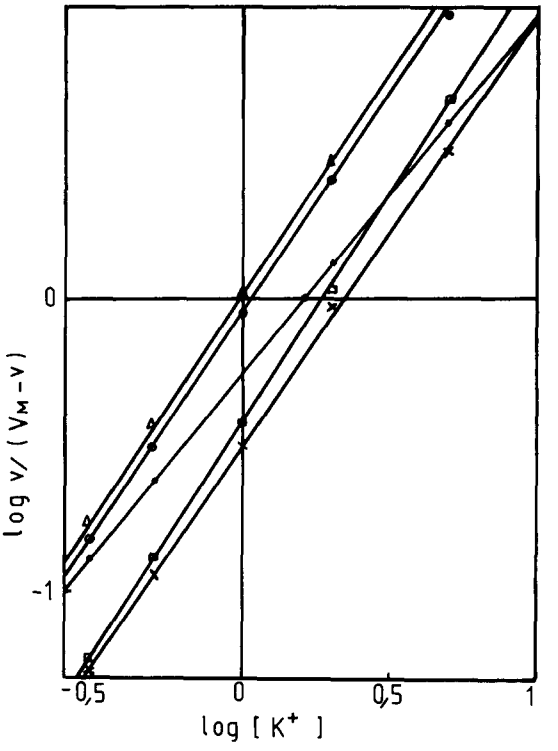

Fig. 3. Determination of Hill's coefficient (n_H) and affinity constant for K^+ by Hill diagrams. Operating conditions described under Fig. 2. Symbols $(Na^+ + K^+)$-ATPase: △——△, native; ○——○, treated by saponin; ●——●, treated by deoxycholate; □——□, treated by SDS followed by elimination of SDS; ×——×, treated by SDS.

Na^+. Regardless of the detergent used, no difference in Na^+ enzyme affinity was observed (Table III).

Mg^{2+}. This cation is considered as the regulator ligand of enzymatic activity [22]. Magnesium acts at the regulatory and catalytic sites [22,23]. For the range of concentrations studied (10–500 μM), free Mg^{2+} produced an increase in enzymatic activity with a decrease in affinity for ATP-Mg. The curves $1/v = f(1/[Mg^{2+}_{free}])$ (Fig. 4) demonstrate that free Mg^{2+} acts primarily at the regulatory site. The results in Table III indicate that membranes treated by deoxycholate and SDS have a higher affinity for free Mg at the regulatory

TABLE II

AFFINITY CONSTANT ($K_{0.5}$) AND HILL'S COEFFICIENT FOR POTASSIUM

DOC, deoxycholate. Number of determinations is in parenthesis.

	$K_{0.5}$ (mM)	n_H
Native $(Na^+ + K^+)$-ATPase (3)	1.02±0.05	1.36±0.05
$(Na^+ + K^+)$-ATPase + saponin (10)	1.13±0.09	1.47±0.07
$(Na^+ + K^+)$-ATPase + SDS (10)	1.85±0.24	1.45±0.07
$(Na^+ + K^+)$-ATPase + DOC (3)	1.45±0.11	1.27±0.04
$(Na^+ + K^+)$-ATPase after elimination of SDS (4)	1.86±0.26	1.44±0.09
$(Na^+ + K^+)$-ATPase after elimination of DOC (2)	1.40	1.20

sites. Their elimination did not change this result. Saponin did not modify the behaviour of native membranes.

Study of phosphatase activity

As in the case of $(Na^+ + K^+)$-ATPase, the detergents produced a similar increase in the specific activity of the K^+-dependent phosphatase. Their presence did not seem to modify the affinity of the enzyme for methyl-UMP, which is equal to 0.87 ± 0.1 mM (Fig. 5).

The apparent affinity of K^+-dependent phosphatase for K^+ is comparable for membranes in the native state and after unmasking by saponin. The affinity is approx. 2.5–3 mM. With SDS a reversible decrease in affinity was observed ($K_{0.5} \approx 5$ mM). Due to the sigmoidal pattern of the curves, a high degree of cooperativity ($n_H \approx 1.4$) can be concluded for the different preparations (Fig. 6, Table IV).

Phosphorylation and Na^+-ATPase activity

Phosphorylation of $(Na^+ + K^+)$-ATPase in an Na^+ medium and in the presence of ATP and Mg^{2+} produced a relatively stable phosphorylated intermediate [24]. The turnover of the phosphorylated intermediate represents Na^+-ATPase activity. In these studies the enzyme was first equi-

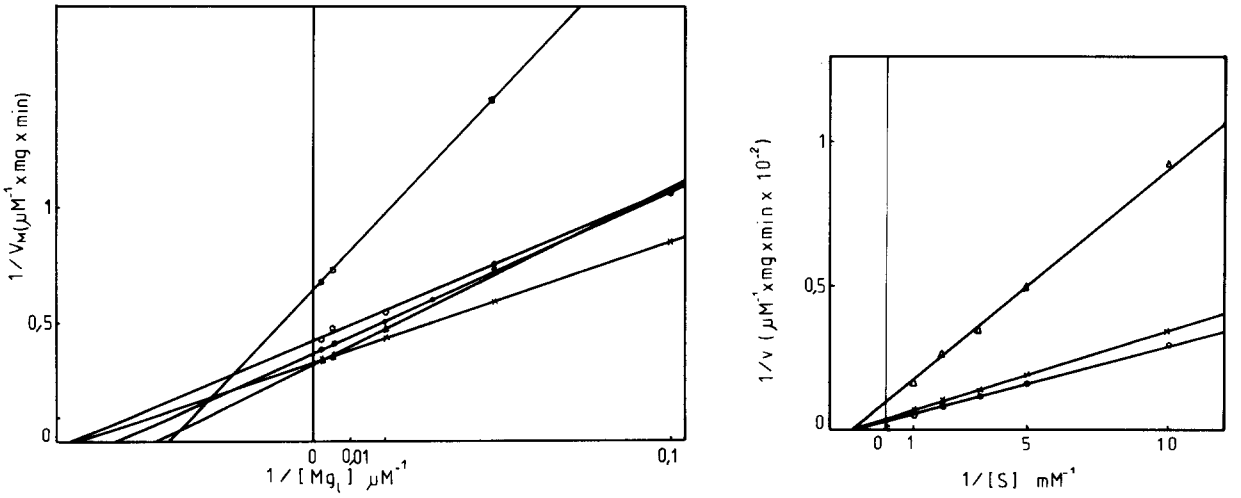

Fig. 4. Lineweaver-Burk plot: $1/V_m = f(1/[Mg^{2+}_{free}])$. Operating conditions were those described for the determination of kinetic parameters. Symbols: $(Na^+ + K^+)$-ATPase: □———□, native (×2); △———△, treated by saponin; ●———●, treated by deoxycholate; ×———×, treated by SDS, ○———○, treated by SDS followed by elimination of SDS.

Fig. 5. Lineweaver-Burk plot: $1/v = f(1/[\text{methyl-UMP}])$. K^+-phosphatase: △———△, native; ○———○, treated by saponin; ×———×, treated by SDS. 50 μl membrane suspension (0.5 mg/ml) were added to the reaction medium (2 ml) containing 1 mM UMP; 5 mM Mg^{2+}; 20 mM imidazole; 160 mM choline chloride (pH 7.5 at 37°C). Concentrations of K^+ were variable. The phosphatase activity was measured as the difference between the activities with and without K^+.

TABLE III

AFFINITY CONSTANT ($K_{0.5}$) AND HILL'S COEFFICIENT FOR SODIUM AND $K_{0.5}$ FOR FREE Mg^{2+}

DOC, deoxycholate.

	Native $(Na^+ + K^+)$-ATPase	$(Na^+ + K^+)$-ATPase + saponin	$(Na^+ + K^+)$-ATPase + SDS	$(Na^+ + K^+)$-ATPase + DOC
$K_{0.5}$ (Na^+) (mM)	3.94±0.04 (4)	3.80±0.31 (3)	3.95±0.22 (3)	4.20±0.55 (3)
n_H (Na^+)	1.77±0.09	1.80±0.12	1.80±0.08	1.70±0.05
$K_{0.5}$ (Mg^{2+}_{free}) (μM)	25 (2)	23 ±0.80 (5)	15 ±1.05 (5)	18 ±1.10 (4)

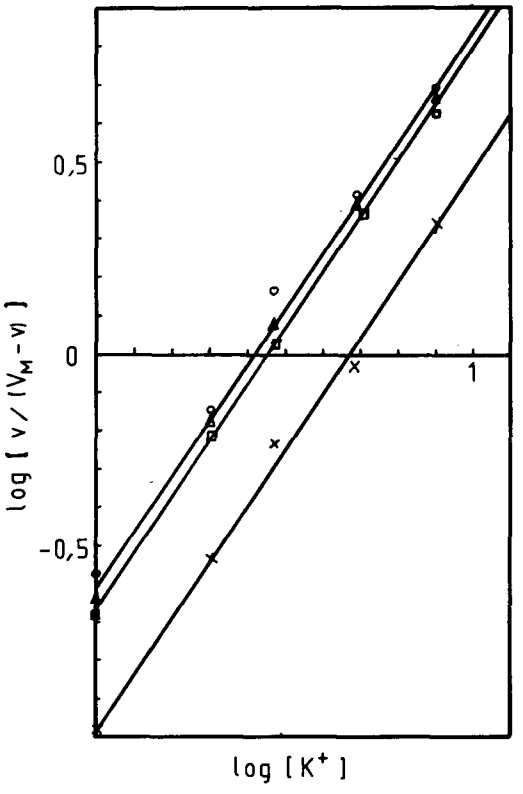

Fig. 6. Determination of Hill's coefficient (n_H) and affinity constant for K^+ of the phosphatase activity. K^+-phosphatase: △——△, native; ○——○, treated by saponin; ×——×, treated by SDS; □——□, after elimination of SDS. Operating conditions described under Fig. 5.

librated with different ligands in the reaction medium. Following addition of ATP, various levels of phosphorylation were obtained according to the detergent used for unmasking. Table V shows that the amount of phophorylated enzyme is higher when experiments are carried out with SDS-treated membranes. Due to standard deviations (S.D.) of the data, it might be thought that these difference are not significant. However, separate analysis of each individual experiment always shows an increase (15–40%) in phosphorylation of SDS-treated enzyme. On the other hand, there is no difference in the $(Na^+ + K^+)$-ATPase maximum velocity whatever the detergent used. We therefore interpret our results as a change in he level of $(Na^+ + K^+)$-ATPase phosphorylation when different detergents are used for enzyme unmasking. In addition, the Na^+-ATPase activity corresponding to these different preparations is lower when SDS is used as the unmasking agent (Table V). Under these conditions, the phosphoenzyme/Na^+-ATPase activity ratio varied with detergents used for unmasking. Native enzyme and membranes unmasked by saponin had the same ratio.

These results for the phosphorylation and Na^+-ATPase activities were the same after elimination

TABLE IV

AFFINITY CONSTANT ($K_{0.5}$) AND HILL'S COEFFICIENT FOR POTASSIUM OF PHOSPHATASE ACTIVITY

	Native K^+-phosphatase	K^+-phosphatase + saponin	K^+-phosphatase + SDS	K^+-phosphatase after elimination of SDS
$K_{0.5}$ (mM)	2.60±0.11 (3)	2.50±0.10 (5)	5.00±0.45 (4)	2.75±0.20 (3)
n_H	1.45±0.05	1.45±0.03	1.40±0.02	1.50 (2)

TABLE V

BINDING OF ^{32}P INDUCED BY [$\gamma-^{32}P$]ATP IN THE PRESENCE OF Na^+ SODIUM-DEPENDENT ATPase ACTIVITY AND BINDING OF OUABAIN INDUCED BY P_i IN THE PRESENCE OF Mg

200 μl membrane suspension (0.5 mg/ml) were added to the reacting solution and then incubated. After 15 s the reaction was started by adding 50 μM [$\gamma-^{32}P$]ATP. The reaction was stopped 18 s later by adding 10% trichloroacetic acid. 60–80 μg protein were incubated in the reaction medium (pH 7.4, 30 mM Tris/3 mM $MgCl_2$/3 mM phosphate/10^{-7} M [H^3]ouabain. After 20 min the reaction was stopped and the reaction medium was centrifuged for 30 min at 60000 rpm, 4°C.

	Native $(Na^+ + K^+)$-ATPase	$(Na^+ + K^+)$-ATPase + saponin	$(Na^+ + K^+)$-ATPase + SDS	$(Na^+ + K^+)$-ATPase after elimination of SDS
Phosphoenzyme (nmol·mg^{-1})	0.053 (2)	0.175±0.019 (5)	0.220±0.043 (5)	0.205±0.021 (4)
Na^+-dependent ATPase (μM P_i·mg^{-1}·h^{-1})	2.02±0.3 (7)	6.1 ±0.52 (10)	3.95 ±0.37 (10)	3.4 ±0.45 (6)
Phosphoenzyme/ Na^+-ATPase activity	0.026	0.028±0.004	0.055±0.006	0.058±0.007
Ouabain-binding induced by P_i (nmol·mg^{-1})	0.16±0.018 (5)	0.165±0.012 (7)	0.158±0.02 (5)	

of detergent. We then studied the capacity of ouabain binding in the presence of P_i and Mg^{2+} as a determination of the amount of unmasked enzyme.

Incorporation of ouabain

The formation of enzyme-ouabain complexes (type II complex) is a slow reaction. The results show identical incorporation of ouabain for the different membranes (native, treated by SDS or by saponin) (Table V). This is seen as an identical percentage of enzyme for each preparations. Moreover, the phosphoenzyme obtained with membranes unmasked by saponin closely corresponds to the amount of enzyme-ouabain complexes.

Discussion

Detergents are necessary for unmasking of the enzyme and its purification. Their action results in modifications of membrane permeability when low concentrations are used [13] and an increase in specific activity. Detergents currently used, such as deoxycholate and SDS, induce partial and sometimes reversible alterations of $(Na^+ + K^+)$-ATPase activity. We looked for a gentle detergent which did not produce apparent alterations of the enzyme. Our choice was saponin, a naturally occurring vegetable compound composed of a steroid structure and a sugar molecule known to act upon the membranes [29].

As reported, the effect of deoxycholate and SDS on the enzyme affinity for ATP-Mg is reversible. This effect also alters the affinity of the enzyme for K^+ (site α) by competition between ATP-K^+ as suggested by Robinson [30]. However, the elimination of SDS and deoxycholate by high-velocity centrifugation does not restore the $K_{0.5}$ for K^+, but suppresses the detergent effect on K_m. Furthermore, the affinity for K^+ of saponin unmasked membranes was not modified by addition of small amounts of SDS (equal to that remaining bound to membranes) in the test medium. There is thus an irreversible alteration of the K^+ sites by deoxycholate and SDS. This could correspond to a localization of ATP-Mg and K^+ sites more ore less accessible for detergents or a K^+-ATP interacton much more complex than simple competition.

The apparent affinity of the enzyme for Na^+ is independent of the extracting agent. This suggest the existence of a conformational state in which ATP-Mg and Na^+ sites are totally independent.

These conclusions are in agreement with the results of Kanazawa, Garay and co-workers [31,32] which showed that there was no interaction between the affinities for Na^+ and ATP during Na^+-ATPase activity. No alteration was induced by the presence of K^+. This lack of interaction can be explained in that Na^+ and ATP stabilize the same conformational state of the enzyme.

Although the precise role of magnesium is unknown, it is unanimously accepted that magnesium is the regulatory agent [22]. At the regulatory sites SDS and deoxycholate induce an increase in the affinity of the enzyme for free Mg^{2+}, as well as an increase in the affinity for ATP-Mg. This might correspond to a functional association between regulatory and substrate sites or a structural analog between regulatory and catalytic sites.

Reversible alterations in $(Na^+ + K^+)$-ATPase activity treated by SDS were found during the study of K^+-dependent phosphatase activity. The effects of K^+ on the phosphatase activity are complex and are modulated by Na^+ and ATP: K^+ alone activates at relatively low-affinity site(s) (α sites). This affinity is reversibly modified by SDS and the degree of cooperativity between sites is not altered by SDS.

Based on the use of SDS in studies of phosphorylation by $[\gamma - {}^{32}P]$ATP and the capacity of fixation of ouabain induced by P_i, it can be seen that SDS modifies the level of phosphorylation of $(Na^+ + K^+)$-ATPase. Even though several authors [25–28] have shown that the purified enzyme incorporates one molecule of P_i and ouabain per molecule of enzyme, some authors [33] – working on determination of the enzyme molecular weight – have shown recently that each catalytic subunit $\alpha\beta$ incorporates one P_i. The results that we obtained with SDS (used as unmasking agent) were closer to those obtained by Peters et al. [33] with purified enzyme. However we observed differences in the results obtained with native membranes and saponin-unmasked membranes. We suppose that the modification in the phosphorylation level observed with membranes unmasked by SDS could be due to the remaining membrane-bound detergent.

The same authors have also shown that SDS stabilizes the phosphorylated intermediate produced from the microsome fraction of beef brain [34]. This might explain why phosphorylation by ATP was overestimated. However, this action was not reversed by the elimination of SDS. This detergent induced an irreversible non-inactivating denaturation which can arise from the stability of bound detergent to protein site. Changes in the enzyme related to the occupation of a second site might explain a consequent reduction in turnover of Na^+-ATPase.

Although little is known about the action of saponin, it seems to affect the properties of $(Na^+ + K^+)$-ATPase to a lesser extent. Comparison of kinetic properties of the enzyme bound to native-state membranes and after treatment by saponin seem to demonstrate that this detergent does not alter $(Na^+ + K^+)$-ATPase. Its use as an unmasking agent seems to present to us the advantage of conserving protein-lipid, protein-protein and lipid-lipid interactions which govern the regulation of $(Na^+ + K^+)$-dependent ATPase activity in the native state.

References

1 Singer, S.J. and Nicolson, G.L. (1972) Science 175, 720–731
2 Skou, J.C. (1965) Physiol. Rev. 45, 596–602
3 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36–52
4 Jørgensen, P.L. and Skou, J.C. (1971) Biochim. Biophys. Acta 233, 366–394
5 Hokin, L.E., Dahl, J.D., Deupree, J.D., Dixon, J.F., Hackney, J.F. and Perdue, J.F. (1973) J. Biol. Chem. 248, 2593–2605
6 Goldman, S.S. and Albers, R.W. (1973) J. Biol. Chem. 248, 867–874
7 Hokin, L.E. (1976) Trends. Biochem. Sci. 233–237
8 Sanderman, H. (1978) Biochim. Biophys. Acta 515, 309–328
9 Bretscher, M.S. (1973) Science 181, 622–629
10 Bangham, J.A. and Lea, E.J.A. (1978) Biochim. Biophys. Acta 511, 388–396
11 Bretscher, M.S. and Raff, M.C. (1975) Nature 258, 43–49
12 Brotherus, J.R., Jost, P.C., Griffith, O.H. and Hokin, L.E. (1979) Biochemistry 18, 5043–5050
13 Kawamura, M., Ohta, T. and Nagano, K. (1980) J. Biochem. 87, 1327–1335
14 Anner, B.M. and Moosmayer, M. (1975) Anal. Biochem. 65, 305–309
15 Magnusson, R.P., Portis, Jr A.R. and McCarty, R.E. (1976) Anal. Biochem. 72, 653–657
16 Phillips, R.C., Philip-George, S.J. and Rutman, R.J. (1963) Biochemistry 2, 501–512
17 Titus, E.O., Holland, W.W., Brown, E.A. and Hart, W.M., Jr. (1973) Anal. Biochem. 54, 40–46
18 Jørgensen, P.L., Hokin, L.E. and Dahl, J.L. (1973) J. Biol. Chem. 248, 2557–2567

19 Hansen, O. (1976) Biochim. Biophys. Acta 433, 383–392
20 Hansen, O. and Skou, J.C. (1973) Biochim. Biophys. Acta 311, 51–66
21 Lane, L.K., Copenhaver, J.H., Lindenmayer, G.E. and Schwartz, A. (1973) J. Biol. Chem. 248, 7197–7200
22 Robinson, J.D. and Flashner, M.S. (1979) Biochim. Biophys. Acta 549, 145–176
23 Swann, A.C. and Albers, R.W. (1978) Biochim. Biophys. Acta 523, 215–227
24 Sakamoto, J. and Tonomura, Y. (1980) J. Biochem. 87, 1721–1727
25 Schuurmans-Steckhoven, F.M.A.H., Swarts, H.G.P., De Pont, J.J.H.H.M. and Bonting, S.L. (1980) Biochim. Biophys. Acta 597, 100–111
26 Karlish, S.J.D., Yates, D.W. and Glynn, I.M. (1978) Biochim. Biophys. Acta 525, 230–251
27 Forbush, B. and Hoffman, J.F. (1979) Biochemistry 18, 2308–2315
28 Skou, J.C. (1976) in Structural and Kinetic Approach to Plasma Membrane Functions (Nicolau, C. and Paraf, A., eds.), pp. 145–151, Springer-Verlag, New York
29 Segal, R. and Milo-Goldzweig, I. (1978) Biochim. Biophys. Acta 512, 223–236
30 Robinson, J.D. (1975) Biochim. Biophys. Acta 397, 194–206
31 Kanazawa, T., Saito, M. and Tonomura, Y. (1970) J. Biochem. 67, 693–711
32 Garay, R.P. and Garrahan, P.J. (1973) J. Physiol. 231, 297–325
33 Peters, W.H.M., Swarts, H.G.P., De Pont, J.J.H.H.M., Schuurmans-Stekhoven, F.M.A.H. and Bonting, S.L. (1981) Nature 290, 338–339
34 Bonting, S.L., Schuurmans-Stekhoven, F.M.A.H., Swarts, H.G.P. and De Pont, J.J.H.H.M. (1979) in ($Na^+ + K^+$)-ATPase: Structure and Kinetics (Skou, J.C. and Norby, J.G., eds.), pp. 101–103, Academic Press, New York