Biochimica et Biophysica Acta 816 (1985) 349–357
Elsevier

BBA 72643

Electrostatic characteristics of corn root plasmalemma: effect on the Mg^{2+}-ATPase activity

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(Received December 27th, 1984)

Key words: Surface potential; Mg^{2+}-ATPase; Membrane surface; Electrophoretic mobility; Gouy-Chapman model; (Corn root plasmalemma)

The macroscopic surface potential of corn root plasmalemma was determined from the microelectrophoretic mobility of the membrane vesicles. It was compared to a microscopic surface potential obtained from the binding of the ANS probe on its lipidic site. These two potentials are in good agreement whatever the used ionic strengths. Various univalent salts were shown to depolarize the membrane surface via the aspecific screening effect which was predicted by the Gouy-Chapman model. The addition of 50 mM KCl increased the apparent affinity of the ATPase for MgATP. It was assumed that the electrostatical repulsion of MgATP (valency = −1.5 at pH 6.5) was responsible for the difference between the apparent K_m and the intrinsic one. The macroscopic surface potential values were used to calculate the intrinsic K_m of the enzyme and led to the same value whatever the univalent salt concentration was. Another method of calculation of the intrinsic K_m was used, which did not rely on surface potential measurements. The intrinsic K_m values calculated by this way had the same value as the ones estimated from the experimental determinations of the surface potential. This result implies that the microscopic surface potential in the vicinity of the ATPase catalytic site has the same value as the two other potentials. Membrane proteins were extracted and adsorbed on latex beads, which had the same electrophoretic mobility as the plasmalemma vesicles. The proteic and lipidic charges thus create an electrostatic surface which appears uniformly smeared in the presence of univalent salts. In the presence of $MgCl_2$, both the surface potential of the protein-coated beads and the potential sensed by MgATP near its catalytic site on the vesicles were zero. Nevertheless, the macroscopic surface potential of the vesicles remained negative. These results pointed out the heterogeneity of the surface charge density which was induced by Mg^{2+}. They suggest that the uniformity which was observed in the absence of divalent ion was not the result of a cancellation of local heterogeneities by averaging effects. This uniformity seems to result from identical local charge densities above the lipidic and proteic parts of the membrane.

Introduction

The electrostatic control of membrane enzymes with ionized substrates (NADH, NADPH, glucose

Abbreviations: ANS, 8-anilino-1-naphthalenesulphonate; CP^+, *N*-cetylpyridinium; Mes, 4-morpholineethane sulphonate; SDS, sodium dodecyl sulphate.

6-phosphate,etc.) has recently been demonstrated [1–5]. This effect results from the modifications of substrate concentrations induced locally by the negative surface potential. It manifests itself as a difference between the intrinsic K_m (calculated from the surface substrate concentrations) and the apparent one (calculated from the bulk concentrations). In the case of the bovine brain ($Na^+ + K^+$)-ATPase [6,7] and the sarcoplasmic Ca^{2+}-ATPase [8], evidences of electrostatic control by modifications of the surface concentrations of the inorganic ions have been presented. Since MgATP is an anion, the valency of which is -1.5 at pH 6.5 and -2 at pH 7.4 [9], the electrostatic control of ATPases may also occur via MgATP repulsion according to the negative surface potential. This effect has been demonstrated on corn root plasma membrane [10], and been observed for the bovine heart mitochondrial ATP-ADP antiporter integrated in liposomes [11].

One of the most used plant plasma membrane markers is the K^+-stimulated Mg^{2+}-ATPase activity [12–15]. Its ionic selectivity is rather poor [13], and evidences have been presented for its association with more than one membrane fraction [16–20]. Since this Mg^{2+}-ATPase is suspected to function as an electrogenic proton pump, part of its activation by K^+ could correspond to a dissipation of the electrical transmembrane gradient [21–22]. On the other hand, the K^+ salt concentration generally used (50 mM) for stimulating the Mg^{2+}-ATPase in biochemical works [12–15], is typically used for inducing large surface depolarization in biophysical studies [23]. Furthermore, this screening effect is not ion-specific, and concerns all the cell membranes because of their negative surface charge. In this context, the electrostatic characteristics of the plant plasma membranes and their effect on the Mg^{2+}-ATPase activity need to be evaluated.

Material and Methods

Membrane preparation. Corn seeds (*Zea mays* L., var INRA 508) were surface-sterilized for 15 min with 3% calcium hypochloride, soaked in water and germinated for 2 days on damp paper towels. For the first experiments (batch 1), the seedlings were grown for 4 days in the dark at 25°C on aerated 0.2 mM $CaSO_4$. In the subsequent work, another batch of seeds was used (batch 2), giving a lower level of Mg^{2+}-ATPase stimulation by K^+. It was then necessary to shorten the culture time to 2 days for retrieving the precedent percent of stimulation by K^+. As shown below, the intrinsic K_m for MgATP was 50% lower in the new conditions, and the electrostatic characteristics were not modified (see Table II, batch 2). The plasma membrane fraction was prepared from excised roots as described by Leonard and Hotchkiss [24]. The final plasma membrane-enriched fraction was resuspended in 2 mM Tris-Mes buffer, (pH 6.5), 0.25 M sorbitol, 20% v/v glycerol, 1 mM dithiothreitol and was stored in liquid N_2.

ATPase solubilization. Membranes were incubated for 15 min at 15°C in 25 mM Tris-Mes buffer (pH 6.5) and 1 $mg \cdot ml^{-1}$ lysophosphatidylcholine (4 mg/mg protein). After centrifugation ($100\,000 \times g \times 60$ min at 15°C) the supernatant was used for ATPase assay.

ATPase assay. ATPase activity was measured according to Leonard and Hotchkiss [24]. The medium (1 ml final volume) contained variable amounts of ATP (Mg salt), 15 or 25 mM Tris-Mes (pH 6.5), 20–25 μg protein and univalent cations added as chlorides when indicated. Incubation time was 30 min, except for the measurement of solubilized ATPase activity or when substrate concentration was less than 1 mM. In these cases, the assay time was reduced in order to keep hydrolysis of ATP below 20%. The reaction was stopped and the liberated inorganic phosphate was assayed by adding 2 ml of a freshly prepared solution containing 0.375 M H_2SO_4, 0.75% $(NH_4)_2Mo_7O_4$, 3% $FeSO_4$ and 0.75% SDS. The absorbance was read at 740 nm after 10 min and corrected for spontaneous hydrolysis measured in the absence of membrane. The method of Ames [25] was used when a better sensitivity was needed for ATPase assay (Fig. 4). The Mg^{2+}-ATPase activity of the plasmalemma fraction was oligomycin-insensitive, 30–50% stimulated by 50 mM KNO_3, 50–70% stimulated by KCl, and KCl-insensitive in the presence of 20 μM vanadate, which induced 85% inhibition at 0.2 mM (data not shown). These characteristics are similar to those described for the same material by Dupont et al. [26] and O'Neil et al. [27]. Proteins were estimated according to Lowry et al. [28].

Electrostatic potential measurements. Two types of surface potentials were measured, namely the so-called microscopic and macroscopic ones [29]. The fluorescent anionic probe ANS was used to determine the microscopic potential above its lipidic binding site according to the previously described method [30–31]. The microelectrophoretic mobility of membrane vesicles was measured using a Rank Brothers Mark II apparatus [32]. It was used to calculate the macroscopic potential of the whole surface with the help of the Helmoltz-Smoluchovsky relation [33]. It was assumed that the charges are located at the membrane solution interface, and that macromolecules protruding from the interface exert no hydrodynamic drag. The surface potential of the proteins was determined on the solubilized fraction used for ATPase assays, precipitated by 0.35 M $(NH_4)_2SO_4$ and washed by centrifugation. The pellet (approx. 1 mg proteins) was resuspended in 0.25 ml Tris-Mes buffer (pH 6.5). A small amount of latex beads (1 μm diameter) was added to an aliquot of this protein suspension and incubated for a few minutes. After dilution with Tris-Mes buffer to a final volume of 5 ml, the microelectrophoretic mobility of these beads coated with the proteins [34,35] was used to estimate the surface potential of the proteic component of the membrane surface. We verified that increasing amounts of proteins did not modify the microelectrophoretic mobility value. Occasionally detected proteic aggregates had the same microelectrophoretic mobility than the coated beads. Surface charge densities were calculated from the surface potentials using the classical Gouy-Chapman relation [23], and expressed as elementary charges per square angström.

Results

Surface potential

Membrane vesicles. The distribution of the macroscopic surface potential was Gaussian in 25 mM Tris-Mes (pH 6.5) (Fig. 1). The mean ($\pm$ standard error for 168 vesicles) was -22.4 ± 0.8 mV, which corresponded to a surface charge density of $-0.52 \cdot 10^{-3}\ e \cdot Å^{-2}$. Adding 50 mM KCl did not change the surface charge density ($-0.51 \cdot 10^{-3}\ e \cdot Å^{-2}$), but depolarized the membrane

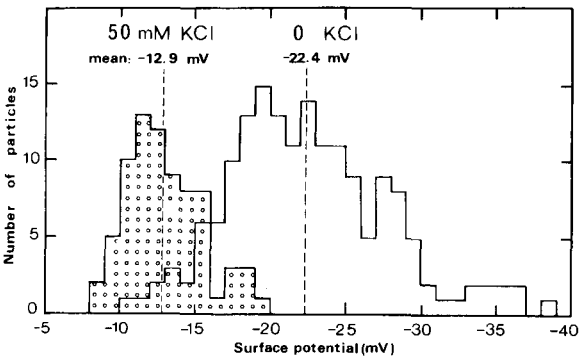

Fig. 1. Distribution of the surface potential values of plasmalemma vesicles. The surface potential was determined from the microelectrophoretic mobility of the vesicles in 25 mM Tris-Mes buffer (pH 6.5), with 0 or 50 mM KCl. Both these distributions could be adjusted to normal distributions as follows: 0 KCl, mean $m = 22.45$ mV, standard deviation $s = 5.30$ mV, observed Pearson's chi-square = 5.93, chi-square for $p = 0.95$ with 7 degrees of freedom = 14.1; 50 mM KCl, $m = 12.95$ mV, $s = 2.49$ mV, observed chi-square = 4.38, chi-square for $p = 0.95$ with 7 degrees of freedom = 14.1.

surface (-12.9 ± 0.6 mV for 74 vesicles). The values of the microscopic surface potential determined from the ANS method in 15 mM Tris-Mes (pH 6.5) were -20 mV (no KCl) and -11 mV (50 mM KCl). In the same conditions, the potential values obtained from microelectrophoresis were -25 ± 0.8 mV in the absence of KCl and -14.2 ± 0.6 mV in the presence of 50 mM KCl.

The ability of various univalent cations to depolarize the membrane surface was studied at pH 6.5 with 50 mM alkali cation chlorides or Tris-Mes (Table I). No clear-cut selectivity appeared.

Adding $MgSO_4$ resulted in a depolarization of the vesicle surface (Fig. 2). The maximum depolarization corresponded to a 50% decrease of the surface charge (Fig. 2, inset).

The cationic surfactant CP^+ may be used at very low concentrations for inserting positive charges in membranes [1–3,10]. It annihilated the surface potential of the membrane vesicles at 25 μM.

Proteins adsorbed on latex beads. When the latex beads were treated by the supernatant used for ATPase assays (after solubilization by lysophosphatidylcholine), their surface potential and their surface charge density were -11 mV and $-0.25 \cdot$

TABLE I

EFFECTS OF UNIVALENT CATIONS ON Mg^{2+}-ATPase ACTIVITY AND MICROELECTROPHORETIC POTENTIAL OF PLASMALEMMA VESICLES

The basal medium contained 15 mM Tris-Mes (pH 6.5). The surface charges corresponding to the $Tris^+$, Li^+, Na^+, K^+, Cs^+ and Rb^+ treatments were (10^{-3} $e \cdot Å^{-2}$) −0.491, −0.429, −0.435, −0.517, −0.437 and −0.435, respectively. The analysis of variance gave an observed F of 5.7 ($F_{0.01} = 9.0$).

	Added cations (50 mM chlorides)						
	None	$Tris^+$	Li^+	Na^+	K^+	Rb^+	Cs^+
ATPase activity ($\mu mol \cdot h^{-1} \cdot mg^{-1}$ protein)							
0.6 mM Mg-ATP	5.0	9.3	9.9	10.1	10.2	11.1	–
3.0 mM Mg-ATP	10.0	11.5	14.3	16.4	17.3	18.5	–
Microelectrophoretic potential (mV)	−25.0±1.3	−13.3±0.9	−11.7±0.8	−11.9±0.6	−14.0±1.1	−11.8±0.6	−11.9±0.6
(number of particles)	(50)	(104)	(50)	(130)	(105)	(110)	(110)

10^{-3} $e \cdot Å^{-2}$ in 25 mM Tris-Mes (pH 6.5) (Table II). After precipitation with 0.35 M $(NH_4)_2SO_4$, the washed proteic pellet conferred a surface charge density of $-0.39 \cdot 10^{-3}$ $e \cdot Å^{-2}$ to the beads. This charge was totally neutralized upon addition of 6 mM $MgSO_4$ (Table II).

ATPase activity of membrane vesicles

Effects of KCl and of the ionic strength. The ATPase activity was measured on membranes as a function of MgATP concentration in 15 mM Tris-Mes (pH 6.5). The values of the kinetic parameters K_m and V_m were determined from Scatchard plots (Fig. 3) and are nearly identical to the ones reported for the same material [24]. The addition of 50 mM KCl to the 15 mM $Tris^+$-Mes^- (Tris is totally protonated at pH 6.5) stimulated the activity both by decreasing K_m and increasing V_m. The replacement of 4 mM $Tris^+$ by 4 mM K^+ (K^+ + $Tris^+$ = 15 mM) only slightly affected K_m, but was as effective as 50 mM KCl in increasing V_m. The kinetic parameters obtained in the batch 1 and 2 experiments are given in Table II.

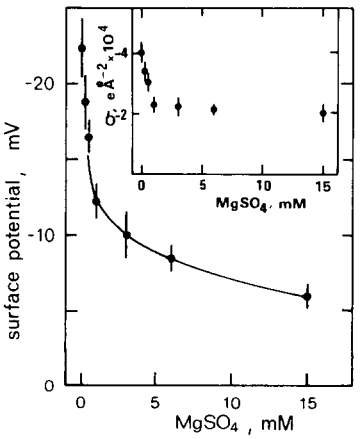

Fig. 2. Effect of $MgSO_4$ on the surface potential of plasmalemma vesicles which was determined from their microelectrophoretic mobility. The medium contained 15 mM Tris-Mes (pH 6.5). Inset: effect of $MgSO_4$ on the surface charge density of the particles. The vertical bars are the standard deviations ($p = 0.95$).

The stimulating effect of 50 mM KCl on the Mg^{2+}-ATPase was studied at a low MgATP concentration (0.3 mM) and with various Tris-Mes concentrations (Fig. 4). The percentage of stimulation of the enzymic activity upon KCl addition decreased from 200 to 15% when the ionic strength of the buffer increased from 5 to 50 mM (Fig. 4, inset). Such ionic strength effect of the buffer has been already described for oat root plasmalemma Mg^{2+}-ATPase [36].

Ionic selectivity. The selectivity of the effect of the cations on the Mg^{2+}-ATPase was appreciated by adding various chloride salts (50 mM) to 15 mM Tris-Mes (pH 6.5) (Table I) and using 0.6 mM MgATP or 3 mM MgATP. There was no clear-cut selectivity at 0.6 mM MgATP and the percentage of stimulation was high (approx. 100%). At 3 mM MgATP, the percentage of stimulation

TABLE II

ELECTROSTATIC PARAMETERS OF PLASMALEMMA VESICLES AND PLASMALEMMA PROTEINS, AND Mg^{2+}-ATPase K_m FOR MgATP

Except when indicated, the media comprised 15 mM Tris-Mes (pH 6.5). n.d., not determined. K_{ma}, the apparent K_m calculated from bulk MgATP concentrations. K_{mi}, the intrinsic K_m.

Ionic treatment	Material	Electrostatic characteristics from						K_m for MgATP	
		electrophoresis		ANS		K_m for MgATP		K_{ma} (mM)	K_{mi} (mM)
		mV	10^{-3} $e \cdot Å^{-2}$	mV	10^{-3} $e \cdot Å^{-2}$	mV	10^{-3} $e \cdot Å^{-2}$		
Batch 1									
0 KCl	vesicles	−25.0	−0.454	−20.0	−0.358	−25.0	−0.454	1.18	0.29 [b], 0.23 [d]
0 KCl	proteins [a]	−11.0	−0.250	n.d.	n.d.	−11.1	−0.252	0.54	0.29 [b]
50 mM KCl	vesicles	−14.2	−0.523	−11.0	−0.403	−14.0	−0.516	0.64	0.29 [b]
0 KCl, 6 mM $MgSO_4$	proteins [a]	0	0	n.d.	n.d.	−0.6	0	0.33	0.33 [b]
50 mM KCl, 6 mM $MgSO_4$	proteins [a]	0	0	n.d.	n.d.	0	0	0.29	0.29 [b]
Batch 2									
0 KCl	vesicles	−22.8	−0.411	n.d.	n.d.	−20.2	−0.362	0.72	0.20 [b], 0.23 [d]
50 mM KCl	vesicles	−13.9	−0.511	n.d.	n.d.	−10.9	−0.400	0.43	0.20 [b]
50 mM KCl	proteins [e]	−10.7	−0.392	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0 KCl, 6 mM $MgSO_4$	vesicles	−8.5	−0.229	n.d.	n.d.	0	0	0.20	0.20 [c]
50 mM KCl, 6 mM $MgSO_4$	vesicles	−6.5	−0.271	n.d.	n.d.	0	0	0.20	0.20 [c]
0 KCl, 6 mM $MgSO_4$	proteins [e]	0	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
50 mM KCl, 6 mM $MgSO_4$	proteins [e]	0	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0 KCl, 25 μM CP^+	vesicles	0	0	n.d.	n.d.	0	0	0.61	0.61 [b]
50 mM KCl, 25 μM CP^+	vesicles	0	0	n.d.	n.d.	0	0	0.59	0.59 [b]

[a] Solubilized and maintained in 1 $mg \cdot ml^{-1}$ lysophosphatidylcholine, 25 mM Tris-Mes.

[b] Calculated from the apparent K_m and the measured surface potential (microelectrophoresis).

[c] Calculated under the assumption that the surface potential above the catalytic site is zero (as the one measured on the protein-coated beads).

[d] Calculated from Relation 3, applied to the apparent K_m values in 0 KCl and 50 mM KCl.

[e] Solubilized as for (a), then precipitated by 0.35 M $(NH_4)_2SO_4$, washed and resuspended in 15 mM Tris-Mes.

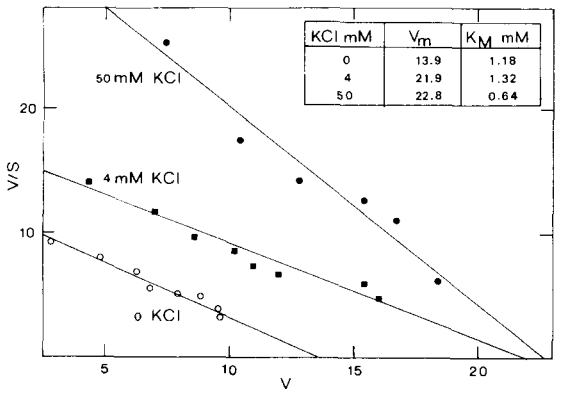

Fig. 3. Scatchard plots of the Mg^{2+}-ATPase activity (V, μmol/mg protein per h) of plasmalemma vesicles which was assayed at pH 6.5 as a function of the MgATP concentration (mM) (batch 1 experiments). The medium contained 15 mM Tris-Mes, or 15 mM Tris-Mes and 50 mM KCl, or 11 mM Tris-Mes and 4 mM KCl.

was lower (15–85%), but a selectivity sequence was observed.

Effect of CP^+ and $MgSO_4$. In conditions ensuring the electrostatical neutrality of the membrane surface (25 μM CP^+), titrations with MgATP revealed that the affinity constant was independent of KCl and equal to 0.6 mM (Table II). The effect of CP^+ on V_m was evaluated from the activity at 3 mM MgATP in the presence of 50 mM KCl and 0.1 $mg \cdot ml^{-1}$ lysophosphatidylcholine, for CP^+ concentrations varying from 0 to 50 μM. There was a progressive inhibition of the activity (25% at 25 μM CP^+, data not shown). In the presence of 6 mM $MgSO_4$ (condition ensuring the electrical neutrality of the solubilized proteins, but not that of the vesicles) (Fig. 2), the K_m was equal to 0.20 mM (batch 2) and insensitive to the

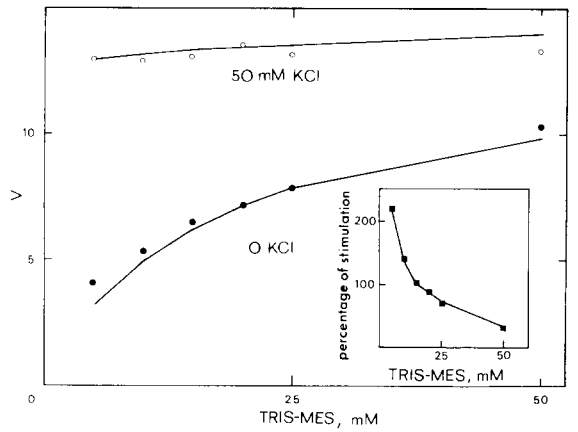

Fig. 4. Effect of the buffer ionic strength on the Mg^{2+}-ATPase activity of plasmalemma vesicles (V, μmol/mg protein per h). The media contained 0.3 mM MgATP and 0 or 50 mM KCl (batch 2 experiments). Inset: percentage of stimulation of the activity by 50 mM KCl. The curves are calculated from the Gouy-Chapman model and from relation 4 with the following parameters: 50 mM KCl: $V_m = 39$ μmol/mg protein per h, $K_{mi} = 0.2$; 0 KCl: $V_m = 27$ μmol/mg protein per h, $K_{mi} = 0.2$ mM. The surface charge density which was used to calculate the surface potential was $-0.432 \cdot 10^{-3}$ $e \cdot Å^{-2}$.

addition of 50 mM KCl (Table II).

Solubilized ATPase activity. After treatment with 1 mg · ml^{-1} lysophosphatidylcholine and 60 min centrifugation at $100000 \times g$, 50% of the total proteins were recovered in the supernatant. The specific Mg^{2+}-ATPase activity of the supernatant was 10-fold higher than that of the membrane suspension in the absence of KCl. The surface potential of the protein-coated latex beads or of the protein-lysophosphatidylcholine micelles was annihilated by adding 6 mM $MgSO_4$ (Table II). In these conditions (25 mM Tris-Mes (pH 6.5), 6 mM $MgSO_4$), the stimulation by 50 mM KCl was very slight (13% at 3 mM MgATP), and the K_m was approx. 0.3 mM either in the absence or in the presence of KCl (batch 1) (Fig. 5). The reference value measured in the absence of KCl and $MgSO_4$ was $K_m = 0.54$ mM.

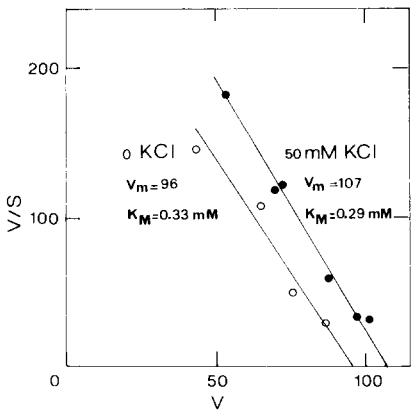

Fig. 5. Scatchard plots of the solubilized plasmalemma Mg^{2+}-ATPase activity (V, μmol/mg protein per h) as a function of the MgATP concentration (mM) (Batch 1 experiments). The medium contained 25 mM Tris-Mes (pH 6.5), 6 mM $MgSO_4$ in excess of MgATP, and 0 or 50 mM KCl. The procedure for the solubilization is described in the text.

Analysis and Discussion

Surface potential

The fact that the calculated surface charge densities remained constant (approx. $-0.5 \cdot 10^{-3}$ $e \cdot Å^{-2}$) (Tables I and II) indicates that the surface depolarizations of membrane vesicles induced by various 50 mM univalent salts were the ones predicted by the Gouy-Chapman model. The adequacy of the Gouy-Chapman model for describing the surface electrostatics of lipidic bilayers and, in some cases, biological membranes has been shown [23,31,37]. On the contrary, Mg^{2+} appeared to depolarize the membrane surface via both screening and masking effects (Fig. 2). The shape of the curve on Fig. 2 (inset) suggested a binding of Mg^{2+} on a finite number of sites.

Electrostatic control of the affinity for MgATP

The surface concentration of the anion MgATP (valency = -1.5 at pH 6.5) is related to the bulk concentration and the surface potential by the Boltzman relation:

$$(ATP)_s = (ATP)_b \cdot \exp(1.5e\psi/kT) \quad (1)$$

where (ATP) stands for MgATP concentration, the subscripts s and b refer to the surface and the bulk phases, ψ is the surface potential and e, k, and T have their usual meaning.

Introducing Relation 1 in the classical Michaelis-Menten relation gives the following expression for the apparent affinity constant:

$$K_{ma} = K_{mi} \cdot \exp(-1.5e\psi/kT) \quad (2)$$

where K_{ma} and K_{mi} are the apparent and intrinsic Michaelis constants, respectively.

Relation 2 predicts that $K_{ma} = K_{mi}$ when $\psi = 0$. This condition was fulfilled in the presence of 6 mM Mg^{2+} with protein-coated beads. In this case, the measured K_{ma} was approx. 0.3 mM (batch 1, solubilized ATPase), and represented a first estimation of K_{mi}. In the case of membrane vesicles in the presence of 25 μM CP^+, $\psi = 0$ and K_{ma} was approx. 0.6 mM both in the absence of KCl and in 50 mM KCl (Table II, batch 2). The insensitivity of K_{ma} to KCl at $\psi = 0$ is in accordance with the hypothesis of an electrostatical effect of the salt on the affinity for MgATP. Nevertheless, the observed K_{ma} probably does not measure the intrinsic affinity of the native ATPase, since the maximal rate of the latter is partially inhibited by the surfactant, suggesting conformational modifications. The same situation was described by Krämer for the bovine heart mitochondrial ATP/ADP antiporter in the presence of cetyltrimethylammonium [11].

The macroscopic potential was used as an estimation of ψ for calculating K_{mi} from Relation 2 in the cases of non-zero potential. From data of Table II (membrane vesicles, batch 1), K_{mi} is 0.29 mM both in the absence of KCl and in 50 mM KCl (Table II). For the solubilized ATPase of batch 1 in the absence of KCl and $MgSO_4$, the calculated K_{mi} is again 0.29 mM.

In the case of the membrane vesicles of batch 2, the application of Relation 2 gives $K_{mi} = 0.20$ mM in both the absence of KCl and in 50 mM KCl (Table II).

A second method was used, which did not rely on the measurements of the surface potentials, but gave K_{mi} from the comparison of two estimations of K_{ma} (K_{ma1} and K_{ma2}) at two concentrations of monovalent salt (C_1 and C_2). This method, which relies on the assumption that the Gouy-Chapman relation holds, and that there is no binding, has been described elsewhere [31] for estimating the intrinsic affinity of membrane for the surface probe ANS. Applied to the present case, it leads to:

$$K_{mi} = (A \cdot K_{ma1} - K_{ma2})/(A - 1) \qquad (3)$$

with

$$A = (K_{ma2}/K_{ma1})^{1/3} \cdot (C_1/C_2)^{1/2}$$

This relation was applied to the results of Fig. 2 (Table II, batch 1; 0 Mg^{2+}, $C_1 = 15$ mM, $K_{ma1} = 1.18$ mM, and $C_2 = 65$ mM, $K_{ma2} = 0.64$ mM). It gave $K_{mi} = 0.28$ mM, which was in good accordance with the estimations obtained from the microelectrophoretic data (Relation 2). The same agreement between the two methods was observed for batch 2 in the absence of Mg^{2+}. (Both Relation 2 and Relation 3 applied to the results of Table II gave K_{mi} = approx. 0.2 mM.) Finally, Relation 2 was used with the K_{mi} values obtained from Relation 3, and the different values of K_{ma} for calculating the microscopic surface potential in the vicinity of the ATPase catalytic site (Table II, treatments with 0 Mg^{2+}). As expected from the concordance of the two methods for estimating K_{mi}, these calculated surface potentials were in agreement with the ones experimentally determined.

The effect of ψ on the ATPase activity can be explicited by introducing Relation 2 into the Michaelis-Menten equation:

$$v = V_m \cdot (ATP)_b/(K_{mi} \cdot \exp(-1.5e\psi/kT) + (ATP)_b) \qquad (4)$$

The results in Fig. 3 show that KCl stimulates the ATPase activity by modifying both the affinity for MgATP and the maximal rate. Relation 4 indicates that the electrostatic control of the affinity for MgATP can be well detected at low substrate concentrations. This was the rationale for using 0.3 or 0.6 mM in the experiments of Fig. 4 and Table I. On the other hand, the effect of the cations on V_m was estimated from the measurements at 3 mM MgATP. Relation 4 was used together with the Gouy-Chapman model for analysing the effect of the ionic strength of the buffer on the stimulation by 50 mM KCl (0.3 mM MgATP). This procedure led to a good fit of the data (Fig. 4).

Homogeneity of the surface charge distribution

The validity of the estimation of K_{mi} from the macroscopic surface potential (Relation 2) depends on the assumption that the potential at the shearing plane in microelectrophoretic migration is a reasonable estimation of the potential in the vicinity of the catalytic site. There is good agreement between the K_{mi} values calculated from the

macroscopic surface potential in 0 or 50 mM KCl (approx. 0.3 mM and 0.2 mM for batches 1 and 2, respectively). Furthermore, the alternative determination of K_{mi} (Relation 3), which does not rely on surface potential measurements gives the same K_{mi} value as the precedent one. In other words, the microscopic surface potential at the catalytic site (calculated from Relations 3 and 2) is the same as the macroscopic one and also the same as the microscopic potential above the ANS lipidic site. Similar agreement between electrophoretic data and a molecular approach of the surface potential have been reported by Lau et al. [38] for phospholipid bilayers. Finally, the constancy of the K_{mi} value suggests that (i) 50 mM KCl can induce surface potential shifts resulting in a 2-fold increase of the affinity for MgATP, (ii) the surface potential is uniform in these experiments (0 Mg^{2+}), in spite of the complexity of the membranes. The same conclusion has been reached for liposomes [30,38].

Origin of the potential at the catalytic site

Some proteins are known to experience the electric field of the charges born by the lipidic moiety of the membrane [1,3–5,11]. Thus, the fact that ψ is the same above the ATPase as above the lipids may indicate that the latter determine the electrostatic conditions perceived by the proteins. Alternatively, the proteins may bear the same net charge as the lipids. The effects of the ions on the apparent affinity of the ATPase solubilized with neutral lysophosphatidylcholine (Table II) suggests that proteins create their own electrostatic environment similar to the one of the whole membrane surface. This has been directly shown by measuring the electrophoretic mobility of protein-coated latex beads (Table II).

Further indications on this point were obtained in the presence of Mg^{2+}: (i) Mg^{2+} was able to totally depolarize the membrane proteins but not the whole membrane, (ii) it reduced the value of the apparent K_m for MgATP of the membrane ATPase to that of the intrinsic K_m, and (iii) rendered it KCl-insensitive. These results suggest that in the presence of Mg^{2+}, the potential was zero above the catalytic site, while negative elsewhere. The fact that Mg^{2+} masks the net negative charge of the proteins but not the one of the lipids indicates that both these membrane components have their own dissociated groups.

Conclusion

The uniformity of the surface charge density in the absence of Mg^{2+} results from the fact that identical net surface charges are created by different ionized groups in the proteic and lipidic parts of the membrane. The same conclusion had been reached from the comparison of liposomes and microsomes of horse bean roots [5]. The uniformity of the surface charge of natural membranes has already been described for various membranes from animal cells [1,11], and for plant root microsomes [5].

Our results show that part of the typical stimulation of the plant plasma membrane Mg^{2+}-ATPase by K^+ is due to an aspecific electrostatic effect, acting on the apparent K_m and corresponding to a change of the substrate concentration near the catalytic site rather than on the structure of the latter. Another part of the stimulation corresponds to a more specific effect of cations on V_m. The electrostatic effect on the apparent K_m can be correctly described using the macroscopic electrostatic characteristics of the whole membrane, because the membrane surface charge is uniform. Nevertheless, it was possible to show that in some circumstances (presence of Mg^{2+}, solubilization of the protein), the ionized groups of the protein are responsible for this interaction.

Acknowledgement

The authors are greatly indebted to Suzette Astruc for her valuable assistance.

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