The negative surface charge density of plasmalemma vesicles from wheat and oat roots

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Plasmalemma vesicles were isolated in a sucrose-containing medium from wheat and oat roots and the net negative surface charge density was determined with 9-aminoacridine fluorescence [Chow, W.S. and Barber, J. (1980) J. Biochem. Biophys. Methods 3, 173–185]. The outer surface of the vesicles (measured in the presence of sucrose) had densities of -16 to -20 mC·m^{-2} and -29 mC·m^{-2} for wheat and oat roots, respectively. The inner surface – presumed to be the cytoplasmic side and calculated from the values measured in the presence and absence of sucrose – was more negative, and its size depended on the salt status of the roots.

9-Aminoacridine *Membrane asymmetry* *Plasmalemma* *Surface charge density* *Surface potential*

1. INTRODUCTION

All biological membranes contain charged groups on their surfaces derived from both proteins and lipids. At neutral pH most membranes have a net negative charge, the isoelectric point being 4.1–4.7 for thylakoid membranes [1] and 4.8–5.4 for rat liver mitochondrial membranes [2]. Many biological processes are governed by electrostatic interactions with these membrane charges – the pH optimum and kinetics of membrane-bound enzymes [3–7], membrane–membrane interactions like stacking or fusion, the binding of charged proteins like cytochrome *c*, and the binding of inorganic ions (e.g., Ca^{2+} or Mg^{2+}) either required for enzyme activity [8] or needed for maintaining the conformation of the membrane. In chloroplasts electrostatic interactions play a very significant role in many processes as reviewed in [9,10]. In plant mitochondria electrostatic effects have also been described [7,8,11,12]. No such information, however, is available on the plasmalemma isolated from plant cells.

All the ions required by the plant are taken up through the cell wall and across the plasmalemma of the cells of the plant root. Other processes involving charged molecules like the reduction of Fe^{3+} [13] and the oxidation of NADH [14] also take place on the outer surface of the plasmalemma of root cells. Here clearly is a place where electrostatic interactions could be of potential significance. However, the uptake of ions like K^+ is governed not only by its concentration in the root growth medium, but also by its concentration inside the plant. Plants of high K^+ status take up K^+ at a slower rate than plants of low K^+ status [15,16]. Likewise many plant species exhibit the so-called 'cation equivalent constancy' [17], which means that a decrease in K^+ content is compensated for either by an equivalent increase in Na^+

Abbreviation: (DM)Br_2, *N,N,N,N',N',N'*-hexamethyldecane-1,10-diamine bromide

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content or by about half the molar increase in Mg^{2+} content (see [18]).

Thus, there are clear indications of a regulation of processes at both sides of the plasmalemma of root cells by cations. This regulation could be through electrostatic interactions with membrane charges. It was the purpose of the present investigation to determine the surface charge density of the plasmalemma from plants for the first time and to see if species or salt status affected this important membrane property.

2. MATERIALS AND METHODS

Oat (*Avena sativa* L. cv. Brighton) and wheat (*Triticum aestivum* L. cv. Drabant) were grown in darkness and low salt solution as in [19]. In a second series of experiments, winter wheat (cv. Hildur) was grown in a medium as in [20], ± 1 mM KCl ('high K' and 'low K' in table 2, respectively). In all cases, roots were harvested after 7 days and the purified plasmalemma fraction isolated as in [21] with the following modifica-

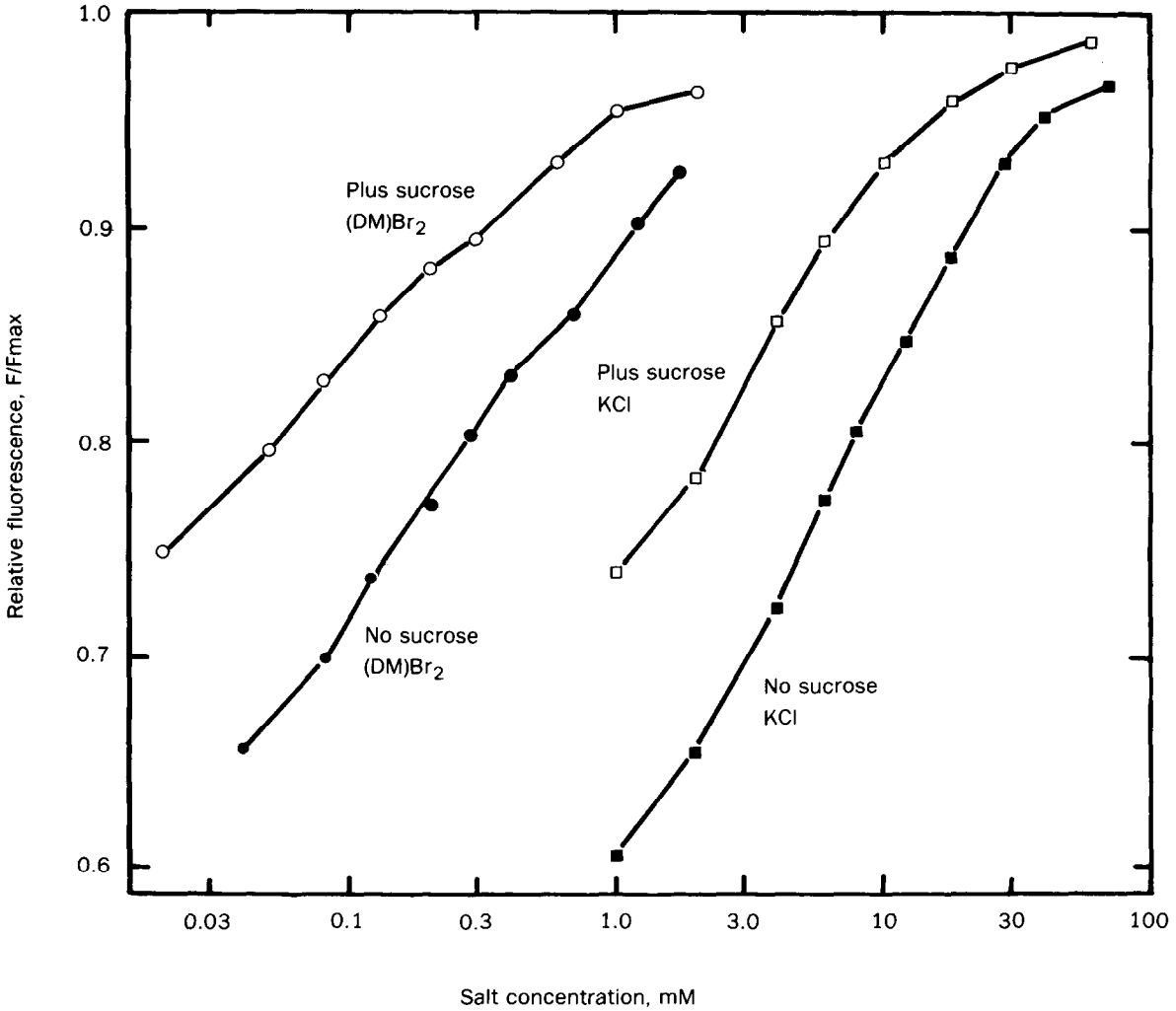

Fig.1. The relative fluorescence of 9-aminoacridine in the presence of plasmalemma vesicles from wheat roots and different concentrations of KCl and $(DM)Br_2$. The wheat roots were grown as in [19]. F_{max} was the fluorescence reached by adding 20 mM $MgCl_2$ at the end of the experiments. Vesicles at 50 μg protein · ml^{-1} were present in all experiments. The results from this figure were used in table 1 to calculate C_1^+, C^{2+} and the surface charge density of the vesicles.

tions: the homogenate was filtered through a 60 μm nylon net; phase system composition was 6.5% (w/w) dextran T500, 6.5% (w/w) PEG 4000, 0.25 M sucrose, 5 mM K^+-phosphate, 4 mM KCl (pH 7.8); isolated plasmalemma vesicles were resuspended in the preparation medium without EDTA. These vesicles are of a uniform polarity (see section 4).

Jerusalem artichoke (*Helianthus tuberosus* L.) mitochondria were isolated as in [8].

Protein was determined as in [22] after solubilising the membranes with 0.5% (w/v) deoxycholate.

The K^+ concentration in the assay buffer was determined by atomic absorption spectrophotometry.

The fluorescence of 9-aminoacridine was measured in a Perkin-Elmer fluorescence spectrophotometer model 204-A by exciting at 355 nm (10 nm slit) and measuring at 456 nm (10 nm slit). 9-Aminoacridine at 20 μM was added to 5 mM Mops (pH 7.0) ± 0.3 M sucrose and the fluorescence adjusted to 75 units on the recorder. The vesicles (32–108 μg protein · ml^{-1}) were added, as well as 50 μM EDTA to remove all bound bivalent cations [8]; this brought the fluorescence down to a minimum. The fluorescence was now brought back up by titrating with either KCl or $(DM)Br_2$. At the end of each experiment 20 mM $MgCl_2$ was added to give the maximum fluorescence release (F_{max}). The relative fluorescence values were plotted (see fig.1) and the figure used to determine concentrations of KCl and $(DM)Br_2$ which gave the same relative fluorescence. The surface charge density (σ) was calculated from these values as in [23].

3. RESULTS

Plant protoplasts are ruptured by a lowering of the sucrose concentration by 0.25 M [24]. We have used this property of the plasmalemma to study either the outer surface of the plasmalemma vesicles (measured in the presence of 0.3 M sucrose) or both sides of the ruptured vesicles (measured in the absence of sucrose). Fig.1 and table 1 show the results of typical experiments where the surface density was determined on plasmalemma vesicles isolated from low-salt roots. In the absence of sucrose, the addition of the vesicles to the weak buffer caused a larger quenching of fluorescence than when the same amount of vesicles were added to a sucrose-containing medium (fig.1). The titrations with either KCl or $(DM)Br_2$ gave S-shaped curves (on a semi-logarithmic plot) similar to those in [23]. When the surface charge densities were calculated using corresponding concentrations of KCl and $(DM)Br_2$ from fig.1, increasing values were obtained with increasing salt concentrations (table 1) as also found in [23]. However, the values were consistently higher in the absence of sucrose. Due to the form of eq.3 in [23], σ cannot be calculated as long as the KCl concentration added is less than or equal to the concentration of K^+ in the medium. For this reason low F/F_{max} values are not suitable. Likewise, the error in determining the salt concentration that gives a certain F/F_{max} becomes very high above F/F_{max} = 0.90. For this reason F/F_{max} = 0.85 was used for all further comparisons (except where noted in table 2).

The σ of intact oat root plasmalemma vesicles was higher than for vesicles from wheat roots (table 2). In the absence of sucrose, all plasmalemma vesicles, irrespective of species or growth con-

Table 1

The calculation of the net negative surface charge density on plasmalemma vesicles from wheat roots using 9-aminoacridine fluorescence

F/F_{max}	C_1^+	C_2^+ (mM)	C^{2+}	σ ($mC \cdot m^{-2}$)
With sucrose				
0.75	1.2	1.2	0.021	–
0.80	2.4	1.2	0.054	−12
0.85	3.8	1.2	0.12	−15
0.90	6.6	1.2	0.34	−17
0.95	15.1	1.2	0.92	−24
Without sucrose				
0.70	3.3	1.5	0.08	−15
0.75	5.1	1.5	0.15	−19
0.80	7.7	1.5	0.27	−23
0.85	12.2	1.5	0.60	−24
0.90	21.0	1.5	1.2	−30

C_1^+ and C^{2+} were calculated by linear interpolation from fig.1. C_2^+ (the background concentration of K^+) was measured on the assay medium using atomic absorption spectrophotometry. σ was calculated as in [23]

Table 2

The net negative surface charge densities of plasmalemma vesicles isolated from oat and wheat roots

Fraction	Surface charge density ($mC \cdot m^{-2}$)		
	Outside[c]	Both sides[d]	Inside[e]
Wheat root plasmalemma			
Low salt[a]	−16 ± 1 (2)	−25 ± 1 (2)	−34 ± 0 (2)
Low K^{+}[b]	−21 ± 4 (3)	−26 ± 2 (4)	−32 ± 6 (3)
High K^{+}[b]	−19 ± 1 (3)	−21 ± 3 (4)	−24 ± 7 (3)
Oat root plasmalemma	−29 ± 1 (2)	−33 ± 2 (2)	−38 ± 5 (2)
Jerusalem artichoke mitochondria (F/F_{max} = 0.80)	−33 ± 0 (2)	–	–

[a] Roots grown as in [19]
[b] Roots grown as in [20]
[c] Measured in the presence of 0.3 M sucrose
[d] Measured in the absence of sucrose
[e] Calculated from [c] and [d] using eq.1

Values are means ± SE (number of independent preparations)

ditions, gave higher σ than in the presence of sucrose. Assuming that all vesicles are ruptured in the absence of sucrose, the measured σ without sucrose (σ_-) is simply the arithmetic mean of the charge of two surfaces (σ_i and σ_o) of equal area:

$\sigma_- = \frac{1}{2}(\sigma_o + \sigma_i)$, which gives

$$\sigma_i = 2\sigma_- - \sigma_o \quad (1)$$

The inner surface had almost the same σ in vesicles from wheat and oat roots grown under identical conditions. However, if the K^+ status of the roots was varied, the σ of the inner side was affected significantly (table 2). The σ of Jerusalem artichoke mitochondria is also shown in table 2 for comparison since electrostatic interactions have been described for their membranes [7,8,11,12].

4. DISCUSSION

The estimation of the negative surface charge density of plasmalemma vesicles from roots and Jerusalem-artichoke mitochondria through the use of 9-aminoacridine fluorescence [23] proved to be a convenient and reproducible method (table 2). The values found for plasmalemma vesicles and mitochondria fall within the range reported for thylakoid membranes estimated by the 9-aminoacridine technique [10]; they are considerably higher than surface charge densities of thylakoid membranes (5–10 $mC \cdot m^{-2}$) [10] or amyloplasts (5 $mC \cdot m^{-2}$) [25] determined by particle electrophoresis. Although it is known that the latter technique underestimates the surface charge density by measuring the zeta potential at the plane of shear instead of the true surface potential, the 9-aminoacridine technique may overestimate the charge density by monitoring local charge densities instead of an overall average (see [10,23] for details).

The phase separation technique separates membrane vesicles on the basis of surface properties so that, for example, inside-out and right-side out thylakoid vesicles [26] or submitochondrial particles [27] can be separated. The plasmalemma vesicles used here are therefore of a uniform polarity. Our unpublished observations show that while glucan synthetase II – which participates in cell wall synthesis [28] – is readily measured on intact vesicles, the activity of the Mg^{2+}-dependent ATPase is only apparent after the vesicles have been ruptured by sonication, detergents or in a hypo-osmolar medium. Thus, it is reasonable to assume that the inner surface of the plasmalemma

vesicles used here originally faced the cytoplasm of the root cells and that the outer surface faced the apoplasm and the cell wall. The vesicles are therefore right-side out.

The outer surface of plasmalemma vesicles from oat roots has a significantly higher surface charge density than that of vesicles from wheat roots grown under the same conditions (table 2). The precise ecological significance of such a difference is not clear at present, but it adds to the list of differences between oat and wheat roots (see [29]).

The charge densities on the inner, presumably cytoplasmic surface of wheat and oat root vesicles appear to be very similar. However, when wheat roots were grown under different conditions it is of particular interest to note that the charge density of the inner side was affected; it was significantly higher when the roots were grown under low K^+ conditions than when more K^+ was available (table 2). The possibility that the intracellular cation concentration regulates cation uptake (see section 1) through changes in membrane charges on the cytoplasmic side of the plasmalemma merits further investigation.

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