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Ion channels in vacuoles from halophytes and glycophytes

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The electrical properties of the vacuolar membrane (tonoplast) of a halophyte, sugar beet, and a glycophyte, tomato, have been investigated using the patch-clamp technique [(1981) Pflügers Arch. 391, 85–100]. Voltage-dependent ion channels were analyzed using isolated membrane patches. Both species displayed channel activities which were nonselective between sodium and potassium. Beet tonoplast channels displayed inward rectification (65 pS and 10 pS for negative and positive potentials, respectively), while tomato tonoplast channels showed a constant conductance (25 pS) in the range −80 to +80 mV potentials. The observed low channel conductance at positive potentials in halophytes would prevent a significant loss of the Na^+ accumulated in the vacuole through the operation of the Na^+/H^+ antiport [(1987) Physiol. Plant. 69, 731–734], while channel rectification in glycophytes would have no physiological significance.

Ion channel; Patch clamp; Halophyte; Glycophyte

1. INTRODUCTION

The ionic relations of the vacuole of plant cells are still very inadequately known; this is true for both glycophytes and halophytes. It is likely that the tonoplast plays an important role in controlling the ionic concentrations in the vacuole particularly for these halophytes which accumulate high concentrations of sodium chloride in their cell vacuoles. In general the tonoplast membrane potential is about +20 mV (vacuole positive) and there is a pH difference of the order of 2 units (vacuole more acidic). The electrochemical potential difference for protons, $\Delta\mu H^+$ ($\mu H^+_{vac} >> \mu H^+_{cyt}$) is generated by electrogenic proton pumps on the tonoplast driven by the hydrolysis of ATP [3] and, perhaps, by the hydrolysis of pyrophosphate [4]. The situation for ions other than protons is far from clear. For instance, their electrochemical potential differences are not well known. But we can say that, with a tonoplast potential of +20 mV, if sodium, potassium and – say – chloride are in passive equilibrium then, their vacuolar concentrations would be about one half, one half and twice their cytoplasmic concentrations, respectively. Since rough values for cytoplasmic Na^+, K^+ and Cl^- concentrations are < 20 mM, 200 mM, < 20 mM, respectively, rough values of equilibrium vacuolar concentrations would be < 10 mM, 100 mM, < 40 mM. (Nitrate would also be in very low vacuolar concentrations at equilibrium). For osmotic reasons alone all of these ions are likely to be at higher vacuolar concentrations and thus all of them, except possibly K^+ in some cases and maybe Na^+ for glycophytes, must be actively transported into the vacuole.

Such active ion transport would be expected to be secondary, using the electrochemical potential difference for H^+, the protonmotive force, as the source of energy. One such secondary active transport, the Na^+/H^+ antiport in beet, has been fairly well characterized [5,6]. But there is as yet no definite information about a possible K^+/H^+ antiport system and the clear necessity to postulate porters for the inward fluxes of anions such as Cl^- and NO_3^- is still just that: a postulate.

Such anion porters would be of considerable interest for they would be strongly electrogenic; they must be A^-/H^+ antiporters and thus carry two

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negative charges into the vacuole, but there is sufficient energy available from the protonmotive force for the accumulation of univalent anions to a level approaching 1000 times greater concentrations in the vacuole than in the cytoplasm. The negative charge carried in raises no difficulties; at flux equilibrium the influx of each ion equals the efflux which for all the ions, other than H^+, would be expected to be via ion channels.

This paper is concerned with an aspect of ion transport at the tonoplast other than active transport; this is the existence of passive ion transport via channels in the membrane. Membrane channels for the passive passage of ions, of various degrees of specificity, seem to be ubiquitous. Thier 'function' in most cases is not quite clear, but in general a degree of passive permeability to the major ions would seem to be required for the physiological functioning of all membrane-bound compartments at the cellular and sub-cellular levels. Such (passive) channels of course act as leaks and to a more or less greater extent clearly affect the results of the active-transport systems.

2. MATERIALS AND METHODS

Vacuoles were isolated by osmotic shock of protoplasts obtained from cell suspension cultures of sugar beet (*Beta vulgaris*) and tomato (*Lycopersicum esculatum*) [6]. Cells were incubated at 30°C for 1.5–2 h in a medium containing 2% cellulase Y-C, 0.1% pectolyase Y-23 (both enzymes from Seishin Pharmaceutical Co., Ltd, Tokyo, Japan), 0.5% BSA, 0,5 mM $CaCl_2$, 0.4 M sorbitol, and 25 mM Mes-KOH buffer, pH 5.5. Protoplasts were separated from digestion medium by centrifugation at 800 rpm for 5 min, and purified in a 25% Percoll discontinuous gradient (1200 rpm, 10 min). Lysis of protoplasts was carried out by mixing one volume of purified protoplasts containing 0.4 M sorbitol, 0.2 mM $CaCl_2$, and 15 mM Tris-Mes buffer, pH 5.5, with three volumes of lysis medium (15 mM Tris, 2 mM EDTA, 2 mM EGTA, 50 mM KCl, pH 8.0), at 4°C for 20 min. Released vacuoles were stabilized by adding an equal volume of 0.7 M mannitol, 50 mM KCl, 3% Ficoll, and 15 mM Tris-Mes buffer, pH 8.0. Vacuoles were finally isolated by layering on top of the vacuole suspension 2–3 ml of a medium containing 0.4 M mannitol, 50 mM KCl, 5 mM Tris-Mes buffer, pH 8.0 and centrifugation at 800 rpm for 30 min at 4°C. Vacuoles were recovered at the top of the Ficoll gradient.

Isolated tonoplast patches were obtained by initially sealing the patch pipette against the vacuole and breaking the underlying membrane by a pulse of 1 V and 30 ms. Once in the whole vacuole configuration [12], the pipette was pulled away from the vacuole, and thus, obtaining an outside-out patch [1]. Singe-channel recordings were made with a 8900 patch clamp amplifier (Dagan Corporation), low pass filtered at 1 kHz with a two pole active filter and data were recorded on FM tape. For subsequent analysis, data were low-pass filtered (Bessel), digitized and processed with the PAT V 6.0 program developed by J. Dempster (University of Strathclyde, Glasgow, Scotland) in a PCII-286 computer.

3. RESULTS

The tonoplast of sugar beet and tomato cells displayed voltage-dependent single-channel activity. Fig.1 illustrates the single-channel current on tonoplast patches of both sugar beet (fig.1a) and tomato (fig.1b) cells. In sugar beet, negative pipette potentials elicited channel activity with a conductance of 65 pS. In contrast, positive pipette potentials only elicited small channel openings, of conductance 10 pS (note the change in scales). In tomato patches, single-channel openings increased proportionally with the voltage applied, between −80 mV and +80 mV, with a constant conductance of 25 pS. Single-channel recording in tomato patches showed that the channels were in the open state most of the time. The constant amplitude of the brief deflections observed were assumed to correspond to current changes due to channel closings. The fact that the tomato channels were open almost all the time, could explain the high level of noise observed in these recordings.

The current-voltage relation of single channels for membrane patches from sugar beet vacuoles bathed in K^+ solutions on both sides is shown in fig.2. The patches are orientated so that the cytoplasmic side is facing the bathing solution; thus the voltage (of the pipette electrode) is that between the vacuole and the cytoplasm, i.e. V_{vc} in conventional symbols. Clearly these channels rectify: with symmetrical 100 mM K^+ solutions the conductance is about 65 pS at negative potentials and less than, or about, 10 pS for positive voltages. The I-V curve for asymmetrical K^+ solutions (100 mM K^+_{vac}/10 mM K^+_{cyt}) shown in the same figure cuts the voltage axis at about −35 mV. The average zero current potential is −40 mV indicating that the channels are cation channels with a selectivity for cations over anions of the order of 6:1. Although the channel activity is completely inhibited by 10 mM TEA (tetraethylammonium) or 5 mM Ba^{2+}, two well known inhibitors of K^+ selective channels in animal cells [7,8] (data not shown), the current-voltage relation when there is a K^+ solution on one side and a Na^+ solution on the

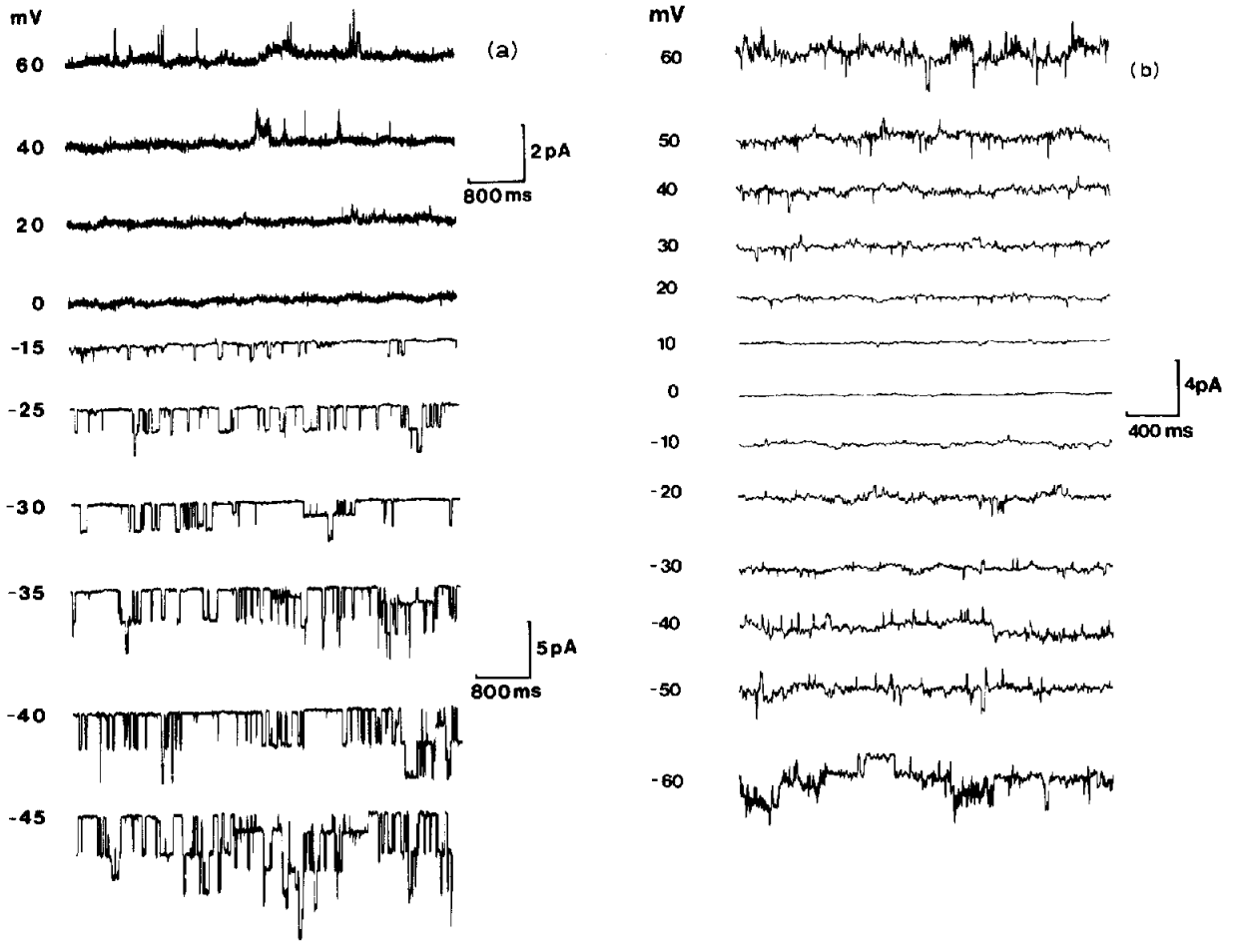

Fig.1. Single-channel recordings from vacuolar membranes of (a) sugar beet and (b) tomato cells. The membrane potential of isolated outside-out tonoplast patches was continuously polarized to the values shown on the left of the individual traces. Solutions were: 100 mM KCl, 2 mM $KgCl_2$, 0.1 mM $CaCl_2$, 5 mM Tris-Mes, pH 7.5, in the pipette and the bath, and adjusted to a final osmolarity of 550 mOsmol with mannitol. Temperature was 22°C.

other is identical with that for symmetrical K^+ solutions (fig.2). These observations suggest that these channels are nonselective between K^+ and Na^+. The absence of an effect of DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid), an inhibitor of Cl^- channels [9], on the *I-V* curves confirms that the current is carried, mostly, by the cations (fig.3).

Single channels in membrane patches from tomato vacuoles do not rectify (fig.4). This is true for patches bathed by symmetrical or asymmetrical K^+ solutions or by patches bathed by K^+ solutions on one side and Na^+ solutions on the other (fig.4). In all cases the single-channel conductance is about 29 pS at all voltages between −80 mV and +80 mV with solutions of concentrations of about 100 mM. The reversal potential in a 100/10 mM solution is −25 to −30 mV, again indicating a cation-selective channel with a cation/anion selectivity of about 4:1. These tomato ion channels are also nonselective between K^+ and Na^+, although the addition of 5 mM Ba^{2+} to the bathing solution, totally inhibited the single-channel currents (data not shown).

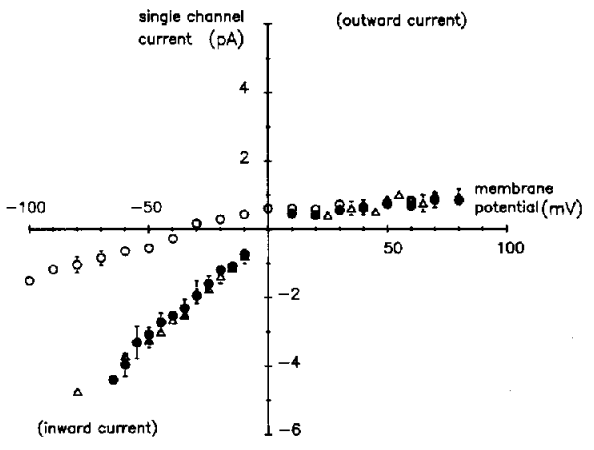

Fig.2. Singe-channel current as a function of vacuolar membrane potential in sugar beet. Under symmetrical 100 mM KCl (●), the *I-V* relationship is linear between −80 mV and 0 mV, with a reversal potential of 0 mV, and the slope giving a conductance of 65 pS. At pipette positive potentials, the conductance decreased to 10 pS. With 100 mM KCl in the pipette and 10 mM KCl in the bath (○), the reversal potential shifted towards a negative potential of −40 mV (average value). The conductance under asymmetrical K^+ concentrations, decreased to 20 pS. Substitution of 100 mM Na^+ for 100 mM K^+ in the bath (△) did not change either the conductance of the channel, or its reversal potential. In addition to the different concentrations of K^+ and Na^+, pipette and bath solutions also contained 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 5 mM Tris-Mes, pH 7.5, and mannitol to give a final osmolarity of 550 mOsmol. Points are the mean ± SE (n = 8).

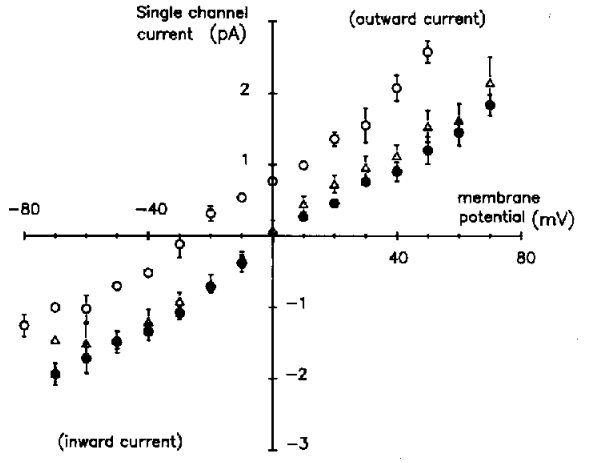

Fig.3. Single-channel current as a function of vacuolar membrane potential in tomato. The *I-V* relationship of tonoplast patches exposed to symmetrical solutions of 100 mM KCl (●) was linear between −80 mV and 80 mV, with a conductance of 29 pS, with a reversal potential of 0 mV. Establishing a K^+ concentration gradient across the patch (100 mM K^+_v/10 mM K^+_c) (○), shifted the zero membrane current to a potential of −30 mV. The conductance of the channel however, was not altered by the reduction of external KCl. Substitution of Na^+ for K^+ (100 mM K^+_v/100 mM Na^+_c) (△), yielded a linear *I-V* relationship with a reversal potential of 0 mV and a conductance of 29 pS. Pipette and bath solutions always contained 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 5 mM Tris-Mes, pH 7.5, and adjusted to an osmolarity of 550 mOsmol with mannitol. Points are the mean ± SE (n = 8).

4. DISCUSSION

The striking feature of these results is that the sugar beet (a halophyte) tonoplast cation channels rectify, i.e. their conductance is much higher when the vacuole is negative with respect to the cytoplasm; while in the physiological range of positive tonoplast potentials, the conductance is much lower. In the glycophyte, tomato, there is no such rectification. Such channel behaviour seems 'appropriate' for the two groups of plants. Halophytes, of the sugar beet type, must accumulate Na^+ to high concentrations in the vacuole, with a probable concentration ratio of more than 10:1. Such a concentration ratio, together with the positive membrane potential, results in a large outward (vacuole to cytoplasm) driving force of about 100 mV equivalent electrochemical potential difference. If the passive conductance were appreciable, an electrochemical

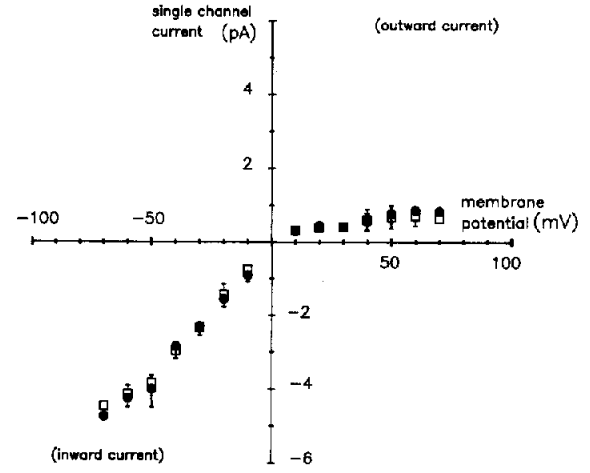

Fig.4. Effect of DIDS on the inward rectifying currents of sugar beet tonoplast. The voltage-dependence of the single-channel currents of sugar beet tonoplast patches, exposed to symmetrical KCl (●), was not altered by the addition of 100 μM DIDS (□). The data for the DIDS experiments are the mean ± SE (n = 4).

potential difference of 100 mV could not be maintained by the Na^+/H^+ antiport. (Note that the energy available for the antiport is about 150 mV, from the protonmotive force created by the proton pump(s).) Glycophytes seem to have no Na^+/H^+ antiport and thus a rectifying behaviour of the channels would have no relevance. Whatever the conductance, Na^+ and K^+ would be distributed passively; although it is true that one would expect, on osmotic grounds, that Na^+ and/or K^+ would be at a somewhat higher electrochemical potential in the vacuole; this point needs some attention and resolution.

We are making measurements on the conductance of the whole vacuolar membrane, to obtain the conductance per square metre of tonoplast; but we can estimate rough figures for these conductances from the average number of channels per 1 μm^2 patch, from the channel conductance and from the ratio of open/closed times. Such estimates yield physiological conductances for the sugar beet tonoplast of the order of 1 $S \cdot m^{-2}$ and 20–50 times bigger for the tomato tonoplast. This beet conductance is of the same order of magnitude as that reported for negative potentials by Hedrich and Neher [10] for the so-called SV (slow vacuolar) type of ion channel, although they reported a single-channel conductance of about 80 pS.

We see no rectification of the tomato tonoplast channel conductance, nor, by calculation, should there be any rectification of the total ionic conductance of the whole tonoplast.This behaviour is different from that reported by Hedrich et al. [11] for the vacuoles of the glycophyte, maize (*Zea mays*).

Whether in beet or in tomato, the channels are not selective between cations, at least not between Na^+ and K^+, nor are they completely cation/anion selective for the reversal potentials for 100/10 mM KCl solutions of less than -54 mV (the figure obtained using K^+ activities rather than concentrations). This indicates some anion, presumably Cl^-, conductance of between 1:4 for tomato and 1:6 for sugar beet of that of K^+. However, the absence of an effect of DIDS (fig.3), does not suggest much Cl^- conductance; also in experiments where a Cl^- concentration gradient was established across the membrane patch (100 mM Cl^-_v/10 mM Cl^-_c) while keeping K^+ at 100 mM on both sides, a reversal potential of 0 mV in the *I-V* curve was obtained (data not given), thus indicating a low Cl^- premeability. It may be that the ion channels observed in these experiments are the only types in the vacuolar membrane and they serve for passive transport of both K^+ and Na^+ and, to a lesser extent, anions such as Cl^-. The apparently lower anion conductance is consistent with a lower leak to give the high anion concentrations produced by the hypothetical, but apparently necessary, anion pumps in the tonoplast.

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