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Electrical Properties of the Plasma Membrane of Microplasmodia of *Physarum polycephalum*

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Summary. Microplasmodia of *Physarum polycephalum* have been investigated by conventional electrophysiological techniques. In standard medium (30 mM K^+, 4 mM Ca^{++}, 3 mM Mg^{++}, 18 mM citrate buffer, pH 4.7, 22 °C), the transmembrane potential difference V_m is around −100 mV and the membrane resistance about 0.25 Ω m². V_m is insensitive to light and changes of the Na^+/K^+ ratio in the medium. Without bivalent cations in the medium and/or in presence of metabolic inhibitors (CCCP, CN^-, N_3^-), V_m drops to about 0 mV. Under normal conditions, V_m is very sensitive to external pH (pH_o), displaying an almost Nernstian slope at $pH_o = 3$. However, when measured during metabolic inhibition, V_m shows no sensitivity to pH_o over the range 3 to 6, only rising (about 50 mV/pH) at $pH_o = 6$. Addition of glucose or sucrose (but not mannitol or sorbitol) causes rapid depolarization, which partially recovers over the next few minutes. Half-maximal peak depolarization (25 mV with glucose) was achieved with 1 mM of the sugar. Sugar-induced depolarization was insensitive to pH_o. The results are discussed on the basis of Class-I models of charge transport across biomembranes (Hansen, Gradmann, Sanders and Slayman, 1981, *J. Membrane Biol.* **63**:165–190). Three transport systems are characterized: 1) An electrogenic H^+ extrusion pump with a stoichiometry of 2 H^+ per metabolic energy equivalent. The deprotonated form of the pump seems to be negatively charged. 2) In addition to the passive K^+ pathways, there is a passive H^+ transport system; here the protonated form seems to be positively charged. 3) A tentative H^+-sugar cotransport system operates far from thermodynamic equilibrium, carrying negative charge in its deprotonated states.

Key words *Physarum polycephalum* · membrane potential · electrogenic pump · cotransport · Class-I model

Introduction

An ideal biological object for membrane studies should have the following features in the same, native, developmental state: unicellular (for uniform membranes), spherical (for reference to membrane area), large (for puncture with micropipettes), suitable for mass production (for biochemical studies), physiologically relevant (for comparison with other subjects), genetically accessible (for picking appropriate mutants). None of the present standard objects in membrane biology nearly fulfills this combination of advantages. However, microplasmodia of the slime mold *Physarum polycephalum* seem to satisfy all of these requirements.

During the past ten years, *Physarum polycephalum* has repeatedly been demonstrated to be a powerful model system in eukaryotic physiology, biochemistry and genetics (for reviews *see* [2, 9, 18, 27, 33]). Only a basic description of its electrophysiological properties is still missing, although electrical phenomena could be observed in the course of some investigations of protoplasmic streaming, the motile system, development and chemotaxis of its vegetative states, macro- and microplasmodia [1, 16, 17, 22, 24]. For those investigations, extracellular recordings of currents and potential changes seemed to provide sufficient information [1, 22].

In an independent study [20], some electrical membrane properties of *Physarum polycephalum* have been investigated on a model system, using cytoplasmic droplets of macroplasmodia, which form a membrane when kept in solutions with caffeine. The question is whether these preparations are representative for membranes of intact, native cells. In fact, those results are in good agreement with ours, as far as equivalent experiments have been carried out.

Recently, some progress has been achieved in understanding the principles of ion transport across biomembranes – in particular of electrogenic pumps and cotransport in eukaryotic plasma membranes [12, 13, 26]. The aim of this study is to provide some basic data about the electrophysiological behavior of the plasma membrane of intact microplasmodia of *Physarum polycephalum* and to discuss these results on the basis of the theory of the reaction kinetics for ion transport across biomembranes [13].

The conclusions reached are consistent with the current concepts of diffusion, electrogenic H^+ pumps

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and cotransport in nonanimal cell membranes [15, 19, 29, 31, 35]. In addition, it is demonstrated that, even in the absence of a detailed knowledge of the current-voltage relations of the plasma membrane, considerable insight can be achieved about the electrically charged states of all three types of transport mechanism.

Materials and Methods

Culture of Plasmodia

Microplasmodia were kindly provided by Dr. Th. Schreckenbach. They were grown on semi-defined medium [4] and kept in the dark on an orbital shaker. For experiments, we used two- or three-day-old cells. In order to facilitate puncturing, the cells were washed from extracellular secretion three times in standard medium (SK, Table 1) and kept (unless stated otherwise) for up to 15 hr before measurement under white light in aerated Erlenmeyer flasks at room temperature (22 ± 2 °C). Experiments were also performed at room temperature.

Media

Table 1 gives the composition of the media used. SK medium (similar to that in [28] contained 30 mM K^+, 4 mM Ca^{++}, 3 mM Mg^{++}, 14 mM Cl^- and 18 mM citrate, buffered at pH 4.7. In some experiments, mannitol or sorbitol was added for maintenance of the osmotic state; however no differences were found whether or not adjustment of osmolarity was made.

Experimental Chamber

A square-shaped Lucite® frame (10×10 mm inner width, front and rear wall 5 mm high, side walls only 1 mm high for lateral access of microelectrodes) was mounted to a Plexiglass plate on the microscope (Leitz, Labolux 2). For perfusion of the chamber with medium during the experiments, fresh medium under hydrostatic pressure (tanks hung about 0.4 m above the chamber) entered via an inlet in the front wall near the bottom of the chamber and was removed by a water jet pump, sucking the medium with a surplus of air through an adjustable outlet on one side of the chamber. A small piece of coverslide was kept on top of the chamber by an extra micromanipulator, thus providing good microscopic control, maximum space for manipulations and little vibration during perfusion. The drop of medium between the glass and the bottom of the chamber had a volume of about 0.3 ml. The time constant for bulk fluid exchange (measured by the change of the tip potential of a microelectrode upon a change of the ionic concentration of the medium) was about 4 sec. A further delay (some sec) of the medium exchange next to the membrane is probably due to a slower exchange through the mucopolysaccharide layer, which readily regenerates after washing.

Two different arrangements were used to fix the cells for puncturing. Big plasmodia (up to 100 µm in diameter and 500 µm length) were pushed with the microelectrode against one of four steps (150 µm high) of a Plexiglass socket on the bottom of the chamber. With this method, there was only access from one side for microelectrodes; therefore, it was only used for simple voltage recordings. For multiple electrode impalements, small plasmodia were sucked with a 20-ml syringe onto a special micropipette (inflated tip, broken to a hemispheric opening of about 50 µm diameter) (Felle, *personal communication*). This micropipette could also be moved and adjusted by a micromanipulator. This arrangement enabled access to the cell from almost any direction by more than one microelectrode. Furthermore, protoplasmic streaming could be mechanically controlled by the vacuum of the holding micropipette. After successful impalement, the cells were released from the holding pipette (turnoff of the vacuum and removal of the holding pipette). So the medium could freely reach the entire cell surface and the cells were sufficiently fixed by the impaled microelectrode(s).

Electrical Apparatus

Standard glass microelectrode techniques with Ag/AgCl half-cells were used. The micropipettes were filled with 3 M KCl and 1 mM EDTA. Their resistance in SK was 10-100 MΩ and their tip potentials in SK less than 15 mV. The transmembrane voltage was recorded between the cell interior and the external medium over a differential FET operational amplifier system [10] by a

Table 1. Composition of the external media used[a]

Name	K^+	Na^+	Ca^{++}	Mg^{++}	Cl^-	Ci	Os	pH	V_m	±SD
SK	30	0	4	3	14	18	0	4.7	− 83.5	17
0.5 SK	15	0	2	1.5	7	9	0	4.7	− 88	9
0.5 SKO	15	0	2	1.5	7	9	34.5	4.7	− 88	10
0.1 SK	3	0	0.4	0.3	1.4	1.8	0	4.7	−107	7
0.1 SKO	3	0	0.4	0.3	1.4	1.8	62	4.7	−105	9
SKo	30	0	0	0	14	18	0	5.1	− 43	25
SNa	0	30	4	3	14	18	0	4.7	− 73	19
SNao	0	30	0	0	14	18	0	5.1	− 15	8
pH 3.2	40	0	4	3	31.9	13.6	0	3.2	− 33	9
pH 4.0	40	0	4	3	22.4	13.6	0	4.0	− 62	17
pH 4.7	40	0	4	3	16.8	13.6	0	4.7	− 79	12
pH 5.7	40	0	4	3	4.4	13.6	0	5.7	−103	10
pH 6.8	40	0	4	3	0	13.6	0	6.8	−118.5	10

[a] Concentrations in mM; measured internal potential, $V_m \pm$SD in mV. Ci: citrate buffer, Os: added osmoticum (sorbitol or mannitol). Glucose, sucrose, inhibitor media: *see text.*

pen chart recorder. In order to detect excess noise or hum, the voltage was also monitored by an oscilloscope.

Current pulses were applied to the interior of the cell by a function generator over a 100 MΩ resistor in series with a separate current-injecting microelectrode. This current could only leave the measuring chamber by a FET ammeter with virtual ground. In order to detect progressive changes of the repetitive current pulses (due to larger, spontaneous changes of the resistance of the current-injecting electrode), the current was continuously monitored as well.

Results

Membrane Voltage under Standard Conditions

The average membrane voltage V_m measured with one inserted electrode under steady perfusion of the measuring chamber with standard medium SK (*see* Table 1), was -83.5 mV (SD: ± 17 mV, $n=43$ microplasmodia). No significant differences in V_m could be found, a) when the cells were kept in dark or light, b) over a period of up to 15 hr after washing of the mucopolysaccharide layer, c) whether two- or three-day-old cells were used, and d) whether protoplasmic streaming took place or was stopped by the vacuum of the holding pipette.

After a successful impalement, stable membrane potentials (changes of V_m by less than 2 mV over 60 sec in the steady state) could be recorded for up to 3 ksec. Frequently, especially when no EDTA was in the microelectrode, the potential spontaneously dropped to a value between -15 and 0 mV. This loss of the recording of the membrane potential is probably due to resealing of the membrane around the electrode tip, the remaining potential possibly reflecting the surface potential just outside the membrane. It was often possible to regain the stable, high potential recording by careful repuncturing with the same electrode.

Spontaneous oscillations of V_m in *Physarum polycephalum* have been reported [22]. They occurred also in our experiments with an amplitude of 5 to 10 mV and with a period of 50 to 200 sec, reflecting the rhythm of shuttle streaming. However, these oscillations could only be observed when the medium was not flowing.

In order to estimate the genuine membrane potential E_m electrical leakage of the membrane around an impaled electrode had to be considered. As mentioned above, impalements with one electrode resulted in an average recording of V_m of -83.5 mV; with two electrodes - as used for resistance measurements - the average was $V'_m = -71.2$ mV (SD: ± 8.1 mV, $n=5$) under the same conditions. We can estimate the true membrane potential E_m by extrapolation to "zero" impalements. Linear circuit analysis - as a first approach - yields

$$E_m = \frac{V_m \cdot V'_m}{2V'_m - V_m}. \quad (1)$$

Therefore, the genuine membrane potential E_m is expected to be -101 mV.

Membrane Resistance

For determination of the membrane resistance r_m a separate current-injecting electrode was impaled in addition to the voltage-recording electrode. For these experiments, spherical microplasmodia were used (50 to 100 µm in diameter), which are supposed to lack [7] the invaginations of the plasma membrane [37]. For these cells the membrane area could be determined. The voltage response to small current pulses (1 to 10 nA, 10 sec) yields an input resistance in the range of 100 MΩ. During continuous recordings of V'_m and the input resistance (in intervals of 20 sec) in individual cells, the input resistance increased with V'_m when spontaneous changes in V'_m occurred. An example of this relationship is shown in Fig. 1.

Assuming the changes of voltage and resistance are due to a varying leak, application of linear circuit analysis predicts that the observed relationship between voltage and resistance should be described by a straight line passing through the origin. This procedure resulted in a good fit to the data (Fig. 1), and enabled, by extrapolation to the genuine membrane potential E_m, estimation of the "genuine" membrane resistance r_m. Numerical analysis of 4 cells yielded a value for r_m of $0.25 \pm 0.07\ \Omega\ m^2$.

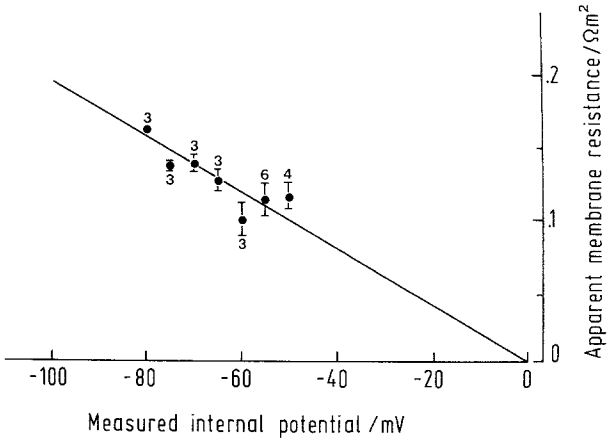

Fig. 1. Example from a single cell, of the correlation between measured internal potential and input resistance (converted to membrane resistance), using two inserted electrodes. Number of samples from 5-mV intervals of a continuous recording marked on points ±SEM. Straight line through origin marks relationship assuming variation in voltage and resistance are due to variable leak

External Ions

In order to characterize the membrane with respect to the specific influence of different ionic species on its electrical properties, changes of membrane potential have been recorded on changing the ionic composition of the external medium. The internal cation concentrations in macroplasmodia are 30 mM K^+, 2 mM Na^+, 4 mM Ca^{++} and 7 mM Mg^{++} [23]; internal pH is taken as 6.1 [8], although a value closer to 7 could be expected as well. This will, however, be of no significance for the conclusions reached in this study. A synopsis of the different media tested and of the resulting membrane potentials is given in Table 1.

K^+, Na^+, Ca^{++}. A possible selectivity between K^+ and Na^+ was examined by partial or total substitution of K^+ in the standard medium by Na^+. Steady-state V_m values have been taken which occurred with the appropriate time constant (10 to 20 sec) for a direct external effect of cations on V_m. The results are given in Fig. 2*A*: no significant changes could be detected. On the other hand, when no bivalent cations were present in the external medium, the membrane potential became much less negative and showed a slightly higher sensitivity for Na^+; for an exchange of K^+ for Na^+ as above, the membrane potential followed the Na^+ concentration with a significant slope of about 10 mV per decade in the range between 1 and 30 mM Na^+ (Fig. 2*B*). It is supposed that Ca^{++} in the external medium is essential for the general integrity of the membrane.

Ionic Strength. When the ionic strength of the external medium was changed by simple dilution of the standard medium, the measured potential increased with a linear slope of about 18 mV per order of magnitude in the range between 30 and 3 mM K^+ (Fig. 2*C*). This general hyperpolarization of the membrane with increasing dilution occurred irrespective of whether or not the change of osmolarity was compensated by appropriate addition of inert sugars like sorbitol or mannitol to the external medium (Table 1).

Protons. The membrane potential of *Physarum polycephalum* turned out to be very sensitive to changes of external pH (pH_o). Figure 3 shows an example from a single cell of the time course of the membrane potential upon several changes of pH_o. The time constant of the voltage response upon addition of protons is about 10 sec; it is assumed to represent the time constant of the medium exchange through the adherent mucopolysaccharide layer.

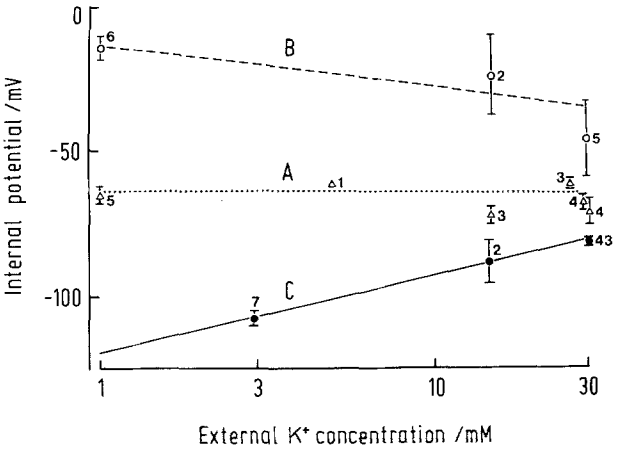

Fig. 2. Effect of external cations on membrane potential. (*A*) △...△: partial substitution of K^+ by Na^+ (sum: 30 mM) under normal conditions. (*B*) ○---○: partial substitution of K^+ by Na^+ (sum: 30 mM), no bivalent cations in medium. (*C*) ●—●: dilution of standard medium, abscissa referenced to K^+ concentration. Means ±SEM; *n* marked on points

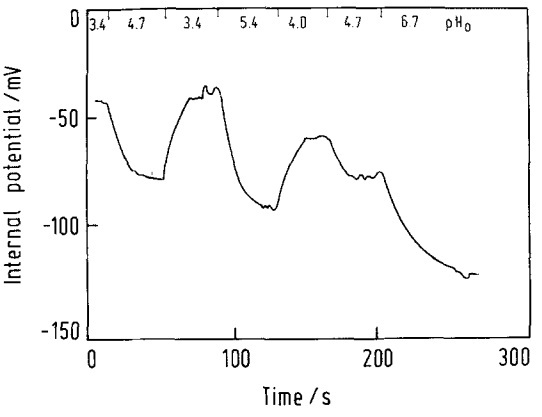

Fig. 3. Example of changes of membrane potential upon changes of external pH. Start of trace at $pH_o = 3.4$; noise on trace during the 10 sec or so prior to each solution change (lag due to tubing distance between medium switch and chamber) are artifacts due to mechanical disturbances

Figure 4*A* shows the average results of V_m versus pH_o. Due to the averaging procedure, the maximum slope between pH_o 3.2 and 4.0 is only about 40 mV/pH. Averaging the individual slopes in this range results in 47 mV/pH; four cells even displayed a slope of 55 mV/pH. Since in these V_m measurements an unspecific leak around the electrode may be involved again, the true slope of $\Delta E_m/\Delta pH_o$ is expected to be even closer to the theoretical value of 58 mV/pH in this range.

Metabolic Inhibitors

The membrane potential of *Physarum polycephalum* is clearly too negative to be explained as being gov-

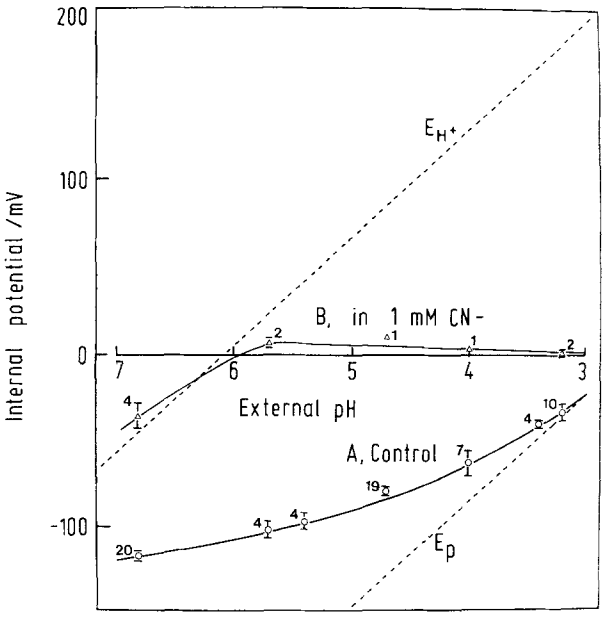

Fig. 4. Effect of external pH on membrane potential. Means ±SEM; n marked on points. (*A*) o——o: normal conditions; (*B*) Δ——Δ: under inhibition of the pump by 1 mM CN^-. Dashed lines have been calculated from the Nernst equation. E_{H^+}: proton equilibrium potential for internal pH = 6.1. E_P: projected equilibrium potential for the pump, referenced to the slope around $pH_o = 3$

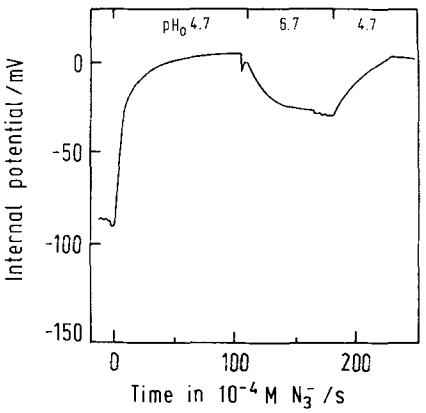

Fig. 5. Example of changes of membrane potential upon exposure to 0.1 mM N_3^- and upon changes in external pH in the presence of 0.1 mM N_3^-. Start of trace: normal conditions ($pH_o = 4.7$); noise on trace during the 10 sec or so prior to each solution change (lag due to tubing distance between medium switch and chamber) are artifacts due to mechanical disturbances

erned by the equilibrium potential of H^+ (*see* Fig. 4) or any other ion. Thus, by analogy with most other eukaryotic nonanimal cells [15, 29, 35] it seems reasonable to expect the involvement of an electrogenic ion pump, carrying positive charge out of the cell and dependent directly on cellular energy metabolism for its activity. Several inhibitors of energy metabolism have, therefore, been tested for their ability to cause a depolarization by withdrawal of metabolic energy from the postulated electrogenic pump. These inhibitors have been used in concentrations expected to be saturating: 0.1 mM iodacetamide, 0.01 mM carbonylcyanide-*m*-chlorophenylhydrazone (CCCP), 1 mM CN^- and 0.1 mM N_3^-.

Iodacetamide, an inhibitor of glycolysis, did not cause any detectable effect (maybe higher concentrations could do so). However, the other three drugs, which are supposed to lower the ATP level by inhibition or uncoupling of respiration, caused a reversible depolarization to about +4 mV. Judged by this value and by the ionic concentrations inside and outside, the membrane potential is now controlled by passive ion diffusion (mainly K^+).

The time course of the depolarization upon exposure to each of these three inhibitors was different: with CN^-, the maximum slope appeared only about 15 sec after its introduction; this delay may be due to slow passage of CN^- through the membrane resulting in a lag before build-up of a saturation concentration at the mitochondria. This delay was not seen with CCCP, probably because CCCP causes a depolarization by creating a proton leak in the plasma membrane, in addition to its effect at the mitochondria [5]. Similarly, with N_3^-, the initial slope was maximum (*see* Fig. 5). After these differences in the initial time course, there was a common depolarization with a time constant of about 20 sec, which may reflect the drop of internal ATP [30]. A slow, spontaneous recovery (up to 50%) was only observed with CCCP after some 100 sec.

These results point to the operation of an electrogenic pump fueled by metabolism. Since the normal membrane potential is sensitive to pH_o, this pump may well carry protons out of the cell. If this is the case, we would expect that with the proton pump not working (i.e. in the presence of inhibitors) the sensitivity of V_m to pH_o should be dramatically changed, and this, indeed, is what is found experimentally. An example from an original recording, showing the response of V_m to pH_o in the presence of N_3^- is given in Fig. 5, and average results from experiments with CN^- are plotted in Fig. 4*B*.

As shown in Fig. 4, the sensitivity of V_m to pH_o is completely lost between pH_o 5.7 and 3.2 with the cells metabolically inhibited. On the other hand, for pH_o more than 5.7, this sensitivity (about 50 mV/pH for V_m, and maybe again even closer to Nernst-slope for E_m) becomes rather high compared with the control (Fig. 4*A*).

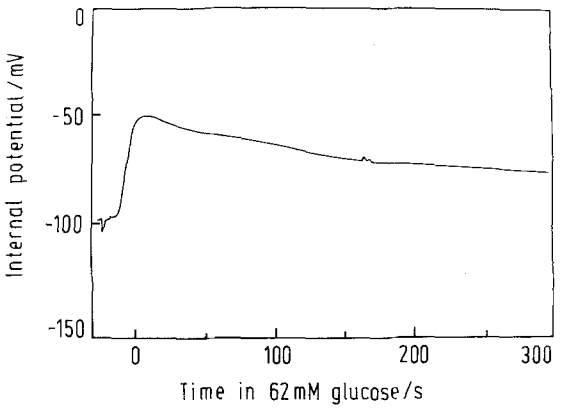

Fig. 6. Rapid depolarization of membrane potential upon exposure of *Physarum* to 62 mM glucose. Time zero marks arrival of glucose in chamber; noise on trace during the 10 sec or so prior to solution change (lag due to tubing distance between medium switch and chamber) is artifact due to mechanical disturbance

Sugar

It is widely accepted (for doubts, *see* Discussion) that plant and fungal membranes maintain a high resting potential in order to facilitate the uptake of nutrients like sugar by electrophoretic H^+ cotransport systems [29]. In these cases, addition of transportable sugar, causes a depolarization. If *Physarum polycephalum* fits into this pattern, sugar-induced depolarizations are expected. Therefore, some preliminary experiments have been carried out.

As mentioned above, inert sugars like mannitol have no effect on V_m. However, glucose and sucrose do cause depolarizations. An example is given in Fig. 6. Upon addition of 62 mM glucose to the medium, the membrane potential drops immediately (i.e. with the time constant of the bathing medium exchange) by about 50 mV; thereafter, partial (about 50%) recovery takes place in some 100 sec with a time course which can be rather complicated.

Addition of only 1 mM glucose causes the same qualitative effect with about half the amplitude. With this concentration, a possible effect of external pH on the glucose-induced depolarization was examined. The sensitivity turned out to be insignificant (-1 ± 4 mV/pH) over the pH_o range from 6.8 to 3.2.

A few experiments with sucrose showed similar effects, although about 30% weaker compared with the effect of glucose at the same concentration (62 mM).

It should be mentioned that, even in the presence of glucose, no effect of light on V_m could be observed.

Discussion

Glucose Uptake and Membrane Potential

Since glucose uptake in microplasmodia of *Physarum polycephalum* has been demonstrated to be inhibited by blue-light [28], an effect of light on the membrane potential seemed possible. However, no immediate effect of light on V_m was detected, either in presence of glucose or in any other of the media used.

Furthermore, this blue-light-sensitive glucose uptake is correlated with an acidification of the medium [28]. From these acidification rates an equivalent proton efflux in the range of 1 μmol m^{-2} sec^{-1} can be calculated, which is close to our calculations (*see below*) of H^+ efflux (0.8 μmol m^{-2} sec^{-1}) driven by the electrogenic pump. However, the lack of any direct light-sensitivity proves the electrogenic pump to be a different entity from the glucose-dependent acidification system. The latter must also be different from a H^+-glucose cotransport system (because of sign) and may consist of an electrically "silent" export of organic acids; and the electrogenic H^+ pump may cause no appreciable acidification of the medium, when the extruded protons leak back into the cell.

Surface- or Transmembrane-Potential

Previous potential recordings of about -50 mV from microplasmodia of *Physarum polycephalum* have been interpreted as surface potentials [16] due to fixed sulfonate$^-$ groups in the mucopolysaccharide envelope [21]. This may be correct for diluted external media in which the surface charge is anticipated to be large. In our experiments, this surface potential is expected to be recorded when the plasma membrane has resealed around the electrode tip. The measured potential is then that of the envelope space, since it is not expected that this resealing process also removes the entire envelope from the electrode tip. Therefore, our recordings of about -10 mV after resealing must reflect the surface potential under the conditions used; and the high negative potentials must be transmembrane potentials.

Further evidence that the high potential recordings are predominantly transmembrane in origin comes from (*i*) the large depolarizing effects of metabolic inhibitors and of sugar (which are difficult to interpret in terms of surface potentials, but easily explainable with present knowledge about the behavior of transmembrane potentials in plants and

fungi) and (*ii*) the depolarizing effects of removal from solution of bivalent cations (which, if anything, would hyperpolarize the surface potential due to the unmasking of surface charges). We do not, however, rule out the possibility that a small component of the measured voltage is due to fixed-charge groups on the envelope; indeed, it may well be the removal of cations from these groups which causes the 30-mV hyperpolarization upon 10-fold dilution of the external medium (Fig. 2).

Voltage Oscillations and Shuttle Streaming

The observation, reported here, that voltage oscillations occur only in unstirred media, can be interpreted in terms of local ionic changes in the external medium. In the light of other findings in this paper, we suggest one of two ions as being responsible. First, periodic efflux of Ca^{++} might well reduce the small surface potential (*see above*) which probably offsets the recordings of transmembrane potential. This view is supported by the observation [25] of higher Ca^{++} efflux during rhythmic contractions, and by the lower potential recordings in this state [24]. Alternatively, it is possible that contractions cause pulsed efflux of H^+ which accumulates just outside the membrane and decreases the transmembrane potential (as in Fig. 4). With sufficient stirring it is expected that this local external accumulation is abolished.

However, the observation that streaming is unaffected under conditions of no voltage oscillations enables us to conclude, along with previous workers [24, 36], that regardless of which ion is responsible, the voltage oscillations represent a peripheral rather than a causative factor in the mechanism of shuttle streaming.

Electrogenic H^+ Pump

The inhibitor results clearly indicate the operation of an electrogenic pump in the plasma membrane of *Physarum polycephalum*. K^+ and Na^+ seem to play a minor role in the electrical properties of the membrane under normal conditions. Bivalent cations in the external medium appear to be necessary to maintain the membrane in intact state.

How can the observation that the membrane potential is normally very sensitive to pH_o be explained on the basis of membrane transport systems? We consider three hypotheses:

1. Electrogenic pump is nonprotonic and a H^+ diffusion pathway, operating in parallel, causes pH_o-sensitivity of V_m. This possibility is ruled out on the basis that inhibition of the pump results in complete loss of pH_o-sensitivity around pH 3.

2. Electrogenic pump transports H^+ out of the cell, but operates as an ideal current source (i.e. is voltage-insensitive over the measured voltage range, such as might be expected for a 1:1 stoichiometry between transported H^+ and ATP, which would generate a reversal potential of -400 to -500 mV [11]. Despite this voltage-insensitivity, changes in pH_o could still lead to very large changes in the saturation current flowing through the pump; in this case the resting membrane potential at each pH_o would be determined mainly by diffusion pathways, the membrane potential coming to rest at the point where the diffusion and pump currents are equal and opposite. Membrane potential should therefore be very sensitive to changes in the magnitude of the diffusion pathways, especially for cations. This sensitivity, however, is rather small (Fig. 2). Therefore we exclude this model as well.

3. The electrogenic pump transports H^+ out of the cell and is sensitive to pH_o and V_m (i.e. it operates fairly close to equilibrium). This hypothesis is in accord with all the observations, in that it predicts that membrane potential will be determined by pH_o-induced changes in pump activity (Fig. 4) irrespective of the nature of the diffusion pathways. The increasing steepness of the V_m response to pH_o as pH_o is lowered suggests that at about $pH_o = 3$, where the slope is almost Nernstian, the pump is very close to equilibrium.

Proceeding further with hypothesis 3, Fig. 4 shows that at $pH_o = 3$, the passive H^+ equilibrium potential E_{H^+} is about 200 mV more positive than the measured membrane potential (around -25 mV). Since the pump is considered to be at equilibrium under these conditions, this energy difference is explained if the transport of one H^+ is coupled to the hydrolysis of 1/2 ATP (with a free energy of ATP around 40 kJ mol^{-1} or 0.4 eV). In other words, H^+ transport can be understood as an electrogenic pump with a stoichiometry of 2 H^+ per ATP (or comparable metabolic energy equivalent).

If at $pH_o = 3$, the pump is in equilibrium at -25 mV, then thermodynamic arguments give its equilibrium potential at normal $pH_o = 4.7$ as -125 mV. For these conditions, the membrane resistance (0.25 Ω m^{-2}) and the membrane potential (-101 mV) have been determined. The pump is not in thermodynamic equilibrium anymore but is shunted by passive pathways. The equilibrium potential of this leak is around ground potential ($+4$ mV under metabolic inhibition of the pump). With these numbers, we can estimate the transport rate of the pump using linear circuit analysis as a first, crude approach [34]. These calculations yield an inner resistance of the pump of 0.3 and 1.3 Ω m^2 for the

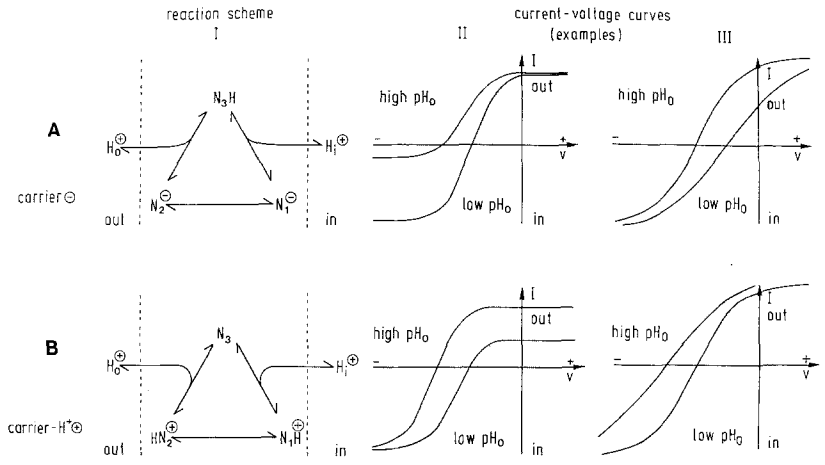

Fig. 7. Alternative reaction kinetic models (Class-I) for H^+ "carrier" across membranes and examples of changes of their current-voltage curves upon changes of external pH (symmetrical saturation currents are no intrinsic feature); I: reaction kinetic schemes; II: change of I-V curve for general case ($k_{12} \times k_{21}$ not small); III: change of I-V curve for special case ($k_{12} \times k_{21}$ small and $k_{23} \gg$ others)

leak. The internally cycling current through the loop of pump and leak would be around 80 mA m^{-2}, corresponding to a pump rate for H^+ of 0.8 μmol $m^{-2}\,sec^{-1}$. This calculated electrical current is about five times larger than measured currents which traverse developing cytoplasmic drops and small plasmodia [1].

In conclusion, it appears that *Physarum polycephalum* possesses an electrogenic proton extrusion pump (stoichiometry: 2 H^+ per cycle) in its plasma membrane, which is close to thermodynamic equilibrium at $pH_o = 3$ and which almost completely controls the electrical properties of the membrane under these conditions, displaying a membrane potential around −25 mV. There are also alternative but more complicated interpretations which require more and specific assumptions. Those should not be discussed here on a merely academic level.

Modelling. The results of the V_m response upon varying pH_o can now be discussed on the basis of the reaction kinetics of Class-I models (with one charge-carrying pathway) of ion transport systems [13]. For this purpose, a 3-state model (*see* Fig. 7) is sufficient. The transport system is portrayed as having two charged states with densities N_1 and N_2 in mol m^{-2} (one inside and one outside for the description of voltage-sensitivity by charge transfer reactions) and one neutral, intermediate state (density N_3). The apparent rate constant which describes the binding of H^+ outside is proportional to the external H^+ concentration. (For our pump, where 2 H^+ are supposed to be bound per cycle, this apparent rate constant should actually be proportional to the square of the external H^+ concentration; however, for the following qualitative considerations, this distinction does not matter.) The rate constants (k_{ij}: from i to j, in sec^{-1}) between the inner and the intermediate state (N_1 and N_3) can be subsumed into one compound pair (k_{13} and k_{31}), because they are considered to be unchanged in the present experiments in which only pH_o and V_m are variables.

Two alternatives have to be discussed: is the unloaded system negatively charged and the protonated form neutral (Fig. 7*A*) or the unloaded form neutral and the protonated state positively charged (Fig. 7*B*)? The change of pH_o will yield quite different changes of the current-voltage relationships for these two cases *A* and *B*, as illustrated in Fig. 7.

The current-voltage relationships of the 3-state model are given by

$$i = zFN \frac{k_{12}k_{23}k_{31} - k_{21}k_{32}k_{13}}{k_{12}(k_{23}+k_{32}+k_{31}) + k_{13}(k_{21}+k_{23}+k_{32}) + k_{21}(k_{31}+k_{32}) + k_{31}k_{23}} \quad (2)$$

where z is the charge, F the Faraday number, N the total density ($N_1+N_2+N_3$) of the system;

$$k_{12} = k_{12}^0 \exp(zu/2) \quad (3a)$$

$$k_{21} = k_{21}^0 \exp(-zu/2) \quad (3b)$$

where k_{12}^0 and k_{21}^0 are the voltage-dependent rate constants at zero voltage, u is the normalized voltage (V_mF/RT) and the factor 2 in the denominator of the exponents stays for a symmetric energy barrier for charge translocation at zero voltage.

We want to discuss the general case (*see* Fig. 7I, II) first. For case *A*, the saturation currents for high positive (i_{s+}) and negative (i_{s-}) voltages are:

$$i_{s+A} = zFN(k_{13}k_{32}/(k_{31}+k_{32}+k_{13})) \quad (4)$$

$$i_{s-A} = zFN(k_{23}k_{31}/(k_{31}+k_{32}+k_{23})) \quad (5)$$

and for case *B*:

$$i_{s+B} = zFN(k_{23}k_{31}/(k_{31}+k_{32}+k_{23})) \quad (6)$$

$$i_{s-B} = zFN(k_{13}k_{32}/(k_{31}+k_{32}+k_{13})). \quad (7)$$

If the external H^+ concentration rises, k_{23} rises in case *A* and k_{32} in case *B*. That means for case *A* that i_{s+} stays constant and i_{s-} increases, yielding a larger amplitude between the saturation currents, and hence an increase of the absolute slope, which is equivalent to the conductance. This case seems to be verified by our results for the pump, since, as shown in Fig. 4, there is an increasing tendency as pH_o is lowered for V_m and E_p to coincide, as expected if the pump conductance is increasing.

In contrast, for case *B*, a lower pH_o would decrease i_{s+} and leave i_{s-} unaffected, yielding a lower slope conductance at low pH_o and a higher one at high pH_o. Such a system would cause E_p and V_m to coincide at high pH_o, when pump conductance is maximal, and Fig. 4 shows that this is certainly not the case.

However, for the passive pathways in the membrane (Fig. 4*B*), the H^+ pathway seems to follow case *B* exactly. As pH_o is raised, so V_m is increasingly well described by E_{H^+}; this would arise from the increasing conductance of the passive H^+ pathway at high pH_o. A similar tendency for equivalency of V_m and E_{H^+} at high pH_o has been described for the alga *Chara corallina* [3]. The passive H^+ pathway is, therefore, thought to consist of a transport system which is neutral in its unloaded state and positively charged in the protonated form (Fig. 7*B* II). By this criterion it seems unlikely that the two H^+ transport systems under consideration (pump and "leak") use the same "carrier" and differ only by the coupling to the energy metabolism; rather, they are supposed to be independent systems. Furthermore, the V_m-pH_o characteristic in Fig. 4*B* excludes the possibility that the passive H^+ pathway is a simple "pore", because pores usually increase their conductance at higher substrate concentrations (more special pores, however, could cause similar V_m-pH_o characteristic, i.e. if they would undergo a major conformal change at high pH_o, or if they were gated by protonation at the external end).

On the other hand, there is a special condition when the conductance changes of carrier systems are opposite to those just described (Fig. 7I, II). This condition is met if $k_{23} \gg$ others (both saturation currents stay constant) and if the product $k_{12}k_{21}$ is relatively small. In this case (*see* Fig. 7I, III), there is a conductance decrease for lowering pH_o in case *A* and an increase in case *B*. Although this possibility cannot be ruled out by the present results, the conditions are so special that situation II seems to be more probable.

It can be shown by analog rationales that the conclusions about the sign of the charge of the charge translocating states are also valid if not H^+ but OH^- is transported.

Glucose-Induced Depolarization

Sugar-induced depolarizations *per se* do not provide conclusive evidence for H^+-sugar cotransport: complementary measurements of sugar transport would be necessary to be sure of this point. Nevertheless, there is no alternative mechanism described at present which would account for such depolarizations. Therefore, we have to discuss – tentatively – our results on the basis of cotransport.

The effect of glucose is evident from our results. The smaller effect of sucrose may reflect a lower affinity of the glucose system to sucrose or a less efficient, separate sucrose system. On the other hand, the results may well point to a decomposition of sucrose by extraplasmatic enzymes, such as α-galacturosidase or invertase, which would result in a glucose effect on the membrane as well.

Under normal conditions, the equilibrium potential of a H^+-glucose cotransport system with a 1:1 stoichiometry can be calculated [31]:

$$E_{co} = RT/F \cdot \ln(C_{H_o^+} \cdot C_{go}/C_{H_i^+} \cdot C_{gi}) \quad (8)$$

where $C_{H_o^+}$ and $C_{H_i^+}$ are the H^+ concentrations outside ($pH_o = 4.7$) and inside ($pH_i = 6.1$), C_{go} the glucose concentration outside (1 mM in our case) and C_{gi} the internal glucose concentration which can be assumed to be a few μM, in accordance with other systems [6]. This equilibrium potential would be more than 150 mV (inside positive). Therefore, the current-voltage relationship of the system must be expected to be saturated (current-source characteristics) in the voltage range investigated. This has been experimentally confirmed in other systems [6, 32].

In this context, we should comment on the current view of the physiological significance of a (energetically expensive) high membrane potential. As mentioned above, it is supposed to be used for the uptake of nutrients by electrophoretic H^+-cotransport. However, H^+-cotransport systems in cells with high membrane potentials [6, 32] behave as ideal current sources in the voltage range investigated; in other words, the transport rates of these H^+-cotransport systems do *not* depend on the voltage. Therefore, according to a suggestion of Dr. D. Sanders (*personal communication*), high membrane potentials seem to be irrelevant for these H^+-cotransport systems. On the other hand, the H^+-sugar cotransport system in *Chlorella*, which is voltage-dependent, seems to operate at rather low membrane voltages [14, 19].

If, again in the case of *Physarum*, the H^+-glucose cotransport system obeys the model in Fig. 7*B*, the glucose-induced proton inward current should be independent of pH_o. As discussed above, at $pH_o = 4.7$ membrane resistance is dominated by that of

the pump. Since, as pH_o is lowered, pump resistance is expected to fall, we would expect a constant glucose current to generate a *smaller* voltage change at low pH_o. This situation was not verified by our results. On the other hand, if the tentative H^+-glucose cotransport system follows the model of Fig. 7*A*II, the glucose-induced H^+ current in our voltage range would rise for lower pH_o. This would provide two counteracting effects of pH_o on glucose-induced depolarizations. For example upon lowering pH_o, the voltage response would tend to increase due to an enhanced H^+ current through the cotransport system, and tend to decrease due to a lowered slope resistance of the pump.

Since, in our measurements, the glucose-induced depolarizations did not change significantly with pH_o, the results support the model of Fig. 7*A* rather than 7*B* to be appropriate for the H^+-glucose cotransport system; i.e. the H^+-glucose entry seems to be electroneutral, wherease the recycling of the unloaded transport molecule ought to carry negative charge.

Conclusions

The above results are consistent with current understanding of the electrically significant entities in "normal" plant and fungal plasma membranes, consisting of a dominant electrogenic H^+ pump, a H^+-sugar cotransport system and leaks of a small conductance.

Physarum polycephalum seems to be a powerful system for further investigations of these transport systems.

The concept of Class-I models [13] has turned out to provide interpretations of electrophysiological observations, even when no current-voltage curves are available and data are limited to simple voltage recordings under different environmental conditions.

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