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Single-channel activity induced in mitoplasts by alkaline pH

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Abstract

Exposure of patch-clamped mitoplasts to alkaline pH induces a reversible conductance increase (Antonenko, Yu. N., Kinnally, K.W. and Tedeschi, H. (1991) J. Membr. Biol. 124, 151–158) which is due to an increase in open probability of a channel activity of 15 pS and larger transitions. The present study defines in more detail some of the characteristics of the channel activity involved in this conductance increase. The results suggest the presence of two channels one slightly cation-selective of approx. 15 pS (referred to here as alkaline-induced cation-selective activity, ACA) and another slightly anion selective of approx. 45 pS (referred to as alkaline-induced anion-selective activity, AAA). The possible implication of these results in relation to other channels and the permeability transitions reported by others using mitochondrial suspensions is discussed.

Key words: Alkaline-induced ion-selective activity; Single-channel activity; Mitochondrial channel; Mitochondrial inner membrane; Permeability; Patch clamp; (Mitoplast)

1. Introduction

We have previously reported a reversible conductance increase induced by alkaline pH in patch-clamped mitoplasts (mitochondria with the outer membrane removed) and also some of its properties such as effects of Mg^{2+} and amphiphilic drugs [1]. At that time we demonstrated the presence of channel activity of 15 pS at alkaline pH and we also observed larger transitions. The present study defines in more detail some of the characteristics of the channel activities involved in this transition. We have identified two different channel activities, one slightly cation selective of about 15 pS in 0.15 M KCl, and another slightly anion selective of approx. 45 pS. The possible implication of these results in relation to other channels and the permeability transitions reported by others using mitochondrial suspensions is addressed in the Discussion.

2. Materials and methods

2.1. Isolation of mitochondria and preparation of mitoplasts

Large mitochondria were isolated from the liver of normal mice by a modification of the method previously described for cuprizone treated mice [2] except that the homogenization medium was generally 230 mM mannitol, 70 mM sucrose, 0.25 mM EDTA, 5 mM Hepes (pH 7.4). After a centrifugation at $150 \times g$ for 80 s, the supernatant was layered on 460 mM mannitol 140 mM sucrose and 0.25 mM EDTA, 5 mM Hepes (pH 7.4) and centrifuged at $800 \times g$ for 3 min. The upper layer close to the interface was collected and recentrifuged at $2600 \times g$ for 5 min to isolate the mitochondria. Occasionally the procedure was carried out using 250 mM sucrose and 500 mM sucrose, respectively, with 0.25 mM EDTA and 5 mM Hepes (pH 7.4). In some experiments, 1 mM EDTA replaced 0.25 mM EDTA. No differences in mitoplasts prepared with the different media were noted. The mitoplasts were prepared by the French press method of Decker

Abbreviations: EDTA, ethylenediaminetetraacetate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate.

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and Greenawalt [3]. The mitochondria in the pellet were resuspended in 15 ml of 460 mM mannitol, 140 mM sucrose, 10 mM Hepes (pH 7.4) and subjected to 2000 psi using a French press to remove the outer membrane. The resulting mitoplasts were centrifuged at $4000 \times g$ for 5 min and resuspended in approx. 3 ml of 0.15 M KCl, 5 mM Hepes (pH 7.4). The mitoplast preparation in media containing EDTA generally produced patches which initially exhibited no resolvable channel activity at pH 6.8 and voltages up to ± 60 mV [4]. Patches exhibiting significant channel activity at pH 6.8 were not used in these experiments. In some patches, some 15 and 45 pS activity was detectable at pH 6.8 (see Results below).

2.2. Chemicals

ATP, glybenclamide and 4-aminopyridine from Sigma were introduced at a concentration of 1 mM for ATP, 4 mM for 4-aminopyridine and 8 μM for glybenclamide by perfusion. ATP and glybenclamide were in either 1 M KCl, pH 8.2 (three experiments) or in 0.15 M NaCl at pH 8.2 (one experiment). 4-Aminopyridine and ATP were in 1 M KCl.

2.3. Patch-clamping procedures

The manipulations were previously described [1]. The resistances of the patches ranged from approx. 3–50 GΩ regardless of KCl concentration. After the seal was formed, inner membrane patches were excised by drawing the pipette away from the mitoplast attached to the slide. The excised patches were most likely in the inside-out configuration (see Ref. [5]).

Generally, the pipette and initial bath medium contained 0.15–1 M KCl, 10 μM $CaCl_2$, 5 mM Hepes (pH 6.8) (however, see special cases below). Generally, 1 ml of external medium was used in the bath and the perfusions were carried out with 5 ml on the bath (matrix) side of the membrane either at pH 6.8 or 8.2. The reference electrode was a Ag, AgCl wire connected to the bath through a 2% agar bridge containing the pipette medium to reduce potential changes at the reference electrode during selectivity experiments. Ionic gradients were obtained by replacing the bath medium by perfusion of the chamber with the test solutions at pH 8.2 after obtaining a gigaseal and a study of the patch under symmetrical conditions. The cations tested were potassium, sodium, lithium, cesium and choline while the anions used were chloride, acetate and glucuronate. For all of these generally a 1 M solution was used. However, for choline chloride 0.7 M choline chloride and 0.3 M KCl were used (with 1 M KCl in the pipette and the agar bridge) and for glucoronate either 0.4 M NaCl, 0.6 M sodium glucoronate (with 1 M NaCl in the pipette and in the agar bridge) or 0.1 M sodium glucoronate, 0.05 M NaCl (with 0.15 M NaCl in the pipette and agar bridge). A direct comparison of K^+ and Cl^- permeabilities was made by introducing a KCl gradient by substituting in the bath medium with 0.25 M KCl and 1.5 M of a non-electrolyte for osmotic balance. Experiments were carried out with sucrose, mannitol and erythritol.

The patch pipettes were fabricated using glass from World Precision Instruments, Sarasota, FL (1B100F4) using a Sachs-Flaming micropipette puller (Model PC84, Sutter Instruments, Novato, CA) with resistances ranging from 20 to 40 MΩ at 0.15 M KCl and which were correspondingly lower at higher KCl concentrations.

The details of the electronics and the software used for analyses of the data has been previously reported [1]. In the present study the sampling rate was at least twice the filtration rate (see legend of figures). In contrast to our previous report [1], the polarity of the voltages (V) is reported in relation to the mitochondrial matrix side so that $V = V_{bath} - V_{pipette}$. All procedures were carried out at room temperature (about 25°C).

2.4. Determination of reversal potentials and calculations

The ratio of permeability constants for cations (P_{cat}/P_K) and anions (P_{an}/P_{Cl}) were calculated from the reversal potentials using the Goldman-Hodgkin-Katz equation after correcting for junction potentials [6] at the agar bridge. The corrections were necessary because of junction potentials arising at the KCl bridge of the reference electrode upon replacement of the KCl of the bath with a solution with a different cation or anion. These produce a junction potential at the reference electrode. We determined experimentally the junction potentials caused by a similar asymmetry by placing the replacement solution at the reference electrode. Where the Cl^- was decreased or absent in replacement solution the appropriate concentration of KCl was introduced at the reference electrode to avoid a potential difference at the AgCl junction. The corrections of the reversal potentials listed with the corresponding concentration at the reference electrode were as follows: 3 mV for 1 M CsCl, 8 mV for 1 M LiCl, -8 mV for 1 M K acetate, -4 mV for 0.05 M NaCl, 0.1 M sodium glucoronate, 3 mV for 0.3 M KCl, 0.7 M choline Cl, -7 mV for 0.4 M NaCl, 0.6 M sodium glucoronate.

Open probability was calculated from current amplitude histograms as the% of the total time the current level occupied the open state. Reported conductances were either calculated from current amplitude histogram peaks and voltage or the slope of the single-channel amplitude vs. voltage plots.

3. Results

We have examined the channel activity induced by alkalinization (pH 8.2) of the matrix side of the membrane at voltages ranging between ±40 mV in 98 patches displaying little or no activity at pH 6.8. In order to resolve individual channel activity of low conductance we used two approaches: increased ionic strength of the solutions [1] or high voltages (e.g., 190 mV) [7] at physiological ionic strength. At 0.15 M concentration of salts (generally KCl), 90% of the patches underwent the current transition we previously reported [1]. On the other hand, at high ionic strength (0.5–2 M KCl) only 66% of the patches ($n = 76$) responded.

3.1. Size of the conductance transitions underlying the effect of alkaline pH

We previously found an 80 pS voltage insensitive increase in conductance (at 0.15 M KCl) when the medium on the matrix side of the patch was changed from pH 6.8 to 8.2. Some of this increase was attributed to an increase in open probability of a ~15 pS channel activity (referred to as the alkaline-induced cation-selective activity, *ACA*). We report here the additional contribution of a ~45 pS activity which is slightly anion selective (referred to as the alkaline-induced anion-selective activity, *AAA*).

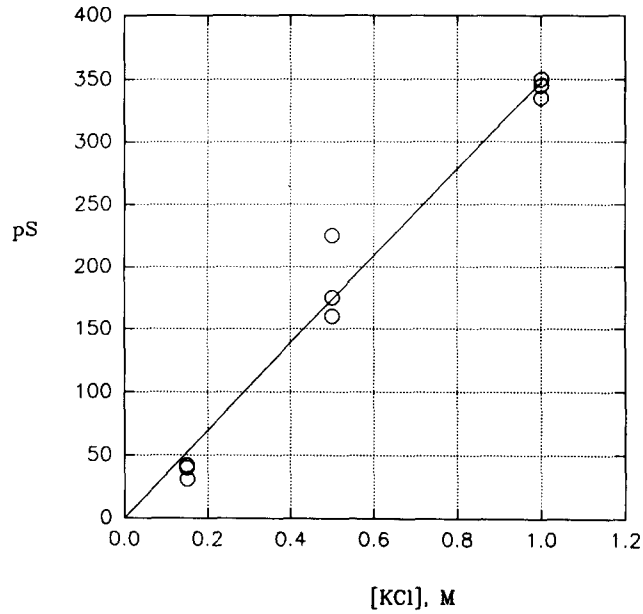

Fig. 2. Single-channel conductance as a function of KCl concentration for AAA. Each point represents a value calculated from the total amplitude diagram at a bin width of 0.4 pA. The KCl concentration was the same in the bath (pH 8.2) and the patching pipette (pH 6.8). The conditions were otherwise the same as in Fig. 1.

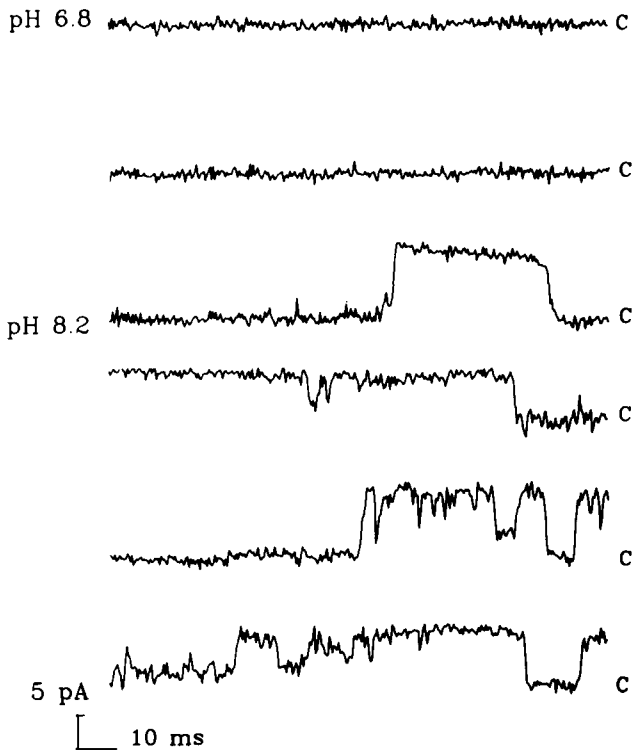

Fig. 1. Current traces of an excised patch illustrating the alkaline pH-induced current transitions. The patch was originally in symmetrical 0.5 M KCl, 5 mM Hepes, 10 μM $CaCl_2$ (pH 6.8). It was then perfused on the bath side (corresponding to the matrix) with the same medium at pH 8.2. The voltage was 40 mV and the data was bandwidth limited at 2 kHz. The upward current transitions from C indicate openings.

Fig. 1 shows the effect of change from a medium at pH 6.8 (Fig. 1, upper trace) to pH 8.2 (lower trace) in a 0.5 M KCl medium at 40 mV. At pH 6.8 little or no channel activity can be observed. Several current levels can be seen at pH 8.2. The predominant transitions in this patch were somewhat higher than those reported previously and correspond to AAA (~220 pS calculated from the total amplitude histogram, not shown, corresponding to 66 pS in 0.15 M KCl). As reported before [1], the activation was reversible and when the pH was returned to 6.8 the channel activity returned to its original level (not shown here). In this patch, the open probability of AAA increased from 0.2 to 0.5 upon alkalinization. While no change in mean open time (3 ms) was observed, the mean closed time decreased from 14 to 9 ms upon alkalinization.

As previously shown [1] for the smaller ACA transitions, the conductance of these larger transition levels increases approximately linearly with KCl concentration (Fig. 2). At 0.15 M KCl AAA was approx. 45 pS. As shown in the figure, the actual conductance level exhibited considerable variability.

In all patches examined (at the appropriate ionic strength or at high voltage, see Section 2) at pH 8.2 the ACA was invariably seen whereas the higher conduc-

tance AAA transitions were absent in approx. 25% of the patches. The experiment of Fig. 3A (in 1 M KCl) in which the voltage was clamped at 43 mV showed several conductance levels including ACA and AAA. In this figure the lowest conductance level (from the amplitude histogram) assumed to correspond to the closed state is labelled C. The dashed lines are spaced 4.5 pA apart (105 pS, i.e., approx. 15 pS at 0.15 M KCl). The trace showed single transitions of ~ 300 pS and intermediate transitions of ~ 100 pS each. In some cases (second and fourth row) the ~ 300 pS transition occurred as three stepwise events. Transitions between level 3 and 1 also took place but less frequently. This information is summarized in the current amplitude diagram of Fig. 3B.

3.2. Ion selectivity of the conductance levels

We examined the ion selectivity of AAA and ACA by perfusing the bath with a substitute medium. In Fig. 4, the dependence of the transition size on voltage in 1 M KCl in both the medium and pipette is shown by the circles. Except for the difference in pH (6.8 in the pipette, 8.2 in the medium), the system was symmetric.

Substitution with potassium acetate (squares) or choline chloride (triangles) in the medium is shown for AAA in Fig. 4A. The reported values have been corrected for junction potentials (see Section 2). The results show a slight selectivity in relation to anions as indicated by the displacement of the curve to negative values, i.e., the channel was less permeable to acetate than to Cl^- (for $n = 4$, $P_{Ac}/P_{Cl} = 0.4 \pm 0.1$, mean ± SD). Similar results were obtained with glucoronate where $P_g/P_{Cl} = 0.38$ ($n = 2$). As expected for this slightly anion-selective channel and indicated by the companion curves (compare circles with triangles), it cannot distinguish between choline chloride and KCl indicating that the permeability constants of the two were approximately the same. This result suggests that it may be anion selective (see below). In the three

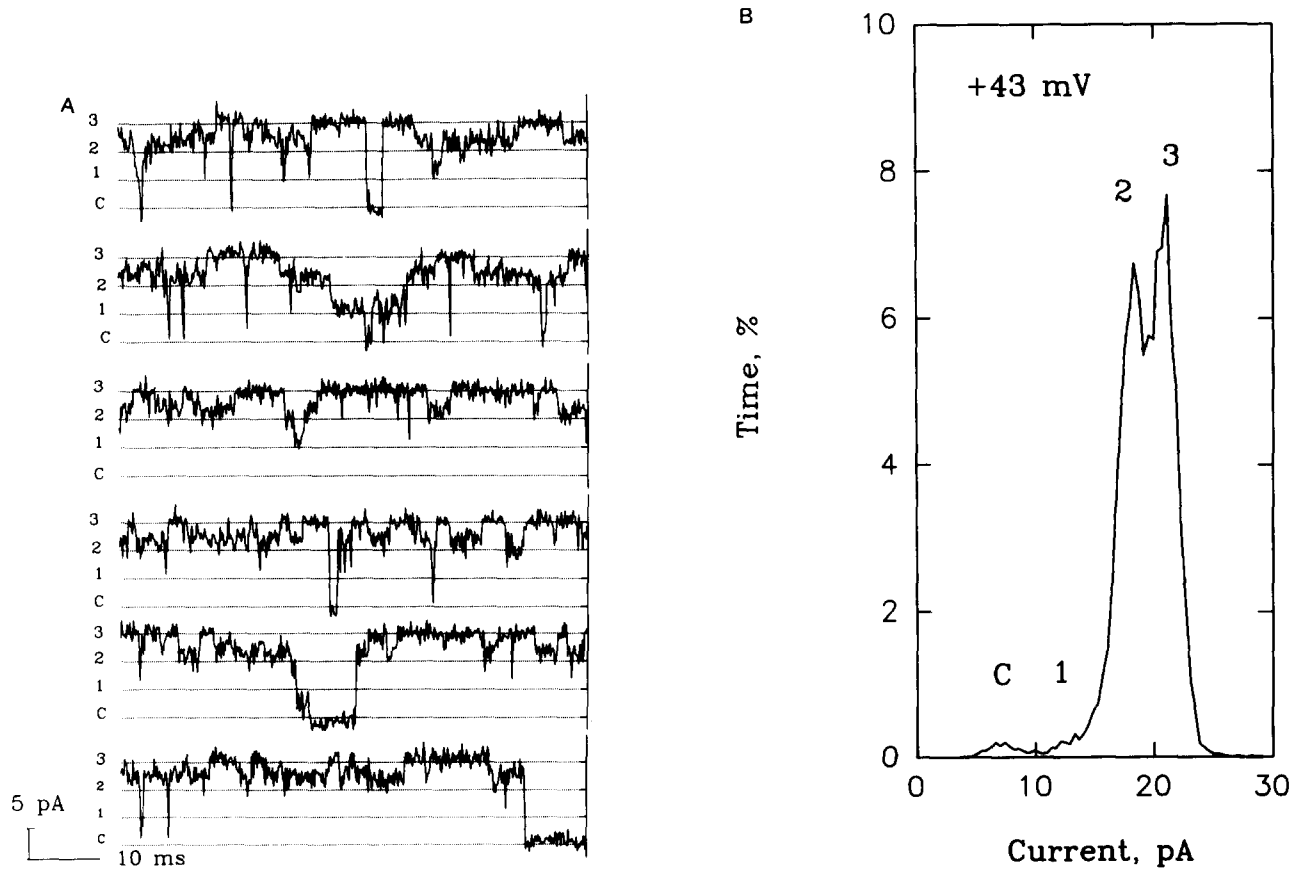

Fig. 3. (A) Current trace induced by alkaline pH at 43 mV after perfusion with 1 M KCl, 5 mM Hepes, 10 μM $CaCl_2$ (pH 8.2) shows conductance substates of AAA. The pipette was filled with the same medium at pH 6.8. The data was bandwidth limited to 4 kHz. The dashed lines in the figure were drawn at 4.5 pA intervals to facilitate the viewing of three open states. (B) Total current amplitude histogram of a 2.0 s trace at a bin width of 0.4 pA. It illustrates the% time occupation of the same four current levels. The open probability was estimated at 0.55 and 0.99 for the 100 and 300 pS activities, respectively, corresponding to the 15 and 45 pS in 0.15 M KCl.

additional cases in which only the cation was substituted there were no significant shifts in reversal potential (generally less than 1–2 mV). These results suggest a permeability ranking of $P_{Cl} > P_{Ac}$, P_g.

Fig. 4B represents the ion selectivity results for ACA, using choline chloride as a substitute for K^+ (see Section 2) and indicates a slight ability to distinguish between cations ($P_{ch}/P_K = 0.5$). We had similar results in other experiments ($P_{ch}/P_K = 0.6 \pm 0.1$ $n = 4$). We generally observed no shift in reversal potential with anion substitutions (in one experiment we found a change in conductance induced by acetate without altering the reversal potential). We found the ratio to be approx. 0.5 for Cs ($n = 2$) and Li ($n = 1$). We generally found the conductance of the channel in symmetrical NaCl to be approx. $18 \pm 6\%$ (mean $\pm$ S.D., $n = 3$) greater than for the equivalent measurement in KCl suggesting that $P_{Na} > P_K > P_{ch} > P_{Cs}, P_{Li}$.

In the presence of ionic gradients, the I–V curves were linear at low voltages. They deviated from linearity at a wider range of voltage (not shown because this range was not studied systematically).

The results obtained with KCl concentration gradients were difficult to analyze because the non-electrolytes used for osmotic balance inhibited channel activity either by maintaining the channels in the open or closed state for long periods. This inhibition was more pronounced with sucrose and mannitol than with erythritol. For this reason, we report the results obtained with erythritol. Furthermore, AAA openings to intermediate states could be easily mistaken for the conductance level ACA, distorting the results. To avoid confusion we selected patches that exhibited predominantly ACA activity. In order to more accurately determine the reversal potential, the mean transition size at each voltage ($n = 4$–6 independent experiments) was used. The reversal potential is ≈ 23 mV which corresponds to P_{Cl}/P_K of ≈ 0.1. For AAA the reversal potentials in two independent experiments were -12 and -16 mV. The corresponding P_{Cl}/P_K is 2.2 and 3.

3.3. Effect of inhibitors

Inoue et al. [8] report a 15 pS channel which is inhibited somewhat by ATP and substantially by 4-aminopyridine and glybenclamide in the presence of ATP. In addition the channel has greater selectivity for K^+ than for Na^+. We found no effect of the inhibitors on ACA in the presence of 1 mM ATP and 8 μM glybenclamide in four independent patches. Similarly we found no effects of 4 mM 4-aminopyridine in the presence of 1 mM ATP (one experiment).

We previously reported on the inhibition of the alkaline pH transition by amphiphilic drugs and Mg^{2+} [1].

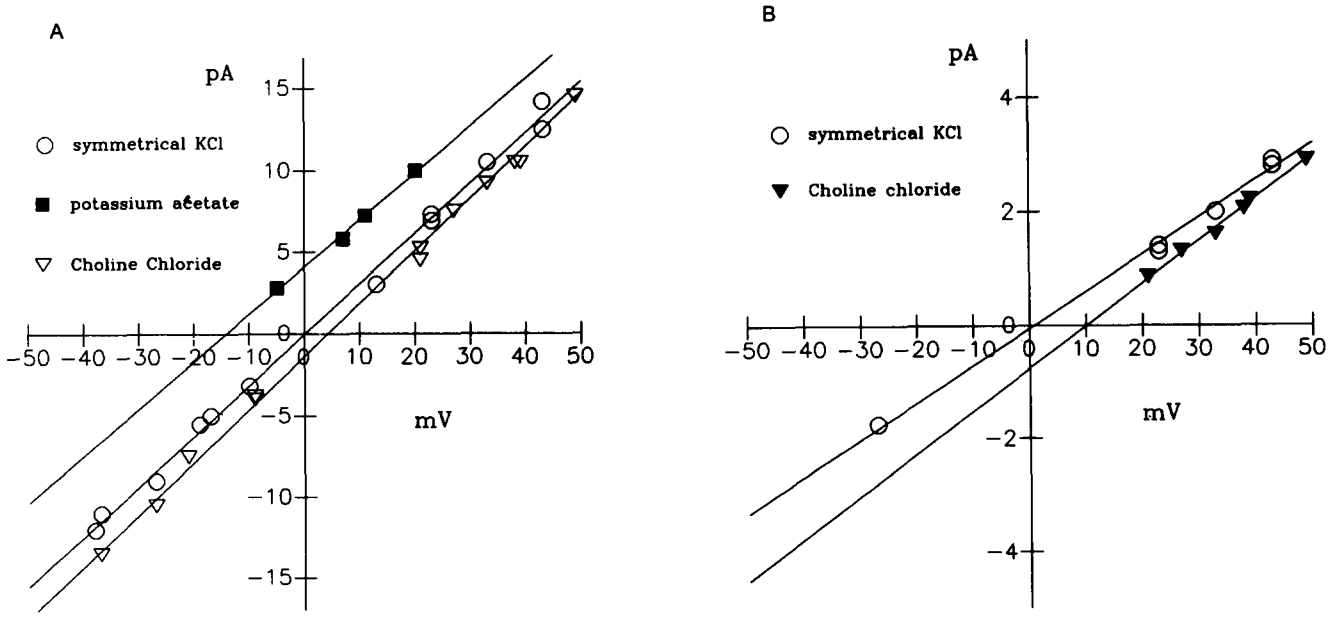

Fig. 4. Single-channel current amplitude-voltage curves obtained from current amplitude histograms with a bin width of 0.4 pA at pH 8.2. (A) AAA current amplitude as a function of voltage (284 pS conductance calculated from the slope of the symmetrical KCl line, corresponding to 43 pS at 0.15 M KCl). The pipette contained 1 M KCl, 5 mM Hepes, 10 μM $CaCl_2$ (pH 6.8). The medium contained either the 1 M KCl medium at pH 8.2 (circles), or the KCl was replaced with 0.3 M KCl, 0.7 M choline chloride (triangles) or 1 M potassium acetate (filled squares). (B) ACA current amplitude-voltage curve (65 pS calculated from symmetrical KCl line, corresponding to 10 pS at 0.15 M KCl) under the same conditions shown in (A).

4. Discussion

4.1. Ion selectivity and single-channel I–V curves

The permeability of the ~ 15 pS channel (ACA) to various cations exhibits some selectivity, whereas the permeability of the ~ 45 pS channel (AAA) exhibits some selectivity for anions.

Typically, the *I–V* curves of AAA were identical or near identical regardless of cation used and conversely those of ACA were the same when KCl of the medium was substituted with another K salt (e.g., K acetate). This would be in agreement with the notion that ACA is cation-selective and therefore relatively impermeable to anions whereas the 45 pS channel is anion-selective and relatively impermeable to cations as generally found in other systems.

This is confirmed in experiments with a KCl gradient. ACA is cation-selective where the permeability to K^+ was seven times greater than that for Cl^-. In contrast, AAA can discriminate between K^+ and Cl^- where the permeability to Cl^- is two to three times greater. This only slight selectivity as to charge may perhaps explain why a substitution of KCl with choline chloride reveals a slight apparent reversal potential (Fig. 4B), suggesting that the higher conductance channel can discriminate between these two cations.

4.2. Possibility of the presence of a ~ 45 pS channel with ~ 15 pS substates

At alkaline pH we have observed single transitions corresponding to approx. 15 and 45 pS in 0.15 M KCl (e.g., see Fig. 3). The lowest conductance level can be interpreted to be the closed state (C). We have also observed stepwise transitions between the totally open (level 3) and the closed level (C) and in some cases a stepwise transition was found to be followed by a closing or opening by a single step when bandwidth limited to 4 kHz. We feel that these observations can be most readily explained by assuming that some of these ~ 15 pS steps correspond to substates of the ~ 45 pS channel. This is supported by current traces (e.g., Fig. 3), selectivity and transition frequency analysis. It is difficult to carry out a detailed analysis because generally the most frequent transition of ~ 15 pS corresponds to the cation-selective channel and not the transition from level 3 to 2. The most compelling evidence for a 45 pS channel with substates is provided however, in many of our experiments, by the frequency of occurrence of the 3 to C transition level which is much greater than the predicted closing of three independent channels. For example, in the experiment of Fig. 3A the resolution of the system was 0.25 ms (bandwidth limited to 4 kHz and 10 kHz sampling). The frequency of the smaller transition was 0.018 ms^{-1} and the observed frequency for the C to 3 transition (for a 2700 ms record) was 0.0013 ms^{-1}. Assuming the C to 3 transition is due to the coincidental simultaneous transition of three independent channels, a transition frequency of $5.8 \cdot 10^{-6}$ is predicted whereas $4.7 \cdot 10^{-3}$ is observed (0.25 ms window). The occurrence of gradual transitions smaller than the 15 pS steps that we see occasionally may indicate the occurrence of other substates of even lower conductance. We found that all the conductance levels observed (i.e., from levels 3, 2 or 1 to C, in the experiment of Fig. 3A) were anion selective and their reversal potential did not differ from each other within experimental error (not shown). In this analysis, the smaller transition level from level 3 to 2 were selected only when clearly associated with the 45 pS transitions. Because of the variability in the size of the AAA (see Fig. 1 or 2), it is difficult to examine precisely the number of conductance states and some of the data are consistent with five substates rather than four.

We interpreted the single 15 pS transition as a separate cation-selective channel (ACA). Differences in the selectivity of substates of the same channel have been observed in the voltage dependent anion channel (VDAC or mitochondrial porin) (e.g., Refs. [9, 10]). However, approx. the same cation selectivity was expressed regardless of whether ACA channel was alone or in the presence or absence of the AAA and as we noted above all the conductance levels of the AAA were found to be anion selective. However, the relationship between the ~ 15 pS ACA channel and the ~ 15 pS-substates of the AAA is far from clear.

4.3. Relation to other channel activities studied by patch clamping

Inoue et al. [8] reported a 15 pS channel with higher selectivity for K^+ than Na^+. Since the 15 pS channel activity (ACA) reported in the present communication does not show the same selectivity or sensitivity to inhibitors (such as ATP and glybenclamide) the two are likely to differ.

We have previously reported on channel activities in the range of 10–20 pS and 45 pS [5] at pH 7.4, sufficiently alkaline to induce some channel activity (see Fig. 2 of Ref. [1]). The 45 pS conductance was found to be slightly anion selective. This observation suggests that the two studies were involved with the same channel.

Zorov et al. [11] have occasionally observed in rat heart mitoplasts a conductance level between 40 and 50 pS which was ascribed to the multiple conductance channel activity (MCC) at pH 7.4. At this time we do not know the possible relationships between these two sets of observations. Szabó et al. [12] have reported a pH dependence of the mitochondrial megachannel

(which most likely corresponds to MCC) similar to that of the AAA and ACA.

4.4. Permeability transitions and channel activity

A variety of important studies of mitochondrial suspensions have observed permeability transitions under specific conditions. In some cases, the transitions were explained by postulating the activation of channels or pores. A so called K^+-uniport is activated by depletion of Mg^{2+} (e.g., Refs. [13,14]) and appears to be relatively unselective, transporting Rb^+, Na^+ and Li^+ [13]. Similarly, alkaline pH and divalent ion depletion (e.g., Refs. [15–19]) activate an anion-uniporter, the so-called inner membrane anion channel (IMAC).

On the basis of these studies our findings of a cation-selective (ACA) and an anion-selective (AAA) channel activity are of considerable interest. Our conditions correspond to those of Mg^{2+} depletion since we have no Mg^{2+} in the medium (which corresponds to the matrix side) and the mitochondria were isolated in the presence of EDTA. In addition, the increase in current induced by alkaline pH was inhibited by Mg^{2+} [1]. We have shown previously that at pH 6.8 the whole patch current is somewhat cation-selective. Alkaline pH shifts the selectivity of the patches toward anionic-selectivity. We have also observed the pharmacology and dependence on Mg^{2+}, or pH of the transition brought about by alkaline conditions [1] and found it to parallel closely that of the anion uniporter (e.g., Ref. [18]). It would be tempting to postulate that the 15 pS channel activity corresponds to the K^+ uniporter and the 45 pS activity to IMAC. However, arguing against this conclusion, the selectivity of IMAC [19] is much greater than that of AAA. For example, the permeability of IMAC to chloride is at least 400 times greater than for glucoronate [19]. For AAA we have found a permeability ratio of 0.3 for glucoronate/Cl^-. In addition, the analysis of patch clamp data is also complicated by the peculiarities of mitochondrial channels which can remain in an open or closed state for prolonged periods without any apparent activity (e.g., see Ref. [11]) as also indicated by the effect of inhibitors on the patches. These drugs reduce the lowest conductance level of the whole patch by as much as 90% (see Fig. 6 of Ref. [1]). In our opinion, it is entirely possible that the 15 pS conductance state is occupied at pH 6.8 often without detectable channel activity.

4.5. Location of ACA and AAA

The study was carried out in mitoplasts, i.e., mitochondria from which the outer membrane has been stripped away by the French press. The results can therefore be ascribed to electrical properties of the inner membrane. In agreement with this interpretation, on occasions ACA and AAA activity were observed in the presence of centum-picosiemen channel (mCS) activity that has been used as a marker of inner membrane location (e.g., Refs. [7,20]).

4.6. Possible functional significance of the inner mitochondrial membrane channels

The abundance of various inner mitochondrial ion channels raises questions (see Ref. [7]) on their significance in the maintenance of electrochemical gradient of mitochondria and in their possible physiological role. The mitoplast membrane is generally of very high resistance and the channels have to be activated by special conditions, e.g., in the case of ACA and AAA by a combination of Mg^{2+}-depletion and alkalinization of the matrix side of the membrane. Therefore the channels will not uncouple mitochondrial oxidative-phosphorylation except under very special conditions. The possible physiological role of the channels remains unclear and all discussions have been so far highly speculative. The findings that some of the channels are sensitive to Ca^{2+} and ATP suggest possible regulatory functions. Beavis [19] suggests a role of IMAC in mitochondrial volume regulation as also proposed by Gunter and Pfeiffer [21] for inner membrane channels particularly during osmotic and metabolic stress. A possible role of some of these channels in thermoregulation has been proposed by Galina Mironova (personal communication).

Note

Recently Hayman et al. (1993) J. Membr. Biol. 136, 181–190 and Hayman and Ashley (1993) J. Membr. Biol. 136, 191–197 reported a 50 pS channel present in cardiac mitoplast membranes reconstituted in planar bilayers. The channel was found to be minimally selective for anions, to lack voltage dependence and to have at least three substates. These properties indicate that it probably corresponds to AAA. There are, however, some differences such as an absence of pH dependence and failure to respond to some drugs (e.g., propranolol or quinine) in the reconstituted membranes.

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