Substitution of Glutamic 779 with Alanine in the Na,K-ATPase α Subunit Removes Voltage Dependence of Ion Transport*

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The effects of changing Glu-779, located in the fifth transmembrane segment of the Na,K-ATPase α subunit, on the phosphorylation characteristics and ion transport properties of the enzyme were investigated. HeLa cells were transfected with cDNA coding the E779A substitution in an ouabain-resistant sheep α1 subunit (RD). Steady state phosphorylation stimulated by Na+ concentrations less than 20 mM or by imidazole were similar for RD and E779A enzymes, an indication that phosphorylation and Na+ occlusion were not altered by this mutation. With E779A enzyme, higher Na+ concentrations reduced the level of phosphoenzyme and stimulated Na-ATPase activity in the absence of K+. These effects were a consequence of Na+ increasing the rate of protein dephosphorylation. In voltage-clamped HeLa cells expressing E779A enzyme, a prominent electrogenic Na+ exchange was observed in the absence of extracellular K+. Thus, increased Na-ATPase activity and Na+-dependent dephosphorylation result from Na+ acting as a K+ congener with low affinity at extracellular binding sites. These data suggest that E779A does not directly participate in ion binding but does affect the connection between extracellular ion binding and intracellular enzyme dephosphorylation. In cells expressing control RD enzyme, Na,K-pump current was dependent on membrane potential and extracellular K+ concentration. However, Na,K-pump current in cells expressing E779A enzyme was voltage independent at all extracellular K+ tested. These results indicate that Glu-779 may be part of the access channel determining the voltage dependence of ion transport by the Na,K-ATPase.

The Na,K-ATPase transports Na+ and K+ across the plasma membrane of eukaryotic cells to establish electrochemical gradients for these ions (1–4). The stoichiometry of ion transport, 3 Na+ outward and 2 K+ inward, also generates a transmembrane electric current (1, 2, 4–7). Recent studies indicate that extracellular ion release and binding steps are voltage dependent, consistent with the hypothesis that extracellular Na+ and K+ approach their binding sites by moving through a narrow access channel in the membrane electric field (8–10). Despite this information, the structure of the protein responsible for ion transport remains unknown. This lack of structural information is a major obstacle in understanding the mechanism of the active ion transport by the Na,K-pump.

A significant effort has been directed at identifying amino acids that participate in cation coordination during transport (11, 12). Logical targets of these studies have been carboxyl residues (Glu-327, Glu-779, Asp-804, Asp-808, Asp-926, Glu-953, and Glu-954) that may be located in the transmembrane segments of the protein (13–24). Site-directed mutagenesis studies have shown that substitutions of amino acids Glu-327, Asp-926, Glu-953, or Glu-954 produce no major changes in cation affinity (16–18, 20) or in the enzyme electrical properties (19). Only Asp-804 and Asp-808 are "essential" for enzyme function (16, 22, 23), and they appear to be involved in K+ coordination.2

Glutamate 779 is located in the putative fifth transmembrane segment of the Na,K-ATPase α subunit. Several studies have proposed a central role for this transmembrane segment in the energy transduction mechanism of the enzyme (13, 14, 25, 26). Chemical modification studies (13, 14) initially identified this residue as involved in cation binding. Site-directed mutagenesis studies showed that the substitution E779L significantly impaired the enzyme such that this heterologous Na,K-ATPase could not support cell growth (16). However, substitutions E779D, E779Q, E779A, and E779K yielded functional enzymes displaying only moderate changes in K1/2 for cation activation of Na,K-ATPase activity (21–23). Surprisingly, E779A enzyme had an increased Na-ATPase activity (ATP hydrolysis in the absence of K+) and a low steady state level of Na+-activated phosphoenzyme (22, 23). Changes in long range interactions within the phosphoenzyme, instability of Na+ occluded conformations, and loss of cation selectivity by the enzyme have been invoked to explain these effects of E779A substitution (22, 23).

On the other hand, these data might also indicate that the removal of the carboxyl group alters the partial reactions associated with external ion binding to and release from phosphorylated enzyme intermediates. To gain a greater understanding of the functional role of Glu-779, we have studied the characteristics of the phosphorylated forms and the electrical properties of the E779A substituted enzyme. Portions of this report have been presented in abstract form (27).

EXPERIMENTAL PROCEDURES

Mutagenesis and Cloning—The eukaryotic expression vector pRC4 containing the sheep Na,K-ATPase α1 subunit cDNA was used. This cDNA was inserted into the expression vector pKC4

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1 Throughout this report the amino acid positions corresponding to the sheep α1 sequence will be used.

2 T. Kuntzweiler, J. M. Argüello, and J. B Lingrel, unpublished results.
cDNA was modified by substitutions Q111R and N122D (RD) to encode an ouabain-resistant form of the enzyme (28). The construct carrying the desired single mutation, E779A, was produced by the method of Kunkel (29) as described previously (30).

**Tissue Culture and Transfection of HeLa Cells—**HeLa cells were maintained in Dulbecco’s modified Eagle’s media supplemented with 10% calf serum at 37 °C in humidified air at 5% CO₂. Cells were transfected using a calcium-phosphate method (31) and selected by inclusion of 1 mM ouabain (28). Ouabain-resistant colonies were isolated from different transfections and expanded into stable cell lines. For electrophysiological experiments, HeLa cells were grown on glass coverslips. Control cells carrying RD enzyme were grown in media containing 1 or 5 mM KCl. The reduction of KCl in the extracellular media increased expression of heterologous enzyme (22, 32–34) without effects on the electrophysiological characteristics of Na,K-pump current.

**Membrane Preparations and Protein Quantification—**Crude membranes from HeLa cells were prepared using a Nal treatment (35) as described previously (36). Heterologous protein was quantified by immunodetection, with sheep-specific monoclonal antibody M8-P1-A3, and anti-mouse horseradish peroxidase-conjugated secondary antibody, using purified sheep enzyme as a standard (34). The antibody M8-P1-A3, which was a generous gift from Dr. James Ball (University of Cincinnati), does not recognize the endogenous human isoforms (37). Na,K-ATPase activity was measured as extracellular K⁺ turnover number per mg of membrane protein, and either 0.01 or 10 mM ouabain. Na-ATPase activity was inhibited by 1 or 5 mM KCl, while 10 mM ouabain inhibited both endogenous human Na,K-ATPase, while 10 mM ouabain inhibited both endogenous human Na,K-ATPase and choline-Cl. Assays were performed at 37°C for 30 min, and the samples were incubated for a further 3 s at 0°C. Stopping solution was then added, and the samples were filtered, washed, and counted as described above.

**Electrophysiological Studies—**Glass coverslips containing HeLa cells were placed in an experimental chamber at 37 °C on the stage of an inverted microscope and superfused with a Hepes-buffered Tyrode’s solution (140 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM dextrose, and 5 mM Hepes, pH (22 °C) 7.4). Cells were whole-cell voltage-clamped using single patch electrodes (∼1.5 MΩ) back-filled with a high Na⁺ intracellular electrode solution (85 mM NaOH, 30 mM CsOH, 85 mM aspartic acid, 20 mM tetraethylammonium chloride, 3 mM MgCl₂, 10 mM MgATP, 5 mM pyruvate acid, 5 mM Tris-creatine phosphate, 5.5 mM dextrose, 10 mM EGTA, 10 mM Hepes, pH (22 °C) 7.35). After establishing a Giga seal, the cells were superfused with a solution containing 145 mM NaCl, 2.3 mM MgCl₂, 2 mM CaCl₂, 0.2 mM CdCl₂, 5.5 mM dextrose, and 10 mM Hepes/NaOH, pH (22 °C) 7.38. All superfusion solutions contained 1 μM ouabain to block endogenous Na,K-ATPase and 0.5 mM DIDS to block an outwardly rectifying chloride current.

**Na,K-pump current due to heterologous Na,K-ATPase activity was defined as the difference current measured in the presence of 1 μM and 10 μM ouabain. However, K⁺ activation of heterologous Na,K-ATPase was measured as extracellular K⁺ sensitive outward current. To activate the Na,K-pump, the KCl concentration in the superfusion solution was increased from 0 to 5.5–50 mM where [Na⁺] / [K⁺] = 145 mM. In some experiments, Na⁺ was replaced by equimolar tetramethylammonium ion. Na,K-pump current data were normalized to total capacitance (pA/pF) calculated from the integral of current elicited by 5 mM depolarizations.

To study the voltage dependence of Na,K-pump current, voltage-clamp pulses were applied from a holding potential of −40 mV to various potentials over the range of −100 to +60 mV for 100 ms at 2 Hz. Whole-cell currents and voltage signals were low-pass filtered at 0.4 and 500 Hz with an 8-pole Bessel filter and then sampled at 1.4 and 1,000 Hz for slow and fast time base records, respectively.

**RESULTS**

Our initial experiments were aimed at the biochemical characterization of the E779A substituted enzyme. The E779A enzyme was expressed in larger quantities than the control RD enzyme (Table I). The specific Na,K-ATPase activity and the turnover number were not affected by the substitution. Table I also shows a large increase in Na,K-ATPase activity observed in the absence of K⁺. These properties of E779A-substituted enzyme are consistent with previous reports (21–23).

**Characteristics of E779A Phosphoenzyme—**In previous studies, E779A-substituted enzyme showed low levels of phosphoenzyme in the presence of ATP, Mg²⁺, and 150 mM NaCl unless oligomycin was present (22). These data suggested that the intermediate E₇[P(Na₃)] is unstable in the E779A enzyme. To better understand this observation, the Na⁺ dependence of enzyme phosphorylation by ATP was studied (Fig. 1). Up to 20 mM NaCl, phosphorylation of these enzymes was similar, while half-maximal phosphorylation estimated to occur at 2.5 and 2.2 mM NaCl for RD and E779A, respectively. Maximum levels of enzyme phosphorylation were similar. At higher NaCl concentrations, E779A phosphoenzyme decreased, while RD

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**TABLE I**

| Expression[^a] | Na,K-ATPase specific activity[^a] | Na-ATPase activity | Turnover number[^a] |
|----------------|----------------------------------|--------------------|--------------------|
| μg / mg         | μmol / min / mg                  | % of Na,K-ATPase    | 1 / min            |
| RD control      | 30 ± 2                           | 8.63 ± 1.5         | 14.2 ± 3.8         | 9533 ± 1481                    |
| E779A           | 78 ± 27                          | 7.61 ± 2.48        | 59.7 ± 1.5         | 9472 ± 2959                    |

[^a]: μg of heterologously expressed Na,K-ATPase per mg of total protein in the membrane preparations.

[^b]: Specific activity (μmol of hydrolyzed ATP by min by mg of expressed Na,K-ATPase) at saturating ligand concentrations.

[^c]: The turnover number was calculated independently for each preparation as the ratio of Na,K-ATPase activity to phosphoenzyme level measured in the presence of oligomycin.

[^d]: Values are the mean ± S.E. of four independent clones; each clone was assayed at least in duplicate.
phosphoenzyme increased to a maximum. The NaCl concentration that produced a half-maximal decrease in phosphoenzyme levels of E779A enzyme was approximately 25 mM, a concentration at which Na\(^+\) binds to extracellular sites (1, 43–46). It should be noted that calculation of half-maximal phosphorylation for E779A enzyme is complicated by the biphasic effects of Na\(^+\). Nonetheless, these results suggest that this mutated enzyme can form a stable phosphoenzyme, but only at low Na\(^+\) concentrations.

To clarify why high Na\(^+\) concentrations decreased the amount of E779A phosphoenzyme, steady state phosphorylation levels were compared in the presence of Na\(^+\), Na\(^+\) plus oligomycin, and imidazole (Fig. 2). As in Fig. 1, phosphorylation of E779A in presence of 50 mM Na\(^+\) was low; however, phosphoenzyme levels for both enzymes were similar in the presence of oligomycin plus 50 mM Na\(^+\). Thus, oligomycin stabilizes the Na\(^+\)-bound phosphoenzyme to a similar degree in both enzymes, in agreement with previous results (22).

Low levels of E779A phosphorylation could be due to an intrinsic instability of the phosphorylated intermediate or a specific action of Na\(^+\), as Fig. 1 might suggest. To distinguish between these possibilities, further experiments took advantage of the finding that imidazole stimulates phosphorylation by ATP in Na\(^+\)-free conditions (47). Phosphorylation of both RD and E779A enzymes reached similar levels in the presence of imidazole (Fig. 2). Thus, low phosphorylation levels of E779A enzyme in the presence of high Na\(^+\) concentrations reflect a direct action of Na\(^+\) on the phosphoenzyme.

The mechanism of this Na\(^+\) effect was examined by measuring enzyme dephosphorylation with ADP, KCl, and NaCl (Fig. 3). Enzyme initially phosphorylated in the presence of 50 mM imidazole (in absence of Na\(^+\)) showed that the E779A phosphoenzyme was sensitive to ADP and KCl to the same extent as the control isofrom. This result suggests that the equilibrium \(E_1P \rightleftharpoons E_2P\) was not affected by the substitution. The substituted enzyme was sensitive to dephosphorylation by 200 mM Na\(^+\), and the rate of this dephosphorylation was comparable with that obtained with 10 mM K\(^+\). This effect of Na\(^+\) upon the E779A phosphoenzyme occurred with low affinity, since 10 mM Na\(^+\) (close to saturating high affinity intracellular sites) did not increase dephosphorylation rate.

**Electrophysiological Characteristics of E779A Enzyme**—One possible mechanism to explain the acceleration of E779A dephosphorylation is that Na\(^+\) can substitute for K\(^+\), albeit with low affinity, to increase enzyme turnover rate. If this is the case, outward current mediated by the Na,K-pump would be observed in the absence of extracellular K\(^+\). To test this possibility, Na,K-pump current was measured in HeLa cells ex-

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**Fig. 1.** Na\(^+\) dependence of phosphorylation by ATP of RD and E779A-substituted enzymes. Na\(^-\)activated phosphorylation was measured at different Na\(^+\) concentrations using RD enzyme (C) and E779A enzyme (●) as indicated under "Experimental Procedures." Data from the RD enzyme were fitted with the Hill equation yielding the following parameters: \(V_{max} = 101 \pm 11\), \(K_{1/2} = 2.5 \pm 0.9\) mM, Hill coefficient = 1.1 \pm 0.3. In this figure, 100% corresponds to 0.53 \pm 0.08 and 0.69 \pm 0.07 nmol/mg of expressed Na,K-ATPase for RD and E779A enzyme, respectively.

**Fig. 2.** Phosphorylation by ATP of RD and E779A-substituted enzymes. Phosphoenzyme levels were measured as described under "Experimental Procedures" in the presence of 50 mM Na\(^+\), 50 mM Na\(^+\) plus 100 mM oligomycin, or 50 mM imidazole. The values are the mean \pm S.E. of results obtained with membrane preparations from three independent clones, each one measured in duplicate.

**Fig. 3.** Effects of ADP, KCl, and NaCl on the phosphoenzyme level of RD and E779A-substituted enzymes. Samples were phosphorylated in the presence of 50 mM imidazole and no added NaCl as indicated under "Experimental Procedures." Phosphoenzyme levels after dephosphorylation in the presence of the indicated ligands are expressed as the percentage of the maximum phosphoenzyme level obtained with 50 mM imidazole. Values are the mean \pm S.E. of results obtained with membrane preparations from three independent clones, each one measured in duplicate.
pressing RD or E779A enzyme under conditions in which contaminating currents were blocked (6).

Fig. 4A shows a typical experiment in which Na,K-pump current was measured in a cell expressing RD enzyme. Increasing extracellular K\(^+\) from 0 to 15 mM elicited a sustained outward shift in membrane current that was rapidly blocked if this maneuver was repeated with 10 mM ouabain. This ouabain-sensitive outward current, representing the movement of positive charge out of the cell, is anticipated for the operation of the Na,K-ATPase (2, 6, 7). Increasing extracellular K\(^+\) from 0 to 15 mM with HeLa cells expressing E779A-substituted enzyme also produced an outward shift in current that was blocked in the presence of 10 mM ouabain (Fig. 4B), a direct demonstration of the functional integrity of this mutated enzyme. However, ouabain produced an inward shift in membrane current. A similar inward shift in current was observed when cells were exposed to 10 mM ouabain in K\(^+\)-free solutions (data not shown). These results suggested that the Na,K-pump continued to carry out electrogenic ion transport even in K\(^+\)-free solution. Ouabain-inhibitable current in K\(^+\)-free solution was found to be 40 ± 5% (n = 7) of total K\(^+\)-activated, ouabain-inhibitable Na,K-pump current.

To determine whether this ouabain-inhibitable current in K\(^+\)-free solution represents uncoupled Na\(^+\) efflux from the cell or an electrogenic Na\(^+\)-Na\(^+\) exchange, experiments were repeated in Na\(^+\)-free superfusion solutions (Fig. 4C). Under these conditions, an increase in extracellular K\(^+\) still produced an outward shift in current, but application of 10 mM ouabain did not result in an inward shift in current. Thus, extracellular Na\(^+\) is required for the mutant enzyme to carry out electrogenic ion transport in the absence of K\(^+\), consistent with an electrogenic Na\(^+\)-Na\(^+\) exchange.

Previous studies (5, 7–10, 48) have demonstrated that electrogenic steps in the ion transport cycle are associated with extracellular ion binding. Under many experimental conditions, these electrogenic steps can control the overall rate of ion transport and thereby confer membrane potential dependence to Na,K-pump current. Therefore, to determine if these electrogenic steps were affected by the substitution E779A, the voltage dependence of Na,K-pump current was examined in cells expressing RD and E779A enzyme. Na,K-pump current amplitude was measured during superfusion with Na\(^+\)-containing salt solutions in the presence of several K\(^+\) concentrations and then calculated by subtracting current at each K\(^+\) concentration from that in K\(^+\)-free solution. The inset of Fig. 5A shows typical traces of difference currents calculated in this manner with 50 mM K\(^+\)-containing solution during voltage clamp pulses to several membrane potentials in cells expressing RD enzyme. With the higher K\(^+\)-containing solutions (5 and 50 mM K\(^+\) in Fig. 5A), the amplitude of the K\(^+\)-activated current in cells expressing RD enzyme increased as membrane potential became less negative. At positive potentials, current amplitude tended to saturate and become independent of membrane potential. At low K\(^+\) concentrations, Na,K-pump current had a biphasic relationship with membrane potential. Current initially increased in amplitude but then decreased as membrane potential became more positive. This membrane potential and extracellular K\(^+\) dependence of Na,K-pump current is similar to that reported for wild-type Na,K-ATPase (7, 48), a clear demonstration that the electrogenic properties of RD enzyme are fundamentally unchanged.

**FIG. 4.** Extracellular K\(^+\) activation and ouabain sensitivity of Na,K-pump current in HeLa cells expressing RD control or E779A-substituted enzymes. Na,K-pump current was measured by applying a 15 mM K\(^+\)-containing extracellular solution with (cross-hatched) or without (solid) 10 mM ouabain at the holding potential of −40 mV. In all records, current spikes represent fast K\(^+\) activation of Na,K-pump current immediately before ouabain inhibition. In all experiments, the voltage clamp electrode was filled with a high Na\(^+\) salt solution. A, continuous current record from an RD cell in Na\(^+\)-containing extracellular solution. B, continuous current record from a E779A cell in Na\(^+\)-containing extracellular solution. C, continuous current record from a E779A cell in Na\(^+\)-free, tetramethylammonium-containing extracellular solution.
Role of Glu-779 in the Na,K-ATPase Voltage Dependence

The voltage dependence of K⁺-activated Na,K-pump current with HeLa cells expressing E779A enzyme (Fig. 5B) was entirely absent at all K⁺ concentrations tested (1–50 mM). These unique results show that substitution E779A has a major impact on the properties of the electrogenic enzyme reaction steps.

The amplitude of Na,K-pump current at 0 mV is expressed as a function of extracellular K⁺ concentration in Fig. 5C. The apparent affinity for extracellular K⁺ activation of Na,K-pump current was decreased 3–4-fold for E779A enzyme without affecting the Hill coefficient. Thus, this replacement has only modest effects on extracellular K⁺ activation of ion transport. A similar decrease in K⁺ activation of Na,K-ATPase activity has previously been reported (21–23).

DISCUSSION

Substitution of alanine for Glu-779 does not alter the attributes of the phosphorylated enzyme per se but does modify the effect of extracellular Na⁺ on the phosphoenzyme. Na,K-pump current in HeLa cells expressing RD enzyme is voltage-dependent in the presence of a wide range of extracellular K⁺ concentrations, similar to wild-type enzyme (6, 7, 48). However, this current is independent of membrane potential when the E779A substitution is introduced. Therefore, this carboxyl-containing residue appears to have a central role in determining voltage-dependent properties of the enzyme.

Effect of Na⁺ on the E779A Phosphoenzyme—Substitution E779A leads to a low steady state level of phosphoenzyme in the presence of ATP and high Na⁺ concentrations and to a higher Na-ATPase activity in the absence of K⁺ (Refs. 22 and 23 and this study). Our results show that maximum phosphoenzyme levels of E779A and RD enzyme can be obtained at low Na⁺ concentrations, similar to those that saturate the intracellular cation binding sites (1). Comparable phosphoenzyme levels for both isoforms can also be obtained when phosphorylation is stimulated with imidazole in the absence of Na⁺. These results indicate that phosphorylation and stability of the phosphoenzyme intermediate are unaffected in E779A-substituted enzyme.

These high levels of phosphoenzyme in the presence of 10–20 mM Na⁺ (without oligomycin) also suggest that substituted enzyme can occlude Na⁺. If the mutated enzyme could not occlude this cation, low steady state levels of phosphoenzyme would be observed at all Na⁺ concentrations. Furthermore, the small changes in apparent Na⁺ affinity observed in this enzyme (Refs. 21–23 and this study) also argue against a major role of Glu-779 in the coordination of Na⁺ or K⁺ in the enzyme.

The dephosphorylation experiments clearly indicate that the measured in K⁺-containing solution at each membrane potential. A, RD cells. Values are the mean ± S.E., n = 11, at the following KCl concentrations: 0.5 mM (○), 1 mM (●), 5 mM (◇), 50 mM (▲). Continuous lines represent fitting of an equation derived from a pseudo 2-state model assuming that Na⁺ and K⁺ must pass through an external high-field access channel to reach their extracellular binding sites in the pump (48). Inset, selected difference current traces for 50 mM extracellular K⁺ . Displayed current traces were elicited by voltage pulses to −100 (bottom trace), −60, −20, 0, +20, and +60 mV (upper trace) from the holding potential of −40 mV. Calibration bars: horizontal, 20 ms; vertical, 0.5 pA/pF. The arrow indicates zero current level. B, E779A cells. Values are the mean ± S.E., n = 10, at the following KCl concentrations: 2 mM (○), 5 mM (●), 10 mM (◇), 50 mM (▲). Lines through the data represent the average current at all potentials tested for each K⁺ concentration. C, extracellular K⁺ stimulation of Na,K-pump current at 0 mM. Data were normalized to the value of maximal current amplitude obtained by fitting a Hill equation. The best-fit parameters were as follows: RD enzyme (○), K½ = 2.1 ± 0.5 mM, Hill coefficient = 1.0 ± 0.2; E779A enzyme (●), K½ = 7.8 ± 1.5 mM, Hill coefficient = 1.0 ± 0.2.

FIG. 5. Voltage dependence of extracellular K⁺ activation of Na,K-pump current. Steady state current-voltage relationships were obtained by subtracting current measured in K⁺-free solution from that measured in K⁺-containing solution at each membrane potential. A, RD cells. Values are the mean ± S.E., n = 11, at the following KCl concentrations: 0.5 mM (○), 1 mM (●), 5 mM (◇), 50 mM (▲). Continuous lines represent fitting of an equation derived from a pseudo 2-state model assuming that Na⁺ and K⁺ must pass through an external high-field access channel to reach their extracellular binding sites in the pump (48). Inset, selected difference current traces for 50 mM extracellular K⁺ . Displayed current traces were elicited by voltage pulses to −100 (bottom trace), −60, −20, 0, +20, and +60 mV (upper trace) from the holding potential of −40 mV. Calibration bars: horizontal, 20 ms; vertical, 0.5 pA/pF. The arrow indicates zero current level. B, E779A cells. Values are the mean ± S.E., n = 10, at the following KCl concentrations: 2 mM (○), 5 mM (●), 10 mM (◇), 50 mM (▲). Lines through the data represent the average current at all potentials tested for each K⁺ concentration. C, extracellular K⁺ stimulation of Na,K-pump current at 0 mM. Data were normalized to the value of maximal current amplitude obtained by fitting a Hill equation. The best-fit parameters were as follows: RD enzyme (○), K½ = 2.1 ± 0.5 mM, Hill coefficient = 1.0 ± 0.2; E779A enzyme (●), K½ = 7.8 ± 1.5 mM, Hill coefficient = 1.0 ± 0.2.
Role of Glu-779 in the Na,K-ATPase Voltage Dependence

The voltage dependence of Na,K-pump current is due to movement of extracellular ions to their binding sites within the protein via an ion access channel (8–10). If this is the case, an alternative interpretation of our data is that the polar side chain of Glu-779 forms a portion of the access channel. Removal of the carboxyl moiety would then alter the channel structure, thereby disrupting the voltage-dependent properties of ion transport.

The residues that form the putative access channel are unknown. Models of the Na,K-ATPase topology propose that the fifth transmembrane segment extends from Tyr-771 to Asn-790 (3) with conserved polar residues on a common face of a putative helical structure. This “polar face,” which contains Glu-779, may be part of an internal path for cation transport through the membrane portion of the protein. Amino acids located near the cytoplasmic end of the helix, such as Ser-775, would be involved in cation coordination (34). On the other hand, residues located toward the extracellular end of the fifth transmembrane segment might provide the lining of the ion access channel. If this is the case, alterations in the polarity of the “channel” residues would be anticipated to alter the accessibility of extracellular ions to their binding sites but not the intrinsic attributes of cation binding. The present results might provide experimental evidence to this model. Thus, Glu-779 may be part of the access channel for extracelluar cation binding to the Na,K-ATPase.

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