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Identification of Asp^{804} and Asp^{808} as Na^+ and K^+ coordinating residues in α-subunit of renal Na,K-ATPase

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Abstract Mutations to Asp^{804} and Asp^{808} in the α-subunit almost abolish Na,K-ATPase activity, but high-affinity binding of [^{3}H]ATP or [^{3}H]ouabain at equilibrium and E_1–E_2 transitions are preserved. Titration of K^+-ion displacement of [^{3}H]ATP or [^{3}H]ouabain shows that the mutations interfere with occlusion of K^+ in the E_2[2K] conformation. Reduced phosphorylation levels or affinities for Na^+ in presence of oligomycin indicate that Asp^{804} and Asp^{808} also contribute to coordination of Na^+ in the E_1P[3Na] form. Demonstration of alternate interactions of Na^+ or K^+ with Asp^{804} and Asp^{808} support the notion of cation binding in a ping-pong sequence in catalytic models of Na,K-pumping.

Key words: Na,K-ATPase; Ouabain; Sodium; Potassium; Mutagenesis; Yeast expression

1. Introduction

Renal Na,K-ATPase is organized in α1β1-units with full activity in the soluble state [1]. The substrate ATP and the inhibitors vanadate and ouabain are bound with a stoichiometry of one ligand per αβ-unit and cavities for occlusion of $2K^+$ or $3Na^+$ ions are formed within the structure of the αβ-unit [2]. The αβ-unit is also the minimum asymmetric unit in membrane crystals of purified renal Na,K-ATPase [3]. The α-subunit may contain 10 transmembrane segments with sites for ATP binding and phosphorylation in a large central domain projecting into the cytoplasm between the fourth and fifth transmembrane segment [4]. Extensive proteolysis have shown that the cation occlusion sites lie in the intramembrane domain [5]. The cation sites of Na,K-ATPase are proposed to bind Na^+ or K^+ ions in a ping-pong sequence [6] and it is of particular interest for understanding the Na,K-pump mechanism to identify amino acid residues that are alternately engaged in coordination of Na^+ or K^+.

Mutations to oxygen carrying residues in the membrane domain of Ca-ATPase of sarcoplasmic reticulum (Glu^{309}, Glu^{771}, Asn^{796}, or Asp^{800}) are known to block transport and occlusion of Ca^{2+} [7]. Mutations to the homologous counterparts in the intramembrane portion of the α-subunit of Na,K-ATPase (Glu^{327}, Glu^{779}, Asp^{804}, Asp^{808}), using the ouabain selection methodology for functional expression in HeLa cells [8,9] or Cos cells [10,11] or expression of a ouabain-sensitive isoform in a ouabain-resistant host [12–14] have provided mixed results. Some mutations to residues Glu^{327}(Asp, Ala), Glu^{779}(Asp, Leu), Asp^{808}(Asn, Ala, Leu) and all described mutations to Asp^{804} are lethal in the sense that they do not allow expression of a functional ouabain resistant Na,K-pump to sustain cell growth in a medium containing ouabain. Other mutations to Glu^{327}(Gln, Leu), Glu^{779}(Ala, Gln) and Asp^{808}(Glu) have some activity and when expressed in ouabain media the cells may display full Na,K-ATPase activity, albeit with altered kinetic parameters. It has therefore been questioned whether Glu^{327} [10] and Asp^{808} [14] are essential residues for active Na,K-transport and only Asp^{804} appears to be important for enzyme function. It is a major problem that the mechanisms of enzyme inactivation have not been determined. Neither lethal nor active mutations have been expressed in amounts sufficient for analysis of ligand binding at equilibrium or other partial reactions required for identification of the possible interactions of Na^+ or K^+ ions with intramembrane carboxylate groups of the α-subunit.

The recently developed system for expression in high yield of Na,K-ATPase in yeast [15] does not distinguish active Na,K-ATPase from lethal mutants with respect to biosynthesis and translocation to cell membranes. The absence of endogenous Na,K-ATPase activity in yeast allows assays not previously achieved for recombinant enzymes such as high affinity [^{3}H]ATP binding to the E_1 form at equilibrium. Here, mutations to Asp^{804} and Asp^{808} in the α1-subunit of pig kidney Na,K-ATPase were expressed at high levels in yeast cells and membranes were prepared for measurements of α-subunit concentration and binding at equilibrium of [^{3}H]ATP and [^{3}H]ouabain. K^+ ion displacement of [^{3}H]ATP and [^{3}H]ouabain binding to the mutations was measured to characterize the ability to stabilize the E_2[2K] occluded conformation. To study interactions with Na^+ ions, phosphorylation from [γ-^{32}P]ATP and Na^+ displacement of [^{3}H]ouabain binding were assayed.

2. Materials and methods

2.1. Site-directed mutagenesis

Site-directed mutagenesis was performed according to Ho et al. [16]. The nucleotide sequences of the mutagenic primers were: 5′-CCT CTG CAT CGA ATT GGG CAC AG-3′ and 5′-CTG TGC CCA ATT CGA TGC AGA GG-3′ (D804E), 5′-CCT CTG CAT CAA CTT GGG CAC-3′ and 5′-CTG CCC AAG TTG ATG CAG AGG-3′ (D804N), 5′-CTT GGG CAC AGA AAT GGT TCC TG-3′ and 5′-CAG GAA CCA TTT CTG TGC CCA AG-3′ (D808E), 5′-CTT GGG CAC AAA CAT GGT TCC-3′ and 5′-GGA ACC ATG TTT GTG CCC AAG-3′ (D808N). Mis-matched nucleotides causing the mutations are underlined. A PCR fragment containing the mutation was subsequently inserted into the expression plasmid pPAP1666. Nucleotide sequences of all PCR fragments were confirmed by dideoxy sequencing.

2.2. Yeast fermentation, membrane preparation, and assay

Computer-controlled fermentation of recombinant yeast cells, isolation of yeast membranes and selective SDS treatment, quantitative determination of heterologously expressed Na,K-ATPase protein, Na,K-ATPase assay, [^{3}H]ATP binding, and [^{3}H]ouabain binding

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were done as described previously [15,17]. K^+ displacement of [^{3}H]ATP or [^{3}H]ouabain and Na^+-dependent phosphorylation were also performed as before [15,17], details are given in figure legends.

3. Results

3.1. Expression in yeast of mutations to Asp^{804} and Asp^{808}

The mutations to Asp^{804} and Asp^{808} almost abolished Na,K-ATPase activity (Table 1). The density of expressed α-subunit protein in yeast membranes was determined by quantitative Western blotting using purified pig kidney Na,K-ATPase as a standard [15]. Data in Table 1 demonstrate that introduction of mutations Asp^{804} or Asp^{808} in the α1-subunit protein did not interfere with quaternary structure as the density of Na,K-ATPase protein in the yeast membranes were in the same range as the capacities for [^{3}H]ouabain and [^{3}H]ATP binding.

Assay of [^{3}H]ouabain binding to wild-type Na,K-ATPase showed that both binding capacities and affinities for ouabain fell in the range observed for wild-type Na,K-ATPase (Table 1). The high affinities for ouabain with K_D values in the range of 7–12 nM (Table 1) can only be achieved if the sites for interaction with Mg^{2+} and vanadate (phosphate) are undamaged since the K_D values are more than 10-fold higher in absence of phosphate or Mg^{2+} [17].

The demonstration of high-affinity ouabain binding to the mutations allowed the use of 2 mM ouabain as a background in assays of [^{3}H]ATP binding to enzymes with mutations to presumptive cation sites. It was observed that the level of [^{3}H]ATP binding to wild-type in presence of 2 mM ouabain was equal to the level of binding at saturating concentrations of KCl (10 mM; data not shown). It is seen from Table 1 that the affinity for [^{3}H]ATP binding at equilibrium remained unaltered after mutations to Asp^{804} or Asp^{808}.

3.2. Interactions of K^+ with mutations to Asp^{804} or Asp^{808}

3.2.1. K^+-ATP antagonism. In the wild-type, K^+ ions displaced [^{3}H]ATP from the E_1ATP form with high apparent affinity ($K_{1/2}(K^+) = 0.11$ mM; Fig. 1) because the ion stabilizes the alternative conformation E_2[2K] with low apparent affinity for ATP ($K_{1/2}(ATP) = 0.2$ mM). This effect of K^+ in the range 0–10 mM was abolished after substitution of Asp^{804} with Asn or Glu. Additional experiments showed that only a partial displacement of ATP (20–30%) was observed at 200–300 mM KCl. The Asp^{808}–Asn substitution also abolished the K^+ induced displacement of [^{3}H]ATP, while the Asp^{808}–Glu mutation gave a 21-fold reduction of the apparent affinity for K^+ ($K_{1/2}(K^+) = 2.3$ mM). Since K^+ is the only ligand required for stabilization of the E_2[2K] conformation, the data show that the carboxylate groups of both Asp^{804} and Asp^{808} contribute coordinating oxygens to K^+ ions in the E_2[2K] complex.

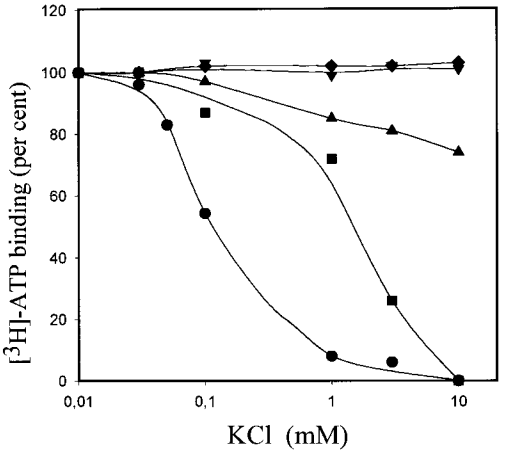

Fig. 1. Effect of KCl on [^{3}H]ATP binding to recombinant wild-type α1β1-Na,K-ATPase (●) and to mutations α1(D804E)β1 (▼), α1(D804N)β1 (▲), α1(D808E)β1 (■) and α1(D808N)β1 (♦). Aliquots of about 200 μg SDS-treated gradient membranes were incubated on ice for 30 min in 10 mM MOPS-Tris, pH 7.2, 10 mM EDTA-Tris, 13 nM [^{3}H]ATP and 0–10 mM KCl. Bound and unbound [^{3}H]ATP were separated by centrifugation at 265 000×*g* for 30 min at 4°C. The supernatant was discharged and remaining buffer removed with a paper towel. Bound [^{3}H]ATP was determined by scintillation counting [16].

3.2.2. K^+-ouabain antagonism. Previous studies have shown that K^+ ions stabilize a conformation with relatively low ouabain affinity (cf., 6). It is seen from Fig. 2 that K^+ displaced binding from wild-type with $K_{1/2}(K^+) = 0.31$ mM. After substitution to Asp^{804} the displacement of ouabain was almost abolished. Instead, the addition of K^+, with low affinity ($K_{1/2}(K^+) = 3$–5 mM), caused a large increase in ouabain binding. The consequence of substitutions of Asp^{808} to Glu or Asn was to shift the position of the displacement curves towards lower apparent affinities for K^+ ($K_{1/2}(K^+) = 3.0$ or 6.7 mM) than for wild-type ($K_{1/2}(K) = 0.31$ mM).

3.3. Na^+ interactions in mutations to Asp^{804} or Asp^{808}

3.3.1. Na^+ dependent phosphorylation from ATP. In the Asp^{804}–Glu mutation, no Na-dependent phosphorylation from [γ-^{32}P]ATP was observed in the range of 0–150 mM NaCl (Fig. 3). The mutation Asp^{804}Asn incorporated phos-

Table 1
Consequences of mutations to Asp^{804} and Asp^{808} for concentration of α-subunit and binding of [^{3}H]ouabain and [^{3}H]ATP

Mutation	Na,K-ATPase (%)	α1β1-unit (pmol/mg prot.)	[^{3}H]ouabain (pmol/mg prot.)	Ouabain K_D (nM)	[^{3}H]ATP (pmol/mg prot.)	[^{3}H]ATP K_D (nM)
D804N	17	15	16	7	12	57
D804E	13	13	16	10	5	67
D808N	0	11	17	7	9	54
D808E	0	9	5	12	9	49
WT	100	11	13	10	7	52

Na,K-ATPases activity was assayed as described in Section 2. Density of αβ1-units in yeast membranes was estimated from scans of Western blots as previously described [15]. Data of binding of [^{3}H]ouabain or [^{3}H]ATP were plotted in Scatchard plots for estimation of capacities and affinities as before [15,17]. The data are mean values of 2 or 3 determinations.

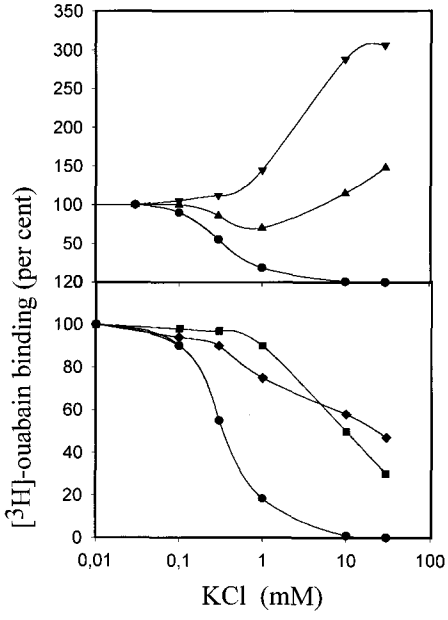

Fig. 2. Effect on KCl on [^{3}H]ouabain binding to recombinant wild-type α1β1-Na,K-ATPase (●) and to mutations α1(D804E)β1 (▼), α1(D804N)β1 (▲), α1(D808E)β1 (■) and α1(D808N)β1 (♦). Aliquots of about 200 μg SDS-treated gradient membranes were incubated at 37°C for 1 h in 3 mM $MgSO_4$, 1 mM EGTA, 10 mM MOPS-Tris, pH 7.2, 10 nM [^{3}H]ouabain and 0–30 mM KCl in exchange for choline-Cl to keep the ionic strength constant. After incubation at 4°C for 20 min, bound and unbound ouabain were separated at 265 000×g for 30 min. Samples were washed twice in ice-cold binding buffer and centrifuged for 10 min at 265 000×g. The amount of bound [^{3}H]ouabain was determined by scintillation counting [15].

phate from ATP to a level of 70% of that of wild type, but the apparent affinity for Na^+ was reduced 26-fold from $K_{1/2}(Na^+) = 0.7$ mM in wild type to $K_{1/2}(Na^+) = 18$ mM in the Asp^{804}Asn mutation, Fig. 3. Substitution of Asp^{808} with Glu reduced the maximum steady-state phosphorylation level to 30% of that in wild type and the apparent affinity for Na^+ was reduced by about 3-fold ($K_{1/2}(Na^+) = 2$ mM; Fig. 3). Exchange of Asp^{808} for Asn abolished steady-state phosphorylation. Since the sites for binding of ATP, Mg^{2+} and phosphate appear to be intact in the mutated proteins, reduction of the steady-state level of phosphorylation for the mutations may reflect altered conditions for coordination of Na^+ ions.

3.3.2. Na^+-ouabain antagonism. In presence of Mg^{2+}, the addition of Na^+ displaced [^{3}H]ouabain from the wild type or the mutations as shown in Fig. 4. The interactions of Na^+ with the ouabain complex of wild type was weak ($K_{1/2}(Na^+) = 6.2$ mM) as compared to the relatively high affinity of Na^+ for phosphorylation from ATP ($K_{1/2}(Na^+) =$ 0.7mM; Fig. 3). Only a 2–3-fold reduction in apparent Na^+ affinity for dissociation of [^{3}H]ouabain are observed for Asp^{804}Glu ($K_{1/2}(Na^+) = 15.5$ mM) and Asp^{808}Glu ($K_{1/2}(Na^+) =$ 15.3 mM). Surprisingly, in both mutations the substitution of Asp for Asn led to even higher apparent affinities for Na^+,Asp^{808}Asn ($K_{1/2}(Na^+) = 2.6$ mM) and Asp^{804}Asn ($K_{1/2}(Na^+) = 5.4$ mM) than in wild type ($K_{1/2}(Na^+) = 6.2$ mM). This suggests that the volume of the residues rather than the charge is important for the relatively weak interactions with Na^+ in the $E_2P[2Na^+]$ intermediate.

4. Discussion

Assays of mutations expressed in yeast cells show that substitutions of Asp^{804} or Asp^{808} by Asn or Glu almost abolished Na,K-ATPase activity and that this may be due to specific interference with cation binding. Analysis of partial reactions provide the first identification of residues that are engaged both in occlusion of Na^+ ions in the phosphoenzyme conformations, $E_1P[3Na]$ and in occlusion of K^+ ions in the $E_2[2K]$ form. Removal of a carboxylate side chain from the intramembrane portion of the protein could conceivably cause structural rearrangements, but unspecific perturbations appear to be limited since the hydrodynamic properties of the (Asp^{808}Asn) mutation were unaffected [15]. Also, the observation that high-affinity binding of ATP and ouabain are preserved shows that the mutant proteins have retained the structure of their binding domains at the cytoplasmic or extracellular surfaces as well as the ability to undergo transitions between E_1 and E_2 conformations (cf., 6).

The titrations with K^+ of [^{3}H]ATP binding or [^{3}H]ouabain binding show that the substitutions to Asp^{804} or Asp^{808} have seriously impaired the ability of the α1β1-unit to assume the $E_2[2K]$ form. In particular the mutations to Asp^{804} and the Asp^{808}Asn mutation were almost completely refractory to K^+, while the Asp^{808}Glu mutation showed a 21-fold reduction in K^+ affinity as determined from the K^+-ATP antagonism. Analysis of sequence homologies for this region (TM 6) shows that the aspartate at residue No. 808 is well conserved among cation pumps. A carboxylate containing side chain is only observed at the position homologous to Asp^{804} in H,K-ATPase [18,19] with counter transport of K^+ like in Na,K-ATP-

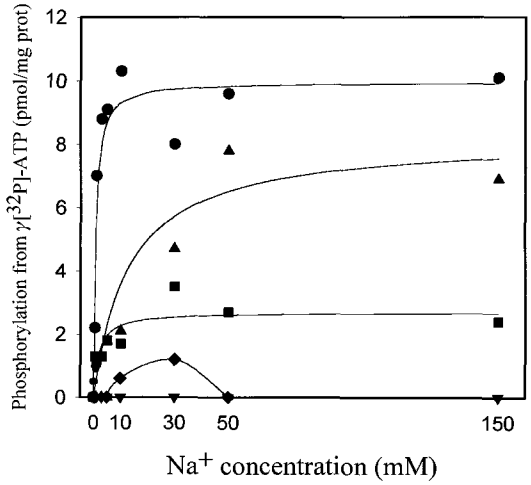

Fig. 3. Na^+-dependent steady-state phosphorylation of recombinant wild-type α1β1-Na,K-ATPase (●) and to mutations α1(D804E)β1 (▼), α1(D804N)β1 (▲), α1(D808E)β1 (■) and α1(D808N)β1 (♦). Aliquots of 100–200 μg SDS-treated yeast membranes were incubated at 20°C for 10 min in 1 ml of 3 mM $MgCl_2$, 10 mM TES-Tris, pH 7.5, 0–150 mM NaCl in exchange for choline-Cl to maintain constant ionic strength, 30 μM oligomycin and protease inhibitors [15]. Samples were placed on ice for 10 min and phosphorylation was initiated by the addition of 2 μM [γ-^{32}P]ATP–Tris. Termination with perchloracetic acid, centrifugation and wash of the phospoenzyme as before [15,17]. Specific phosphorylation was determined as phosphorylation in the presence of NaCl minus phosphorylation in the presence of 10 mM KCl or 2 mM ouabain. Phosphorylation data were fitted according to hyperbolic saturation curves, using the Enzfit program.

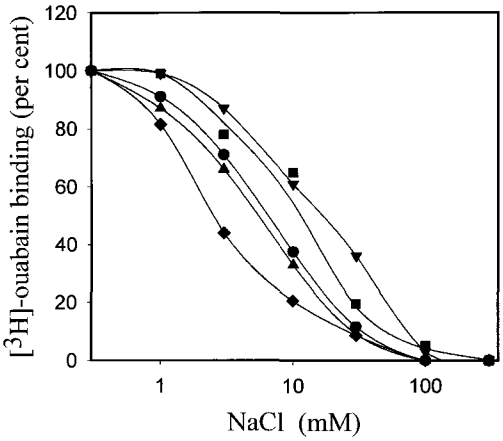

Fig. 4. Effect of NaCl on equilibrium [^{3}H]ouabain binding to recombinant wild-type α1β1-Na,K-ATPase (●) and to mutations α1(D804E)β1 (▼), α1(D804N)β1 (▲), α1(D808E)β1 (■) and α1(D808N)β1 (◆). Aliquots of about 200 μg SDS-treated gradient membranes were incubated at 37°C for 1 h in 3 mM $MgSO_4$, 1 mM EGTA, 10 mM MOPS-Tris, pH 7.2, 10 nM [^{3}H]ouabain and 0–300 mM NaCl. Estimation of [^{3}H]ouabain binding as in Fig. 2.

ase. Asn is found at this position in Ca-ATPase of plasma membranes [20] and sarcoplasmic reticulum [21], while Ala is found in H-ATPase from yeast [22] and plants [23]. This comparison supports the notion that Asp^{804} provides coordinating oxygens for occlusion of K^+ in Na,K-ATPase.

The lowered level or absence of phosphorylation for the mutations could be due to removal of coordinating oxygen atoms required for Na^+-dependent transfer of γ-phosphate from ATP to Asp^{369} or for stabilization of the E_1P[3Na] form. Phosphorylation was performed in the presence of oligomycin to prevent the E_1P[3Na] to the E_2P[2Na] transition. Even in the absence of oligomycin, a change in poise of the conformational equilibrium between the E_1P[3Na] and E_2P[2Na] forms would not in itself alter the level of phosphoenzyme in steady-state, but rather affect the apparent affinity for Na^+. Binding of ATP and ouabain show that the mutations do not affect the sites for ATP, phosphate, or Mg^{2+}. The lowered Na^+ affinity and the reduction of phosphoenzyme levels therefore show that Asp^{804} and Asp^{808} contribute some of the oxygen groups required for coordination of Na^+ in the phosphorylated intermediate E_1P[3Na].

Displacement of ouabain by Na^+ may reflect the formation of a Mg-E_2P[2Na]–ouabain complex [6] with low affinity for ouabain relative to the MgE_2P–ouabain complex and the mutations to Asp^{804} and Asp^{808} had much less influence on these interactions. Since the number of occluded ions are reduced, from $3Na^+$ to $2Na^+$, and the apparent affinity for Na^+ is reduced, fewer oxygen groups are required for coordination of Na^+ in the occlusion cavity in the E2 form than in the E1 form.

Our observations thus show that Asp^{804} and Asp^{808} contribute coordinating oxygens to the occlusion cavity for Na^+ in the phosphoenzyme conformation, E_1P[3Na], and that they, alternately engage in coordination of K^+ ions in the occlusion cavity of the E_2[2K] form. The E_1–E_2 transitions may therefore involve changes in structure of transmembrane helix No. 6 resulting in altered positions and changes in orientation of the acidic side chains of Asp^{804} and Asp^{808}. The identification of Asp^{808} as an important residue for coordination of both Na^+ and K^+ agrees with its position in a consensus sequence D^{808}MVP for cation binding in a number of transport proteins. In Ca-ATPase of sarcoplasmic reticulum or plasma membrane, Ca^{2+} binds to Glu in the fourth (EGLP) and to Asp in the sixth (DMVP) transmembrane segment [20,21,25]. In the FoF1-ATPase of *E. coli* [26] or *P. modestum* [27] H^+ binds to Asp (DAIP) and Na^+ binds to Glu (ESAP) in the intramembrane portion of subunit c.

The results presented also reflect the structural requirements at positions 804 and 808 for maintaining sites for binding of Na^+ in the E_1 conformation and for binding of Na^+ or K^+ in the E_2 form. It appears from the phosphorylation data that the allowed structural alterations are very restricted at the Na^+ site in the E_1 conformation around residue 804 as a considerable increase in side-chain volume completely abolished Na^+ interaction, while removal of the negative charge maintained the Na^+ interaction but reduced the affinity. This contrasts the situation around residue 808 where preservation of side chain geometry seems to be less important than charge conservation. Geometry and charge of residue 804 seem to be essential for K^+ binding as both substitutions completely disrupted the ATP-K^+ antagonism. The observation that conservation of side-chain geometry is more important for the E_2 binding of K^+ than for the E_1 binding of Na^+ could result from a change of surface charge density since the ionic diameter of K^+ (2.6 Å) is larger than for Na^+ (1.9 Å). Charge and geometry also seem to be essential for residue 808. Compared to Na^+ binding in the E_1 form, binding of K^+ ions in the E_2 form seems to be more sensitive to geometry as the reduction in K^+ binding observed for Asp^{808}–Glu is much more severe than the reduction seen for Na^+ binding to the E_1 form. It could be that presence of a larger side chain in the Asp^{808}–Glu mutation interfered with positioning of the larger K^+ ion.

The low affinity of Na^+ for the E_2 form relative to that of the E_1 form and the limited changes in Na^+ affinity observed after mutagenesis of residues 804 and 808 may indicate that the conformational transition from E_1 to E_2 positions these Asp side chains at distances that are less optimal for coordination of Na^+ ions. The fact that the Asp to Asn mutations at positions 804 and 808 slightly increased Na^+ affinity may be due to the larger side chain of Asn. The flexible geometry of the Na^+ sites in the E_2 form may also assure that the extension of side-chain volume at residue 804 or 808 by introduction of a Glu residue only led to slight reductions of Na^+ affinity.

Previous attempts to express Na,K-ATPase with substitutions to Asp^{804} with Asn, Glu or Leu in HeLa [8] or Cos [11] cells failed to produce a functional enzyme since the mutations did not sustain cell growth in a medium containing ouabain. Similarly, the mutation Asp^{808}Asn was inactive. Information about the catalytic properties of these mutations is therefore not available for comparison with the present data. In contrast the mutations Asp^{808}Glu could be expressed as a functional Na,K-ATPase by HeLa cells in ouabain medium with only slightly altered kinetic parameters [14,24]. However, after expression in yeast, the Asp^{808}Glu mutation had very low levels of Na,K-ATPase activity and Na-dependent phosphorylation and the mutation prevented K^+ displacement of ATP binding. A possible explanation for this discrepancy can be that cells growing in medium containing ouabain compensate for a reduced turnover and phosphorylation level by ex-

pression of larger amounts of mutant α-subunits. Over expression of the mutant $Glu^{327}Gln$ protein has been observed in HeLa cells [8].

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References

[1] Brotherus, J.R., Møller, J.V. and Jørgensen, P.L. (1981) Biochem. Biophys. Res. Commun. 100, 146–154.
[2] Vilsen, B., Andersen, J.P., Petersen, J. and Jorgensen, P.L. (1987) J. Biol. Chem. 62, 10511–10517.
[3] Jørgensen, P.L., Skriver, E., Hebert, H. and Maunsbach, A.B. (1982) Ann. NY Acad. Sci USA 402, 203–219.
[4] Karlish, S.J.D., Goldshleger, R. and Jorgensen, P.L. (1993) J. Biol. Chem. 268, 3471–3478.
[5] Karlish, S.J.D., Goldshleger, R. and Stein, W.D. (1990) Proc. Natl. Acad. Sci USA 87, 4566–4570.
[6] Jorgensen, P.L. and Andersen, J.P. (1988) J. Membr. Biol. 103, 95–120.
[7] Clarke, D.M., Loo, T.W., Inesi, G. and MacLennan, D.H. (1989) Nature 339, 476–478.
[8] Jewell-Motz, E.A. and Lingrel, J.B. (1993) Biochemistry 32, 13523–13530.
[9] Feng, J. and Lingrel, J.B. (1995) Cell. Mol. Biol. Res. 41, 29–37.
[10] Vilsen, B. (1993) Biochemistry 32, 13340–13349.
[11] Vilsen, B. (1995) Biochemistry 34, 1455–1463.
[12] Johnson, C.L., Kuntzweiler, T.A., Lingrel, J.B., Johnson, C.G. and Wallick, E.T. (1995) Biochem. J. 309, 187–194.
[13] Kuntzweiler, T.A., Wallick, E.T., Johnson, C.L. and Lingrel, J.B. (1995) J. Biol. Chem. 270, 2993–3000.
[14] Van Huysse, J.W., Kuntzweiler, T.A. and Lingrel, J.B. (1996) FEBS Lett. 389: 179–185.
[15] Pedersen, P.A., Rasmussen, J.H. and Jørgensen, P.L. (1996) J. Biol. Chem. 217, 2514–2522.
[16] Ho, N.S., Hunt, H.D., Horton, R.M., Pullen, J.K. and Pease, L.R. (1989) Gene 77, 51–59.
[17] Pedersen, P.A., Rasmussen, J.H. and Jørgensen, P.L. (1996) Biochemistry, in press.
[18] Crowson, M.S. and Shull, G.E. (1992) J. Biol. Chem. 267, 13740–13748.
[19] Shull, G.E. and Lingrel, J.B. (1986) J. Biol. Chem. 261, 16788–16791.
[20] Adebayo, A.O., Enyedi, A., Verma, A.K., Filoteo, A.G. and Penniston, J.T. (1995) J. Biol. Chem. 270, 27812–27816.
[21] Brandl, C.J., Green, N.M., Korczak, B. and MacLennan, D.H. (1986) Cell 44, 597–607.
[22] Serrano, R., Kielland-Brandt, M.C. and Fink, G.R. (1986) Nature 319, 689–693.
[23] Harper, J.F., Surowy, T.K., and Sussman, M.R. (1989) Proc. Natl. Acad. Sci. USA 86, 1234–1238.
[24] Lingrel. J.B. and Kunzweiler, T.A. (19??) J.Biol. Chem. 269, 19659–19662.
[25] Clarke, D.M., Loo, T.W. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 6262–6267.
[26] Zhang, Y. and Fillingame, R.H. (1995) J. Biol. Chem. 270, 87–93.
[27] Kluge, C. and Dimroth, P. (1993) Biochemistry 32, 10378–10386.