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Assembly of the chimeric Na^+/K^+-ATPase and H^+/K^+-ATPase β-subunit with the Na^+/K^+-ATPase α-subunit

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Abstract

Two sets of chimeric β-subunits were constructed from subunits of *Torpedo californica* Na^+/K^+-ATPase and pig gastric H^+/K^+-ATPase. Five unique restriction sites (*Sna*BI, *Eco*RV, *Mun*I, *Sph*I and *Eco*T22I) were created at equivalent positions of the respective cDNAs and were used as joining points for the construction. One set of chimeras (HxN series) was made by exchanging the 5′ portion of the Na^+/K^+-ATPase β-subunit cDNA with the corresponding portion of the H^+/K^+-ATPase β-subunit cDNA at the respective joining point. Complementary constructs were also prepared (NxH series). In the HxN series, the chimera joined at the *Sna*BI site formed a stable trypsin resistant complex with the Na^+/K^+-ATPase α-subunit, which was functional with respect to ATP hydrolysis and pump current generation, although the activities were less than those of the complex with the Na^+/K^+-ATPase β-subunit. Trypsin resistance decreased for the complex of the chimera joined at the *Eco*RV site. In the NxH series, the chimeras joined at the *Sna*BI site and the *Eco*RV site formed rather trypsin-resistant complexes, but the expressions of the α-subunits were below 50% of the control. The chimeras joined at the *Mun*I, *Sph*I and *Eco*T22I site formed complexes susceptible to tryptic digestion. None of the chimeras in the NxH series were functional. These results suggest that at least two regions of the Na^+/K^+-ATPase β-subunit [*Sna*BI site(Tyr40) to *Eco*RV site(Ile89) and *Eco*T22I site(Cys176) to C-terminus)] are involved in stable assembly with the Na^+/K^+-ATPase α-subunit and that the cytoplasmic domain [N-terminus to *Sna*BI site(Tyr40)] is functionally replaceable with the corresponding domain of the H^+/K^+-ATPase β-subunit. © 1997 Elsevier Science B.V.

Keywords: ATPase, Na^+/K^+; ATPase, H^+/K^+; ATPase, α-subunit; ATPase, β-subunit; Chimeric enzyme

1. Introduction

The enzyme Na^+/K^+-ATPase transports Na^+ and K^+ across the cell membrane and is composed of catalytic α and glycosylated β-subunits (for a review, see Ref. [1]). The α-subunit spans the membrane 10 times [2]; it contains aspartate phosphorylated during the catalytic cycle and the binding sites for ATP, transport cations, and ouabain. The β-subunit spans the membrane once, leaving a short N-terminal domain in the cytoplasm and exposing a large C-terminal domain on the extracellular side [3].

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The β-subunit is an essential component for functional enzyme formation [4–6], and its possible role is to stabilize the nascent α-subunits [7–10]. The β-subunit also modulates the transport activity of Na^+/K^+-ATPase [11–15].

The β-subunit assembles with the α-subunit during or immediately after their synthesis in the endoplasmic reticulum [16]. The segments of the α-subunits involved in the assembly are the extracellular loop between transmembrane segments 7 and 8 [17,18] and possibly the cytoplasmic N-terminal region [15,19]. In the β-subunit, the extracellular domain is important for assembly with the α-subunit: three disulfide-bonded loops [20–23], the proline residue in the third disulfide loop [24] and the C-terminal region [25] in the extracellular domain interact with the α-subunit. The contribution of the N-terminal cytoplasmic and the transmembrane domains of the β-subunit is controversial. Deletions of the cytoplasmic domain or portions of the transmembrane domain have been reported as not affecting the assembly of the β- and α-subunits [26]. On the contrary, Jaunin et al. [27] and Eakle et al. [28] concluded that the cytoplasmic/transmembrane domain is important for the efficient assembly and stability of the resulting αβ complex.

In this report, we constructed a series of chimeric β-subunits between Na^+/K^+- and H^+/K^+-ATPase β-subunits, and studied the effects of various β-subunits on the assembly and the function of Na^+/K^+-ATPase.

2. Materials and methods

2.1. Materials

The enzymes used for site-directed mutagenesis, [U-^{14}C] leucine and [21,22-^{3}H] ouabain, were purchased from Amersham. The restriction endonucleases and T4 DNA ligase were from Takara, Nippon Gene and Toyobo. The enzymes for in vitro transcription were from Ambion. Other chemicals and biochemicals were obtained from Nacalai Tesque, and were of reagent grade or higher.

2.2. Construction of chimeric β-subunits

Plasmids containing cDNA for *Torpedo californica* Na^+/K^+-ATPase β-subunit [22] and the pig gastric H^+/K^+-ATPase β-subunit [23] were subjected to site-directed mutagenesis to create five unique restriction endonuclease sites: *Sna*BI, *Eco*RV, *Mun*I, *Sph*I and *Eco*T22I (Fig. 1). The mutations were verified by restriction mapping and sequencing. This manipulation caused the amino acid replacements Leu41 to Val, Glu125 to Asp, Asp126 to Asn, and Val149 to Ala in the *Torpedo* Na^+/K^+-ATPase β-subunit, whereas in the pig H^+/K^+-ATPase β-subunits it caused Asp95 to change to Glu, Ser151 to Ala and Phe179 to Ile. The resulting β-subunit cDNA (modified β-subunit) were used as parent β-subunits to construct the chimeras and controls throughout the study. The modified Na^+/K^+-ATPase β-subunit

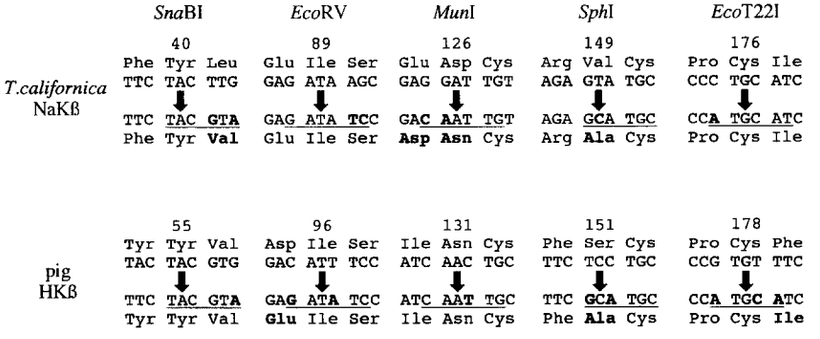

Fig. 1. Site-directed mutagenesis to create restriction sites. The nucleotides of the cDNA coding for *T. californica* Na^+/K^+- and pig H^+/K^+-ATPase β-subunits were changed to create the restriction sites (underlined). The replaced amino-acids after mutations are also shown (bold letters). The β-subunits containing all the created restriction sites (modified β-subunit) were used as parents to construct chimeric β-subunits and as controls of Na^+/K^+- or H^+/K^+-ATPase subunit.

(NN) allowed essentially the same functional expression of the Na^+/K^+-ATPase as the original Na^+/K^+-ATPase β-subunit (data not shown). The modified H^+/K^+-ATPase β-subunit (HH) stabilized the Na^+/K^+-ATPase α-subunit in the same way as the original H^+/K^+ ATPase β-subunit (data not shown). The plasmids coding modified β-subunits (NN or HH) were digested with each of the five endonucleases and *Xba* I, which cut one of the polycloning sites at pSP65. Chimeras were constructed as follows (see Fig. 2). The plasmids were digested with *Sna*BI and *Xba*I; the larger *Sna*BI-*Xba*I fragment from the H^+/K^+-ATPase β-subunit plasmid and the smaller *Sna*BI-*Xba*I fragment from the Na^+/K^+-ATPase β-subunit plasmid were gel-purified and ligated (HsN). Likewise, the larger *Sna*BI-*Xba*I fragment from the Na^+/K^+-ATPase β-subunit plasmid and the smaller *Sna*BI-*Xba*I fragment from the H^+/K^+-ATPase β-subunit plasmid were gel-purified and ligated (NsH). We successively digested the plasmids with each of the other four restriction enzymes and *Xba*I, and we constructed ten chimeric β-subunit cDNAs (Fig. 2).

2.3. Expression in oocytes

The linearized plasmids were transcribed in vitro using SP6 RNA polymerase [29]. The various β-subunit cRNAs (10 or 20 ng/oocyte) were injected into *Xenopus laevis* oocytes (stages IV–V) together with the α-subunit cRNA of the *Torpedo* Na^+/K^+-ATPase (10 or 20 ng/oocyte). In some experiments, we used *Torpedo* Na^+/K^+-ATPase α-subunit cRNA, which was mutated to an ouabain-resistant form (α_{OR}, $K_i = 7 \times 10^{-5}$ M) by site-directed mutagenesis [30]. The oocytes were incubated at 19°C for three days in modified Barth's medium [31] containing 100 μg/ml each of ampicillin, streptomycin, and cefmenoxime.

2.4. Trypsin digestion and immunoprecipitation

The oocytes injected with cRNA were labeled with 0.47 mM [U-^{14}C] leucine (5.55 MBq/ml) for three days at 19°C. About 30 labeled oocytes were mixed with 100 non-labeled and uninjected oocytes, which served as a source of carrier membranes. The microsomes were prepared and digested with trypsin (Worthington; L-1-tosylamide-2-phenylethyl-chloromethyl ketone-treated) at a trypsin: protein ratio (w/w) of 0.01 on ice for 60 min. The digestion was stopped with soybean trypsin inhibitor (Sigma) at an inhibitor: trypsin ratio (w/w) of 100. The digests were brought to 0.5% digitonin and were clarified by centrifugation at 160,000 × *g* for 30 min. Antiserum against *T. californica* Na^+/K^+-ATPase α-subunit

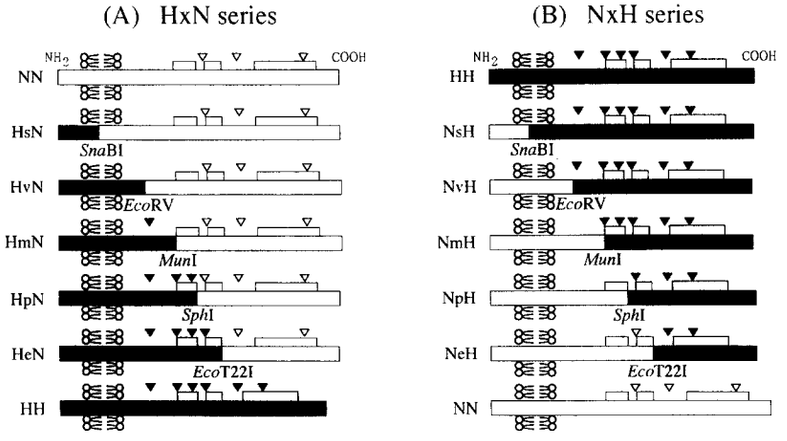

Fig. 2. Construction of chimeric Na^+/K^+-ATPase and H^+/K^+-ATPase β-subunits. Schematic representations of chimeras of the HxN series (A) and the NxH series (B) are shown. These constructions are shown as two capitals and one small letter. The capital letters N and H represent fragments of Na^+/K^+-ATPase and H^+/K^+-ATPase β-subunits, respectively. Small letters are from the restriction sites used as the joining points for chimera construction; s, v, m, p and e represent *Sna*BI, *Eco*RV, *Mun*I, *Sph*I and *Eco*T22I sites, respectively. Na^+/K^+-ATPase (open bars), H^+/K^+-ATPase (closed bars) and their membrane spanning regions are shown schematically. Glycosylation sites are shown as triangles (open triangles, Na^+/K^+-ATPase; closed triangles, H^+/K^+-ATPase). Disulfide-bonded loops are also shown as small open bars.

[32] was added to the supernatant, and the mixiture was incubated for at least 8 h at 4°C. Protein A-Sepharose CL-4B was then added. The resulting immunoprecipitates were resolved by electrophoresis on a 10% polyacrylamide gel containing 0.1% sodium dodecyl sulphate.

2.5. ATPase activity

To assay the ATPase activity, about 200–400 oocytes were homogenized and the microsomal fraction was recovered from the homogenate by centrifugation at 160,000 × *g* for 30 min [32]. The ATPase activity was measured as the ouabain-sensitive formation of inorganic phosphate from ATP by the method of Martin and Doty [33] and was assayed at 37°C in a reaction mixture (0.1 ml) comprising 50 mM imidazole/HCl buffer (pH 7.5), 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$, 1 mM ATP and microsomes (about 30 μg of protein) with and without 1 mM ouabain. When cRNA α_{OR} was used, the activity was assayed with 5 μM and 10 mM ouabain. The reaction was started by adding ATP to a mixture that had been incubated at 37°C for 10 min. The Na^+/K^+-ATPase activity was obtained by subtracting the activity determined with 1 mM and 10 mM ouabain from the activity determined without and with 5 μM ouabain, respectively.

2.6. Pump current measurement

The current–voltage dependencies were determined by conventional two-microelectrode techniques (Axoclamp-2B). From a constant holding potential of −60 mV, rectangular voltage pulses of 200 ms duration and varying amplitude from −150 to +30 mV were applied every second. Currents were recorded at a sampling rate of 1 kHz and steady-state currents were detemined by averaging the current values obtained during the last 50 ms of the test pulses. To reduce non-pump related K^+-sensitive currents, all solutions contained 5 mM $BaCl_2$ and 20 mM tetraethylammonium chloride. Under these conditions, the current generated by the Na^+/K^+-ATPase was determined as the difference between the total membrane current in a solution with or without 2 mM KCl [34].

3. Results

3.1. Stability of αβ complexes

We constructed two series of chimeric β-subunits of Na^+/K^+-ATPase and H^+/K^+-ATPase (Fig. 2). Chimeric β-subunit cRNA was injected into *Xenopus* oocytes together with the cRNA for the *T. californcia* Na^+/K^+-ATPase α-subunit (cRNA α). The oocytes were incubated with [^{14}C]-leucine for three days, then the microsomal fractions were prepared. The translation products in the microsomal fractions were assayed by immunoprecipitation (Fig. 3).

Anti α-subunit antiserum precipitated not only the α-subunit but also the β-subunit, irrespective of chimeric constructs. Since the antiserum is specific for the α-subunit [32], these results indicate that each of the chimeric β-subunits is associated with an α-subunit. The slight differences in mobilities of the β-subunits reflect differences in molecular mass and/or glycosylation.

The amounts of the α-subunit were quantified by using Fuji BAS 2000 and are shown in Fig. 4. The quantitative values of the immunoprecipitated α-subunits are average of three sets of experiments, and the data are normalized to the amounts of the α-subunit of NN.

We examined by limited trypsin digestion whether the αβ complexes were stable. Membrane fractions prepared from ^{14}C-labeled oocytes were digested with trypsin (Figs. 3 and 4). The NN and HsN in the HxN series formed stable complexes with the α-subunit that were resistant to tryptic digestion, suggesting that the cytoplasmic N-terminal domain of the Na^+/K^+-ATPase β-subunit could be replaced with the corresponding domain of the H^+/K^+-ATPase β-subunit without affecting assembly with the α-subunit. However, trypsin resistance of the HvN complex, in which the N-terminal side up to about 30 residues in the extracellular domain was composed of H^+/K^+-ATPase β-subunit, was reduced. The HeN, whose N-terminal side up to the second disulfide loop was substituted by the H^+/K^+-ATPase β-subunit, formed an unstable complex susceptible to tryptic digestion. The bands of the α-subunit of the complexes with chimeras in the NxH series were less dense than those of the corresponding chimeras in the HxN

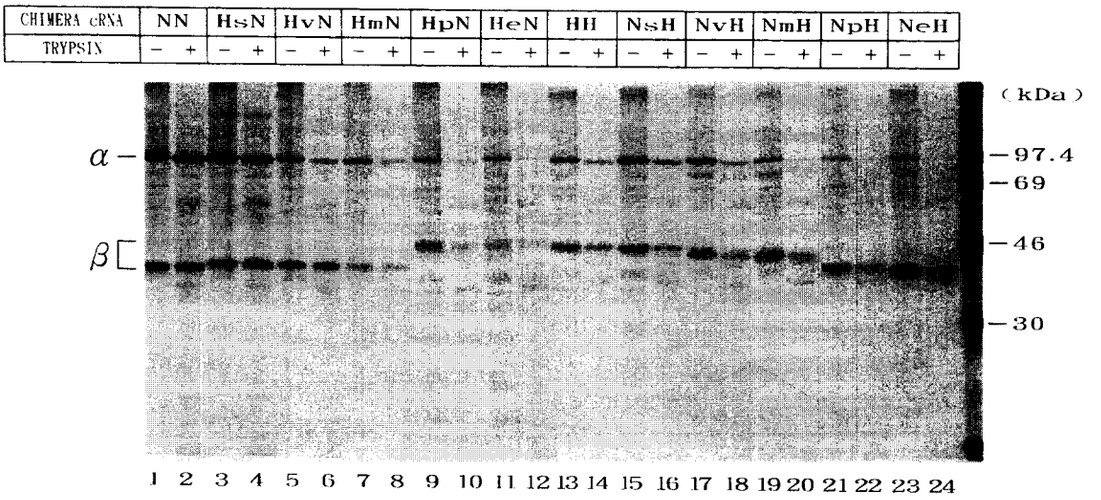

Fig. 3. Fluorographs of microsomes from oocytes injected with cRNAs. Oocytes were injected with cRNA β as shown at the top of each panel together with cRNA α. Microsomal fractions from cRNA-injected oocytes, which had been incubated for three days with [^{14}C] leucine, were incubated for 60 min on ice with (even-numbered lanes) or without (odd-numbered lanes) trypsin. The digestion was stopped with soybean trypsin inhibitor at an inhibitor: trypsin ratio (w/w) of 100. Microsomes were analyzed by immunoprecipitation with anti α-subunit antiserum, followed by gel electrophoresis and fluorography.

series. Among the complexes formed with the NxH series chimeras, the complexes of HH, NsH and NvH were rather resistant to trypsin.

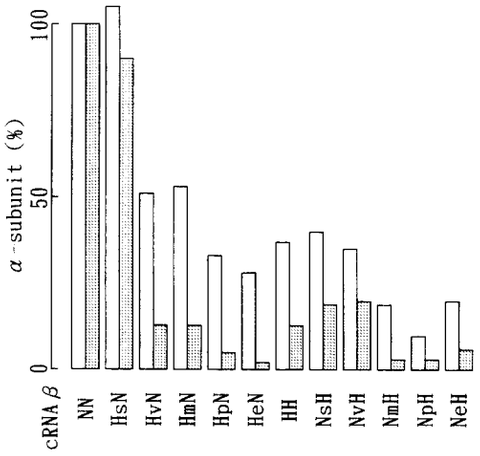

Fig. 4. Trypsin-resistance of the α-subunit in the complex with chimeric β-subunit. The amounts of the α-subunit shown in Fig. 3 were quantified by using Fuji BAS 2000. Data are the average from three sets of experiments and are normalized to the values obtained for NN. Shaded and open bars represent the α-subunit with and without trypsin treatment, respectively.

3.2. Functions of $\alpha\beta$ complexes

We examined whether the complexes are active in ATP hydrolysis and the generation of the pump current. Table 1 shows the results of ATP hydrolysis. The complexes with NN and HsN hydrolyzed ATP, and the activity of HsN was about a half that of NN. The β-subunit affects the affinity for K^+ of Na^+/K^+-ATPase [11–14]. We therefore examined

Table 1
Na^+/K^+-ATPase activity in cRNA-injected oocytes

	HxN series (μmol Pi/mg per h)					
cRNA β	NN	HsN	HvN	HmN	HpN	HeN
	3.20 ± 1.83 ($n = 11$)	1.4	0.0	−0.1	−0.1	−0.1
	NxH series (μmol Pi/mg per h)					
cRNA β	HH	NsH	NvH	NmH	NpH	NeH
	-0.08 ± 0.23 ($n = 8$)	−0.2	0.4	−0.1	0.1	−0.4

Oocytes were injected cRNA β together with cRNA α. Microsomes from cRNA-injected oocytes that had been incubated for three days at 19°C were prepared and assayed as described in Section 2. The data were obtained after subtracting the activity of cRNA-uninjected oocytes in each experiment. The values were averaged from 2–3 sets of experiments except those of NN and HH which were the averages of 11 and 8 sets of experiments, respectively

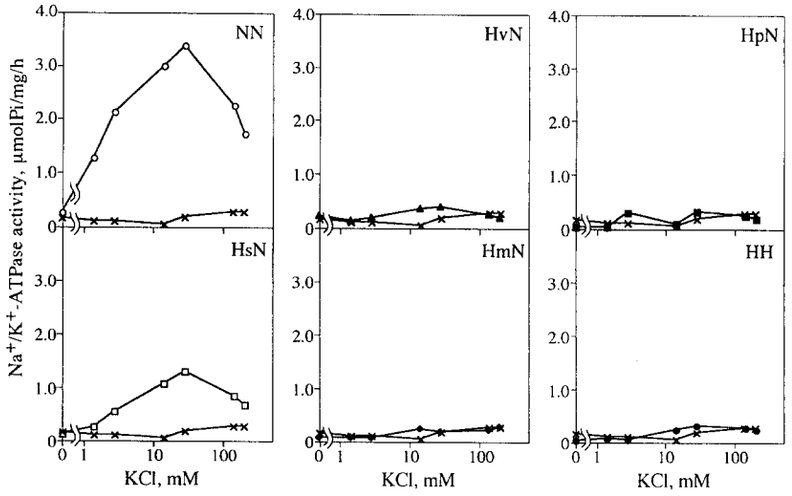

Fig. 5. K^+ concentration dependence of the Na^+/K^+-ATPase activity in microsomes from cRNA-injected oocytes. Oocytes were injected with the cRNA β shown in each panel together with cRNA α_{OR}. Microsomes were prepared and the Na^+/K^+-ATPase activity was assayed. Microsomes from uninjected oocytes (cross) were assayed in parallel.

whether the affinity for K^+ of the chimeric complexes is altered with respect to ATP hydrolytic activity. In this experiment, the ouabain-resistant α-subunit (α_{OR}), constructed from *T. californica* cDNA by site-directed mutagenesis, was used to distinguish exogenous and endogenous ATPase activity. Since endogenous Na^+/K^+-ATPase of oocytes is ouabain-sensitive, the exogenous activity was assayed with 5 μM ouabain, which inhibits over 90% of the endogenous activity. The ouabain-resistant Na^+/K^+-ATPase activities of membrane fractions from oocytes injected with cRNA β for HvN, HmN, HpN and HH, together with cRNA α_{OR}, were at the level of uninjected oocytes for all concentrations of K^+ tested (Fig. 5).

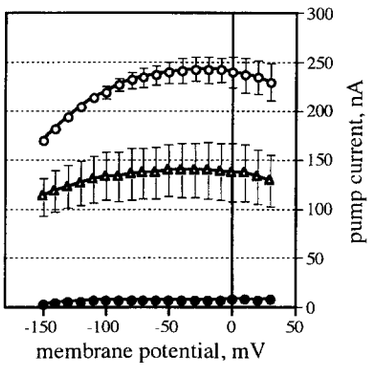

Fig. 6. Voltage dependence of the pump current in cRNA-injected oocytes. Oocytes were injected with cRNA α_{OR} together with cRNA β of Na^+/K^+-ATPase (open circle), H^+/K^+-ATPase (closed circle) or chimera HsN (triangle). Currents were measured with 5 μM ouabain at a KCl concentration of 2 mM.

We conclude that the complexes of the α-subunit with the chimeras, except for HsN, were inactive with respect to ATP hydrolytic activity, although the extent of the activity of the complex with HsN was less than that of the complex with NN. (The small activity of NvH in Table 1 is probably be due to an experimental error, judging that all other chimeras of the NxH series, including HH, are inactive.)

The pump currents generated by exogenous and ouabain-resistant Na^+/K^+-ATPase in oocytes were assayed with 5 μM ouabain (Fig. 6). The oocytes injected with cRNAs for HH plus α_{OR} showed no K^+-stimulated currents over the entire range of membrane potentials tested. The potential dependent pump current of oocytes injected with cRNAs for HsN plus α_{OR} was significant, but lower than that of oocytes with NN plus α_{OR}. These results are consistent with those of the ATP hydrolysis mentioned above in this section. The membrane potential dependence of the pump current of oocytes injected with cRNA for HsN was somewhat different from that of the oocytes with NN, especially at negative membrane potentials.

4. Discussion

In the HxN series, HsN formed stable trypsin-resistant complexes with the α-subunit. The trypsin resistance of the complex was, however, reduced when HvN, HmN, HpN and HeN were used. The discontinuity in stable complex formation along with β-subunit replacements indicates that the region from the *Sna*BI site(Tyr40) to the *Eco*RV site(Ile89) of the Na^+/K^+-ATPase β-subunit, including the transmembrane segment and about 30 residues in the extracellular domain, is not replaceable with the corresponding region of the H^+/K^+-ATPase β-subunit for stable complex formation with the α-subunit of Na^+/K^+-ATPase. However, the chimeras in the NxH series such as NmH, NpH and NeH with the region from the *Sna*BI site to the *Eco*RV site derived from the Na^+/K^+-ATPase β-subunit, could not form stable complexes with the Na^+/K^+-ATPase α-subunit. This means that, in addition to the *Sna*BI(Tyr40) to *Eco*RV(Ile89) segment, another crucial segment(s) should be present for stable complex formation with the α-subunit. The fact that NeH could not form a stable complex suggests that the region from the *Eco*T22I site(Cys176) to the C-terminus has such a crucial segment.

As mentioned above, the sequence between Tyr40 and Ile89 of Na^+/K^+-ATPase β-subunit is one of the essential regions for stable assembly with the α-subunit. Critical residues possibly exist in this region. For example, Arg72 of the *T. californica* β-subunit can be such a residue, since it is conserved among Na^+/K^+-ATPase, but not H^+/K^+-ATPase isoforms. Another candidate is Ala74 with a similar conservation, except that the β_3-isoform of Na^+/K^+-ATPase has a serine in this position. We mutated Arg72 to Gln and Ala74 to Lys in Na^+/K^+-ATPase β-subunit. (In H^+/K^+-ATPase the 72nd and 74th residues are consistently Gln and Lys, respectively.) Both mutant β-subunits formed stable complexes with the α-subunit, having essentially the same ATPase activity as the wild-type complex (data not shown).

Among the NxH series, HH, NsH and NvH formed rather trypsin-resistant complexes with the α-subunit of Na^+/K^+-ATPase (Fig. 4). These complexes must be different in structure from those of the NN and HsN complexes, which is supported by observations [28] that a complex formed with the α-subunit of Na^+/K^+-ATPase and the β-subunit of H^+/K^+-ATPase (heterologus complex) is more sensitive to detergent extraction than the Na^+/K^+-ATPase αβ complex (homologus complex). Previously, we also reported [35] that the heterologus complex has a similar but not exactly the same conformation as the homologus complex.

The complex of HH (heterologus complex) is inactive in ATPase activity (Table 1). Horisberger et al. [36] claim, however, that the heterologus complex is active in pump current generation and that the molar activity of the heterologous complex is at the same level as that of the homologous complex, although heterologous complex formation was less efficient than for the homologous complex. We asssayed the pump current of the heterologous complex under essentially the same conditions as described by Horisberger et al. [36], but could not detect any significant current at any membrane potential tested (Fig. 6). Functional analysis of the complexes of H^+/K^+-ATPase α-subunit with the chimeric β-subunits may help resolve this discrepancy.

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References

[1] L.A. Vasilets, W. Schwarz, Biochim. Biophys. Acta 1154 (1993) 201–222.
[2] R. Goldshleger, D.M. Tal, S.J.D. Karlish, Biochemistry 34 (1995) 8668–8679.
[3] S. Noguchi, M. Noda, H. Takahashi, K. Kawakami, T. Ohta, K. Nagano, T. Hirose, S. Inayama, M. Kawamura, S. Numa, FEBS Lett. 196 (1986) 315–320.
[4] A. Hiatt, A.A. McDonough, I.S. Edelman, J. Biol. Chem. 259 (1984) 2629–2635.
[5] K. Geering, J.P. Kraehenbuhl, B.C. Rossier, J. Cell Biol. 105 (1987) 2613–2619.

[6] S. Noguchi, K. Higashi, M. Kawamura, Biochim. Biophys. Acta 1023 (1990) 247–253.
[7] K. Takeyasu, M.M. Tamkun, K.J. Renaud, D.M.J. Fambrough, Biol. Chem. 263 (1988) 4347–4354.
[8] D. Zamofing, B.C. Rossier, K. Geering, J. Membr. Biol. 104 (1988) 69–79.
[9] K. Geering, FEBS Lett. 285 (1991) 189–193.
[10] P. Jaunin, J.-D. Horisberger, K. Richter, P.J. Good, B.C. Rossier, K. Geering, J. Biol. Chem. 267 (1992) 577–585.
[11] F. Jaisser, C.M. Canessa, J.-D. Horisberger, B.C. Rossier, J. Biol. Chem. 267 (1992) 16895–16903.
[12] D.C. Chow, C.M. Browning, J.G. Forte, Am. J. Physiol. 263 (1992) C39–C46.
[13] S. Lutsenko, J.H. Kaplan, Biochemistry 32 (1993) 6737–6743.
[14] K.A. Eakle, R.-M. Lyyu, R.A. Farley, J. Biol. Chem. 270 (1995) 13937–13947.
[15] A. Shainskaya, S.J.D. Karlish, J. Biol. Chem. 271 (1996) 10309–10316.
[16] M.M. Tamkun, D.M. Fambrough, J. Biol. Chem. 261 (1986) 1009–1019.
[17] M.V. Lemas, K. Takeyasu, D.M. Fambrough, J. Biol. Chem. 267 (1992) 20987–20991.
[18] M.V. Lemas, M. Hamrick, K. Takeyasu, D.M. Fambrough, J. Biol. Chem. 269 (1994) 8255–8259.
[19] Y. Mutoh, S. Noguchi, T. Ohta, K. Higashi, M. Takasugi, A. Kuroiwa, M. Kawamura, Biochem. Int. 26 (1992) 775–785.
[20] M. Kawamura, K. Nagano, Biochim. Biophys. Acta 774 (1984) 188–192.
[21] T.L. Kirley, J. Biol. Chem. 265 (1990) 4227–4232.
[22] S. Noguchi, Y. Mutoh, M. Kawamura, FEBS Lett. 341 (1994) 233–238.
[23] S. Ueno, M. Kusaba, K. Takeda, M. Maeda, M. Futai, F. Izumi, M. Kawamura, J. Biochem. 117 (1995) 591–596.
[24] K. Geering, P. Jaunin, F. Jaisser, A.M. Merilatt, J.-D. Horisberger, P.M. Mathews, V. Lemas, D.M. Fambrough, B.C. Rossier, Am. J. Physiol. 265 (1993) C1169–C1174.
[25] A.T. Beggah, P. Beguin, P. Jaunin, M.C. Peitsch, K. Geering, Biochemistry 32 (1993) 14117–14124.
[26] K.J. Renaud, E.M. Inman, D.M. Fambrough, J. Biol. Chem. 266 (1991) 20491–20497.
[27] P. Jaunin, F. Jaisser, A.T. Beggah, K. Takeyasu, P. Mangeat, B.C. Rossier, J.-D. Horisberger, K. Geering, J. Cell Biol. 123 (1993) 1751–1759.
[28] K.A. Eakle, M.A. Kabalin, S.-G. Wang, R.A. Farley, J. Biol. Chem. 269 (1994) 6550–6557.
[29] J.K. Yisraeli, D.A. Melton, Methods Enzymol. 180 (1989) 42–50.
[30] E.M. Price, J.B. Lingrel, Biochemistry 27 (1988) 8400–8407.
[31] T.B. Gurdon, The Control of Gene expression in Animal Development, Clarendon Press, Oxford, 1974
[32] S. Noguchi, K. Higashi, M. Kawamura, J. Biol. Chem. 265 (1990) 15991–15995.
[33] J.B. Martin, D.M. Doty, Anal. Chem. 21 (1949) 965–967.
[34] L. Vasilets, W. Schwarz, J. Membr. Biol. 125 (1992) 119–132.
[35] S. Noguchi, M. Maeda, M. Futai, M. Kawamura, Biochem. Biophys. Res. Commun. 182 (1992) 659–666.
[36] J.-D. Horisberger, P. Jaunin, M.A. Reuben, L.S. Lasater, D.C. Chow, J.G. Forte, G. Sachs, B.C. Rossier, K. Geering, J. Biol. Chem. 266 (1991) 19131–19134.