Structural analysis of the products of chymotryptic cleavage of the E1 form of Na,K-ATPase α-subunit: identification of the N-terminal fragments containing the transmembrane H_1-H_2 domain

Alexander Ivanov, Amir Askari, Nikolai N. Modyanov*

Department of Pharmacology, Medical College of Ohio, Toledo, OH 43614, USA

Received 10 November 1997

Abstract Chymotryptic cleavage of the Na,K-ATPase in NaCl medium abolishes ATPase activity and alters other functional parameters. The structure of this modified enzyme is uncertain since only one product of selective proteolysis, the 83-kDa fragment of the α-subunit (Ala^{267}–C-terminus) has been identified previously. Here, we applied additional tryptic digestion followed by oxidative cross-linking to identify the products originating from the N-terminal part of the α-subunit. These fragments start at Ala^{72} or Thr^{74} and contain the transmembrane H_1-H_2 domain. Formation of cross-linked product between α-fragments containing H_1-H_2 and H_7-H_{10} demonstrate that the structural integrity of the membrane moiety is preserved. We also determined that secondary cleavage of the 83-kDa fragment leads to the formation of C-terminal 48-kDa α-fragments with multiple N-termini at Ile^{582}, Ser^{583}, Met^{584} and Ile^{585}.

Key words: Na^+,K^+-ATPase; α-Subunit; Chymotrypsin; Trypsin; Cu^{2+}-phenanthroline; Oxidative cross-linking

1. Introduction

Na,K-ATPase is the intrinsic enzyme of the plasma membrane responsible for coupled active transport of Na^+ and K^+ in animal cells. The enzyme consists of α (~112 kDa) and β (~55 kDa) subunits found in equimolar ratio. The catalytic α-subunit contains the ATP-hydrolyzing center on the large cytoplasmic protrusion, and cation occlusion sites within the membrane moiety that most probably contains 10 transmembrane segments [1]. Limited proteolysis of native Na,K-ATPase, along with other approaches, has been used extensively to study the structural organization of the enzyme within the plasma membrane [2–4]. Analysis of functional alterations induced by selective proteolytic splits of the α-subunit which are characteristic of different ligand-induced conformations: Na^+ form (E1) and K^+ form (E2), has provided valuable information on structure-function relationships [2,5–8]. Of particular interest are the studies of Na,K-ATPase subjected to controlled chymotryptic digestion in NaCl medium which abolishes ATPase activity and substantially alters other functional parameters [2,5,6,10]. For example, recently we showed that the chymotrypsin-digested preparation exhibits a twice higher capacity for phosphorylation by ATP than the native enzyme, thus indicating that half of the α-subunits of the native enzyme are dormant with respect to phosphoenzyme intermediate formation [10]. Interpretation of this and other observations with respect to structure-function correlations is complicated, however, due to the uncertainties about the structure of the chymotrypsin-digested Na,K-ATPase. The only product of this cleavage that has been well characterized is the 83-kDa fragment of the α-subunit from Ala^{267} to the COOH-terminus [6,9]. Neither the expected 30-kDa N-terminal fragment containing membrane segments H_1 and H_2 nor any other smaller products of its further digestion were detected previously [9,10]. Based on these observations we suggested that perhaps chymotrypsin splits the Na,K-ATPase α-subunit not only at the Leu^{266}-Ala^{267} bond, but also at a number of other peptide bonds within the 30-kDa N-terminal region, leading to the formation of numerous heterogeneous peptide fragments that are difficult to detect by standard methods [11]. On the other hand, the possibility could not be excluded that relatively short chymotryptic peptides containing membrane segments H_1 and H_2 were released from the postchymotryptic membrane moiety. Partial or complete release of some intramembrane peptides from the membrane moiety of proteolytically dissected Na,K-ATPase has been documented earlier [12–14].

To clarify these uncertainties, we aimed to elucidate the peptide composition of the chymotrypsin-digested Na,K-ATPase. According to the procedure developed in [11,15], the chymotrypsin-digested Na,K-ATPase was subjected to additional extensive tryptic digestion followed by oxidative cross-linking in the presence of Cu^{2+}-phenanthroline complex. Analysis of the cross-linked products has allowed us to determine the structures of the N-terminal part of the Na,K-ATPase α-subunit resulting from controlled chymotryptic digestion. We also determined the structures of the relatively stable products of the secondary digestion of the C-terminal 83-kDa peptide.

2. Materials and methods

The purified membrane-bound Na^+,K^+-ATPase of canine kidney medulla, with a specific activity of 1400–1600 μmol ATP hydrolyzed/mg protein/h, was prepared and assayed as described before [16]. Controlled chymotrypsin digestion was performed essentially as described by Jorgensen and Petersen [5]. The enzyme (0.5 mg/ml) was incubated at 37°C for 1 h in a solution containing 10 mM NaCl, 15 mM Tris-HCl (pH 7.4), and 25 μg/ml of α-chymotrypsin (Sigma, type II, bovine pancreas). The reaction was stopped by the addition of soybean trypsin-chymotrypsin inhibitor (Sigma); 100 μg/25 μg of chymotrypsin. The chymotrypsin-dissected Na,K-ATPase was collected by centrifugation at $160\,000 \times g$ for 1 h at 4°C, and washed with 10 mM Tris-HCl (pH 7.4). Samples were subjected to electrophoresis on SDS polyacrylamide gels (7.5%) at pH 2.4 and stained with Coomassie [11]. For N-terminal sequence analysis peptide material from the 48-kDa band was transferred onto a polyvinylidene difluoride (PVDF) membrane.

Extensive trypsin digestion of native Na,K-ATPase or chymotryp-

*Corresponding author. Fax: (1) (419) 383-2871.
E-mail: NModyanov@Cutter.Mco.Edu

PII S0014-5793(97)01493-2

sin-dissected preparation was done according to Capasso et al. [4] as described before [11,14,15].

For oxidative cross-linking, the postproteolytic membrane preparation was suspended (1.35 mg/ml) in a solution containing 10 mM Tris-HCl (pH 7.4), 0.25 mM $CuSO_4$, and 1.25 mM *o*-phenanthroline, and incubated at 24°C for 15 min. These conditions were chosen after preliminary experiments using the approach described before [17]. The reaction was terminated by the addition of EDTA to a final concentration of 30 mM, followed by incubation for 5 min at room temperature [11,15]. The samples were collected by centrifugation, washed with 10 mM Tris-HCl (pH 7.4), and cross-linked peptides were separated by electrophoresis on 10% tricine/SDS polyacrylamide gels [11,15]. Sulfhydryl reagents were omitted from the buffers used for electrophoresis to preserve the cross-linked products. Stained bands of cross-linked peptides (five lanes) were cut out of the gel, electroeluted with 100 mM NH_4HCO_3 and 0.1% SDS, lyophilized and subjected to additional purification using the same electrophoretic system. Purified cross-linked peptides were transferred from the unstained gel onto the PVDF membrane, detected on the membrane by brief staining with Coomassie and subjected to N-terminal amino acid sequence analysis as described before [18].

3. Results

3.1. Identification of the N-terminal fragment containing the transmembrane H_1-H_2 domain

The primary aim of this study was to identify proteolytic membrane-bound fragments originating from the N-terminal part of the Na,K-ATPase α-subunit after selective chymotryptic cleavage in NaCl medium [2,5,9]. A schematic structure of Na,K-ATPase is shown in Fig. 1. The purified membrane-bound Na^+,K^+-ATPase of canine kidney was incubated with different chymotrypsin concentrations as described in Section 2. Electrophoretic analysis of membrane-bound fraction of the hydrolyzate is shown in Fig. 2. In agreement with previous results [2,5,6,9,11], this digestion did not disrupt the integrity of the β-subunit, and resulted in almost quantitative conversion of the α-subunit to the C-terminal 83-kDa fragment whose N-terminal residue has been determined to be Ala^{267} [6,9], as shown in Fig. 1. Also in agreement with previous observations, staining with Coomassie did not reveal the presence of other well-defined polypeptide bands except the barely detectable band with electrophoretic mobility corresponding to a molecular mass of 48 kDa (Fig. 2, lanes 3–5). No additional smaller peptides were detected when this preparation was resolved on tricine gels (not shown). Peptides present in the 48-kDa band have been identified as products of the secondary digestion of the 83-kDa polypeptide by chymotrypsin as described below.

The above observations suggested the possibility that under the conditions of selective chymotryptic digestion, the N-terminal 30 kDa part of the α-subunit was converted to a number of heterogeneous peptides that were undetectable by Coomassie staining. To attempt the identification of any such

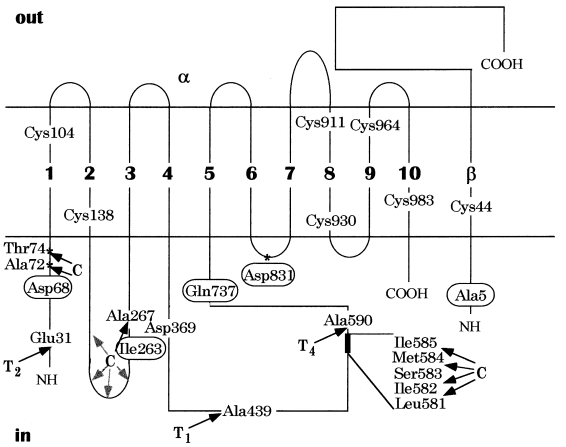

Fig. 1. Topology of Na,K-ATPase and patterns of limited proteolytic digestion. Established (filled arrows) and postulated (shaded arrows) sites of selective digestion of native Na,K-ATPase by chymotrypsin (C). The transmembrane products of extensive trypsin digestion [4] are indicated by circled N-terminal amino acid residues. Indicated intramembrane cysteines are those most likely to be involved in cross-linking reactions of the transmembrane helices of α and β [11,15]. Known sites of limited tryptic digestion of Na,K-ATPase in the E2 conformation are indicated as T1, T2 and T4 [2,9,21]. See text for other details.

peptides, we took advantage of the oxidative cross-linking technique elaborated in previous studies [11,15]. In these studies we had applied this approach to study the structure of the membrane moiety of the extensively trypsin-digested Na,K-ATPase (Figs. 1 and 3, lane 4), and elucidated that two intramembrane Cys residues, which are located in the N-terminal (H_1-H_2) and in C-terminal (H_7-H_{10}) domains of the α-subunit, are in close spatial proximity, and upon incubation with Cu^{2+}-phenanthroline complex form an easily detectable stable disulfide cross-linked product. The cross-linked product was a dimer of a 11-kDa α-fragment containing H_1-H_2 helices and a 22-kDa α-fragment containing H_7-H_{10} helices (Fig. 3, lane 3, band a). Therefore, we used the same strategy to determine if a similar cross-linked product could be obtained from a membrane-bound preparation of chymotrypsin-dissected Na,K-ATPase.

To simplify subsequent identification of expected cross-linked product, the chymotrypsin-dissected Na^+,K^+-ATPase preparation (Fig. 2, lane 5) was first subjected to extensive trypsin digestion in the presence of Rb^+ and EDTA according to established procedures that convert the native Na,K-ATPase to a set of well-characterized [4,11,15] transmembrane fragments of the α-subunit and essentially intact β-subunit (Figs. 1 and 3, lane 4). Electrophoretic analysis of membrane-bound peptides obtained after sequential chymotrypsin and trypsin digestions (Fig. 3, lane 1) revealed the presence of

Table 1
N-terminal sequences of the cross-linked product of the chymotrypsin/trypsin-digested Na,K-ATPase

Sequence	Polypeptide chain region	Yield (pmol)
^{72}Ala-Leu-Thr-Pro-Pro-Pro-Thr-Thr-Pro-Glu-Xaa	α-subunit, H1-H2	20
^{74}Thr-Pro-Pro-Pro-Thr-Thr-Pro-Glu-Xaa-Val-Lys	α-subunit, H1-H2	9
^{831}Asn-Pro-Lys-Thr-Asp-Lys-Leu-Val-Asn-Glu-Arg	α-subunit, H7-H10	32

The cross-linked product designated a* in Fig. 3, lane 2, was sequenced as indicated in Section 2. The sequences were followed for 11 cycles of Edman degradation and were identical to the shown fragments of the canine kidney Na,K-ATPase α_1-subunit [19]. Xaa indicates amino acid residues that were not identified upon sequencing and corresponds to Trp^{82} of the α-subunit. The yield of each sequence was estimated from the average elevation of residues in cycles 2–10.

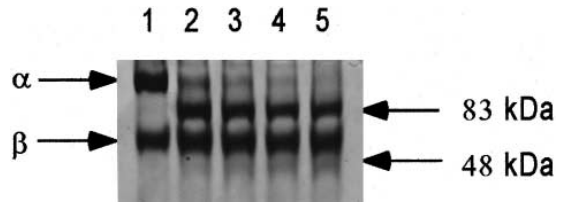

Fig. 2. Selective chymotryptic cleavage of the E1 form of Na,K-ATPase. The Na^+,K^+-ATPase (lane 1) was incubated with chymotrypsin, resolved on SDS gels, and stained with Coomassie blue, as described in Section 2. The digestion was done at chymotrypsin/substrate ratios ranging from 1/50 (lane 2) to 1/20 (lane 5).

the prominent peptide band 11 kDa* which migrates below the 11-kDa band of control sample (Fig. 3, lane 4).

After incubation of the membrane-bound chymotrypsin/trypsin-digested Na,K-ATPase with Cu^{2+}-phenanthroline complex, the intensities of original 22-kDa and 11-kDa* bands significantly decreased, and new bands representing cross-linked products appeared (Fig. 3, lane 2). The most prominent cross-linked product, designated a*, has a slightly higher mobility than the previously identified 11,22 cross-linked product a [11,15] presented in the control sample (Fig. 3, lane 3). Peptide material of band a* was isolated and characterized by N-terminal amino acid sequencing (Table 1).

Three sequences shown in Table 1 were identified based on the known primary structure of canine Na,K-ATPase α-subunit [19]. They were found to be identical to the 22-kDa C-terminal α-fragment starting at Asn^{831} and to N-terminal α-fragments beginning at Ala^{72} or at Thr^{74}. The yield of the C-terminal fragment was equal to the combined yields of N-terminal fragments, thus indicating that cross-linked product contained the C-terminal fragment and one of the two N-terminal fragments in a molar ratio 1:1. Based on the apparent molecular mass of the cross-linked product, it is evident that the identified N-terminal fragments contain transmembrane segments H_1 and H_2.

The N-termini of identified peptides were formed due to cleavages at Asn^{71}-Ala^{72} and Leu^{73}-Thr^{74}, both of which correspond to chymotrypsin specificity, thus indicating that the peptides are products of primary chymotryptic digestion. In addition, it should be noted that digestion of native Na,K-ATPase by trypsin alone generates an analogous α-fragment beginning at Asn^{68} [4,11,15]. We did not detect the presence of such a peptide upon sequencing of the cross-linked product; therefore, additional tryptic digestion did not affect the N-termini of chymotryptic fragments but evidently eliminated variability in the length of the C-terminal parts located beyond the H_2 segment.

Thus, we have identified the major products originating from the N-terminal part of the Na,K-ATPase α-subunit upon controlled chymotryptic digestion. These start at Ala^{72} or Thr^{74} and contain membrane segments H_1 and H_2 (Fig. 1). It is obvious that chymotrypsin cleaves the E1 form of the α-subunit not only at the Leu^{266}-Ala^{267} bond, but also partially or completely at several other peptide bonds within the 30-kDa N-terminal region. Evidently, the H_1-H_2 transmembrane domain in this preparation was not detected in previous studies [2,6,9] because it is present in multiple fragments generated upon partial chymotryptic cleavage of numerous peptide bonds located between the C-terminal boundary of H_2 and Leu^{266}.

It should be noted that in addition to product a* we also detected another cross-linked product designated b* (Fig. 3, lane 2) with a mobility similar to that of 11,22 β cross-linked product b from the control sample (Fig. 3, lane 3), which was identified previously [15]. Formation of the cross-linked product between two pairs of Cys residues from N- and C-terminal α-domains and the β-membrane segment demonstrates that selective chymotryptic digestion does not disrupt the structural integrity of the Na,K-ATPase membrane moiety.

3.2. *Secondary chymotryptic cleavage of the 83-kDa polypeptide; identification of the C-terminal 48-kDa fragment*

As indicated above, when membrane-bound Na^+,K^+-ATPase was subjected to selective chymotryptic cleavage, a small amount of the polypeptide band with electrophoretic mobility corresponding to a molecular mass of 48 kDa was detected, in addition to the well-characterized 83-kDa fragment (Fig. 2). It was noted that the relative amount of the 48-kDa band increased in parallel to the increasing ratio of chymotrypsin/ATPase (lanes 2–5 of Fig. 2). To test the possibility that the 48-kDa band reflects the secondary digestion of the 83-kDa polypeptide by chymotrypsin, the 48-kDa polypeptide band was transferred onto PVDF membrane and subjected to 10 cycles of Edman degradation. The results obtained are shown in Fig. 4. Several overlapping N-terminal sequences were revealed. Detection of approximately equal amounts of the same amino acid residue in four consecutive steps of Edman degradation (for example, Asp in cycles 2–5, Arg in 5–8, etc.) allows us to identify reliable sequences of four individual components of this rather complex mixture. These peptide fragments originated from the partial chymotryptic cleavage of each peptide bond within the sequence ^{581}Leu-Ile-Ser-Met-Ile^{585} of the α-subunit (Figs. 1 and 4). Thus, the 48-kDa fragment has a cluster of highly chymotrypsin-sensitive bonds at the N-terminus and represents, most probably, the entire C-terminal part of the α-subunit, since its electrophoretic mobility correlates well with the calculated molecular mass of

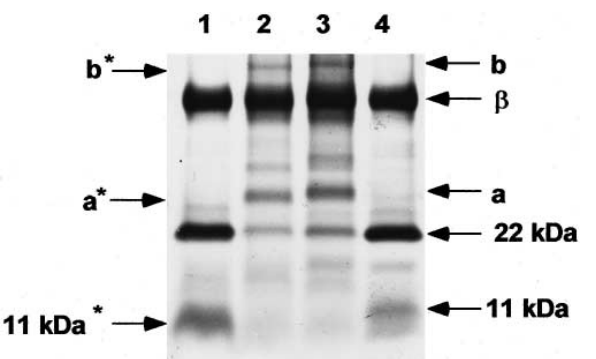

Fig. 3. Cu^{2+}-phenanthroline induced oxidative cross-linking of the membrane-bound preparations of the Na^+/K^+-ATPase obtained after controlled chymotrypsin cleavage followed by extensive trypsin digestion, or after extensive trypsin digestion alone. The membrane samples were prepared, cross-linked, and resolved on 10% tricine gels as described in Section 2. The membrane-bound preparation of chymotrypsin/trypsin-digested enzyme prior to cross-linking (lane 1), and after cross-linking (lane 2). Control sample of enzyme digested by trypsin alone, before cross-linking (lane 4), and after cross-linking (lane 3).

^{582}Ile-Ser-Met-Ile-Asp-Pro-Pro-Arg-Ala-Ala

^{583}Ser-Met-Ile-Asp-Pro-Pro-Arg-Ala-Ala-Val

^{584}Met-Ile-Asp-Pro-Pro-Arg-Ala-Ala-Val-Pro

^{585}Ile-Asp-Pro-Pro-Arg-Ala-Ala-Val-Pro-Asp

Fig. 4. Amino acid sequences identified upon N-terminal analysis of the peptides contained in the 48-kDa product of secondary chymotrypsin cleavage of the Na,K-ATPase α-subunit. The coordinates of the N-terminal residues in polypeptide chain are indicated (see text for details).

about 48 kDa. Absence of any detectable polypeptide bands in the region of about 35 kDa which should correspond to the expected N-terminal part of the 83-kDa polypeptide indicated its more extensive digestion at secondary steps of chymotrypsin action.

4. Discussion

Although the chymotrypsin-digested enzyme has no ATPase or ATP-dependent ion transport activities, it retains the ability to be phosphorylated [5,6], catalyzes ADP-ATP exchange activity [5,6], occludes Na^+ and Rb^+ [5,20], and is responsive to ouabain [6]. However, there are significant differences between the properties of the remaining activities of the chymotrypsin-digested enzyme and the corresponding activities of the native enzyme. For example, while the phosphorylation of the native enzyme by ATP is dependent on Na^+, the chymotrypsin-digested enzyme can be phosphorylated in the absence of Na^+ [6]; and while the phosphorylation by ATP and ADP-ATP exchange activity of the native enzyme are inhibited by ouabain, these activities of the chymotrypsin enzyme are stimulated by ouabain [6]. Also, our recent studies have established that the dormant half of the phosphorylation sites of the native enzyme are unmasked upon cleavage of the α-subunit by chymotrypsin [10]. Clearly, the determination of the structural basis of these contrasting properties of the native and the chymotrypsin-digested preparations is important to the clarification of the relation of the enzyme's structure to its reaction mechanism.

In the past, the functional changes induced by chymotrypsin digestion have been ascribed to the single cleavage of the α-subunit at Leu^{266}-Ala^{267} [5,9]; and in spite of the failure to detect the 30-kDa N-terminal fragment of the α-subunit resulting from this cleavage, the assumption has been made that the altered properties of the chymotrypsin-digested enzyme are due to the disruption of domain-domain interactions caused by this single cleavage [5,9]. That this assumption is not justified is clearly established by the present data showing that the structural changes induced by chymotrypsin are more extensive than a single cleavage. Also ruled out is the possibility considered before [11] that the H_1-H_2 transmembrane domains of the α-subunit may be released from the membrane after chymotrypsin digestion. The present findings clearly indicate that the digested preparation retains these transmembrane domains, and that H_1-H_2 continue to interact with H_7-H_{10} transmembrane domains as they do in the native enzyme [11,15]. To explain the different properties of the native and the chymotrypsin-digested enzymes, therefore, it is necessary to consider the following three structural changes: (1) cleavage at Leu^{266}-Ala^{267}; (2) cleavage at Asn^{71}-Ala^{72} and Leu^{73}-Thr^{74}; (3) partial cleavage of several peptide bonds located between Leu^{266} and the point of exit of H_2 from the membrane. At this time, it is not possible to say which one of the above changes, or which combination of changes, accounts for the interesting properties of the chymotrypsin-digested enzyme.

Formation of the relatively stable C-terminal 48-kDa fragment of the α-subunit upon selective chymotrypsin digestion of the E1 conformation of Na,K-ATPase is of interest with respect to the spatial structure of the enzyme molecule. First, a similar relatively stable C-terminal 48-kDa fragment of the α-subunit with N-terminus at Ala^{590} has been detected upon limited tryptic digestion of Na,K-ATPase in the presence of K^+ (E2 conformation) [21,22]. Second, this highly protease-sensitive region is located at the N-terminal boundary of the highly conservative part of Na,K-/H,K-ATPases and other P-type ATPases extending to the transmembrane segment H_5 (Fig. 1). Third, this conservative sequence contains a number of amino acid residues such as Asp^{710} and Asp^{714}, which are involved in the formation of high affinity ATP-binding sites [1,23].

Taken together, the above observations indicate that part of the large central cytoplasmic loop of the Na,K-ATPase α-subunit located between the protease-sensitive region (Ile^{582}-Ala^{590}) and the membrane moiety (Gln^{737}) exists in both E1 and E2 conformations as a relatively stable, compactly folded, globular nucleotide-binding domain whose N-terminal boundary is marked by a highly protease-sensitive region which is exposed on the surface of the enzyme molecule in both E1 and E2 conformations.

Acknowledgements: This work was supported in part by National Institutes of Health Grant HL-36573.

References

[1] Moller V, J., Juul, B. and le Maire, M. (1996) Biochim. Biophys. Acta 1286, 1–51.
[2] Jorgensen, P.L. (1992) in: Molecular Aspects of Transport Proteins (DePont, J.J.H.H.M., Ed.), pp. 1–26, Elsevier, New York.
[3] Modyanov, N., Lutsenko, S., Chertova, E. and Efremov, R. (1991) in: The Sodium Pump: Structure, Mechanism, and Regulation (Kaplan, J.H. and DeWeer, P., Eds.), pp. 99–115, The Rockefeller University Press, New York.
[4] Capasso, J.M., Hoving, S., Tal, D.M., Goldshleger, R. and Karlish, J.D. (1992) J. Biol. Chem. 267, 1150–1158.
[5] Jorgensen, P.L. and Petersen, J. (1985) Biochim. Biophys. Acta 821, 319–333.
[6] Huang, W.-H., Ganjeizadeh, M., Wang, Y., Chiu, I.-N. and Askari, A. (1990) Biochim. Biophys. Acta 1030, 65–72.
[7] Zolotarjova, N., Periyasamy, S.M., Huang, W.-H. and Askari, A. (1995) J. Biol. Chem. 270, 3989–3995.
[8] Ganjeizadeh, M., Zolotarjova, N., Huang, W.-H. and Askari, A. (1995) J. Biol. Chem. 270, 15707–15710.
[9] Jorgensen, P.L. and Collins, J.H. (1986) Biochim. Biophys. Acta 860, 570–576.
[10] Liu, G., Xie, Z., Modyanov, N.N. and Askari, A. (1996) FEBS Lett. 390, 323–326.
[11] Sarvazyan, N.A., Modyanov, N.N. and Askari, A. (1995) J. Biol. Chem. 270, 26528–26532.
[12] Ovchinnikov, Yu.A., Arzamazova, N.M., Arystarkhova, E.A., Gevondyan, N.M., Aldanova, N.A. and Modyanov, N.N. (1987) FEBS Lett. 217, 269–374.

[13] Lutsenko, S., Anderko, R. and Kaplan, J.H. (1995) Proc. Natl. Acad. Sci. USA 92, 7936–7940.
[14] Liu, L. and Askari, A. (1997) J. Biol. Chem. 272, 14280–14386.
[15] Sarvazyan, N.A., Ivanov, A., Modyanov, N.N. and Askari, A. (1997) J. Biol. Chem. 272, 7855–7858.
[16] Askari, A., Huang, W.-H. and McCormick, P.W. (1983) J. Biol. Chem. 258, 3453–3460.
[17] Periyasamy, S.M., Huang, W.-H. and Askari, A. (1983) J. Biol. Chem. 258, 9878–9885.
[18] Vladimirova, N.M., Potapenko, N.A., Sachs, G. and Modyanov, N.N. (1995) Biochim. Biophys. Acta 1233, 175–184.
[19] Xie, Z., Li, H., Wang, Y., Askari, A. and Mercer, R.W. (1994) in: The Sodium Pump (Bamberg, E. and Schoner, W., Eds.), pp. 49–52, Steinkopff, Germany.
[20] Glynn, I.M., Hara, Y. and Richards, D.E. (1984) J. Physiol. 351, 531–547.
[21] Arystarkhova, E. and Sweadner, K.J. (1996) J. Biol. Chem. 271, 23407–23417.
[22] Abbott, A. and Ball, W.J. (1993) Biochemistry 32, 3511–3518.
[23] Ovchinnikov, Yu.A., Dzhandzhugazyan, K.N., Lutsenko, S.V., Mustayev, A.A. and Modyanov, N.N. (1987) FEBS Lett. 217, 111–116.