Acta Chemica Scandinavica, 1997: 51: 403–406
Printed in UK – all rights reserved

Copyright © Acta Chemica Scandinavica 1997
ACTA
CHEMICA SCANDINAVICA
ISSN 0904-213X

Attempt to Solubilize Na^+/K^+-Exchanging ATPase with Amphiphilic Peptide PD_1

Ursel Soomets,[a,*] Czeslava Kairane,[a] Mihkel Zilmer[a] and Ülo Langel[b]

[a]Department of Biochemistry, Tartu University, EE 2400, Tartu, Estonia and [b]Department of Neurochemistry and Neurotoxicology, Arrhenius Laboratories, Stockholm University, S-106 91, Stockholm, Sweden

Soomets, U., Kairane, C., Zilmer, M. and Langel, Ü., 1997. Attempt to Solubilize Na^+/K^+-Exchanging ATPase with Amphiphilic Peptide PD_1. – Acta Chem. Scand. 51: 403–406. © Acta Chemica Scandinavica 1997.

The effect of the 24-amino-acid-long peptide, PD_1, on rat cerebral cortical Na^+/K^+-exchanging ATPase (EC 3.6.1.37) has been studied. Incubation of the enzyme preparation (25 °C for 10–25 min) with the peptide (10^{-7}–10^{-4} M) did not appreciably affect the activity of the enzyme, only 5–8% activation being registered. On the other hand, PD_1 completely eliminated the cooperative nature of Na^+-binding to Na^+/K^+-exchanging ATPase (n_H decreased from 1.4 to 0.9) and slightly (1.2-fold) decreased the affinity for Na^+. ATP, a substrate of Na^+/K^+-exchanging ATPase, blocked the PD_1-promoted effect on the cooperativity for Na^+. Incubation of cerebral cortical membranes with 5×10^{-4} M PD_1 revealed a shift (from 19.5 °C to 21.4 °C) of the typical break on the Arrhenius plot (15–37 °C). Prolonged incubation of enzyme preparation (25 °C for 1–2 h) with PD_1 (4.5×10^{-4}–0.7×10^{-2} M) followed by centrifugation of the mixture at 53 000*g* for 90 min, resulted in loss of the activity both in the supernatant and the sediment, while the protein content in the supernatant and the sediment remained unchanged. After a short incubation (25 °C for 10 min) with PD_1 (1×10^{-6} M), followed by centrifugation, the full activity of Na^+/K^+-exchanging ATPase in the sediment was restored. These data suggest that peptitergent PD_1 does not solubilize the transmembrane protein Na^+/K^+-exchanging ATPase, although it abolishes the cooperative effect of Na^+.

It is known that Na^+ and K^+ are essential regulators of Na^+/K^+-exchanging ATPase, a transmembrane enzyme, composed of two subunits (α and β) in equimolar ratio.[1] The αβ complex is able to hydrolyze ATP but the normal and allosterically regulated transport of Na^+ and K^+ needs a higher order of αβ complex organisation, apparently the diprotomer $(\alpha\beta)_2$.[2,3] Hence, for cooperativity of such essential allosteric regulators of Na^+/K^+-exchanging ATPase, as Na^+ and K^+, interactions between protomers are probably necessary. Consequently, after the desensibilization of the oligomeric Na^+/K^+-exchanging ATPase enzyme system with detergents (which disturb mainly the interactions between protomers), the specific activity of enzyme should remain practically unchanged, but the cooperativity in the binding of Na^+ and K^+ to Na^+/K^+-exchanging ATPase should change remarkably. We have recently studied the influence of several detergents on the activity and cooperative nature of brain Na^+/K^+-exchanging ATPase and K-pNPPase.[4] These studies reveal that desensibilization of the enzyme preparation with SDS, digitonin or CHAPS reduced both the Hill coefficients of Na^+ and K^+ and the amount of αβ-protomer in the enzyme preparation, whereas the specific activity of Na^+/K^+-exchanging ATPase remained practically unchanged. Hence, these experiments support the assumption that interactions of protomers are necessary for allosteric effects of Na^+ and K^+ on cerebral cortical Na^+/K^+-exchanging ATPase. On the other hand, these experiments showed that under appropriate conditions, SDS, digitonin and CHAPS all exert a desensibilizating influence on such transmembrane enzymes as Na^+/K^+-exchanging ATPase.

Recently, a new, interesting, amphiphilic peptide (also called the first peptitergent), PD_1 was designed and synthesized.[5] Moreover, it was shown that this peptide solubilizes some membrane-bound proteins. We have studied the possibilities of application of this 24-amino-acid-long peptide for solubilisation of rat cortical Na^+/K^+-exchanging ATPase, an enzyme which is very sensitive to changes in lipid environment .

Materials and methods

Peptide synthesis. The peptide PD_1 was synthesized in a stepwise manner in a 0.1 mmol scale Applied Biosystem

* To whom correspondence should be addressed.

Model 431A peptide synthesiser on a solid support using dicyclohexylcarbodiimide–hydroxybenzotriazole activation strategy. *tert*-Butyloxycarbonyl amino acids were coupled as hydroxybenzotriazole esters to a MBHA resin (1.1 mmol amino groups per gram resin, Bachem, Switzerland) to obtain *C*-terminally amidated peptides. The peptide was finally cleaved from the resin with liquid HF at 0 °C for 30 min. Deprotection of the side-chains, cleavage of the peptide and purification on HPLC have been described in detail earlier.[6] The purity of the peptide was >99% as demonstrated by HPLC on an analytical Nucleosil 120–3 C_{18} reversed-phase HPLC column (0.4 cm × 10 cm). The molecular mass of the peptide was determined by a plasma desorption mass spectrometry (Bioion 20, Applied Biosystems), and the calculated value was obtained.

Enzyme preparations and assays. The enzyme preparation of the Na^+/K^+-exchanging ATPase was isolated from the frontal cortex of adult rats (Wistar, 250–300 g) as described earlier.[7] Briefly, the rats were decapitated, the frontal cortex was isolated from the precooled brain and homogenized at 4 °C in medium containing 0.32 M sucrose, 1 mM EDTA, 0.1% DOC and 37.5 mM imidazole–HCl (pH 7.4 at 8 °C). This homogenate was centrifuged for 10 min at 10 000*g* and the clear supernatant was centrifuged for 30 min at 24 000*g*. By resuspension of the final sediment in the above-mentioned buffer (without DOC) the enzyme preparation was achieved.

The activity of the Na^+/K^+-exchanging ATPase was measured as described earlier,[8] the specific activity of Na^+/K^+-exchanging ATPase being expressed as μmol P_i/mg protein per hour. The protein content was determined by the method of Lowry,[9] using bovine serum albumine as a standard. The activity of the Na^+/K^+-exchanging ATPase was a linear function of incubation time and enzyme amount under all experimental conditions.

Other methods. The degree of cooperativity (the Hill coefficients, n_H) and $K_{0.5}$ (sodium or potassium concentration at which the enzyme has half-maximal activity) for rat frontal cortical Na^+/K^+-exchanging ATPase was determined as described earlier.[4,8,10] Briefly, the assay of the activity of Na^+/K^+-exchanging ATPase was carried out at several concentrations of sodium (the concentration of potassium was constant) or potassium (the concentration of sodium was constant) and the values of V_{max} and $K_{0.5}$ were calculated via Lineweaver–Burk plot ($1/v$ vs. 1/[cation]) and checked by means of the Cornish-Bowden method. n_H was obtained from the slope of Hill plot $\{\log(v/V_{max}-v)$ vs. $\log[Na^+]$ or $\log[K^+]\}$.

The temperature dependence of the Na^+/K^+-exchanging ATPase activity was studied in the range 15–37 °C and the data were represented as Arrhenius plots ($\log v$ vs. 10^3 K/T).

Results

In order to examine the influence of the PD_1 on the activity of rat cerebral cortical Na^+/K^+-exchanging ATPase, the enzyme preparation (140 μg of protein) was incubated with PD_1 (10^{-7}–10^{-4} M) for 10–25 min at 25 °C and the remaining ATPase activity was measured. PD_1 showed practically no remarkable effect on the specific activity of rat cerebral cortical Na^+/K^+-exchanging ATPase (Table 1). In all of the following experiments, the preincubation time was 25 min and the concentration of PD_1 was 10^{-4} M (Table 1). Analysis of the effect of different concentrations of Na^+ or K^+ on the activity of cerebral cortical Na^+/K^+-exchanging ATPase, preincubated with PD_1, revealed that this peptide completely eliminates the cooperativity for Na^+ (n_H for Na^+ decreases from 1.4 to 0.9, Table 1, Fig. 1), causes a certain increase in the affinity (decreases $K_{0.5}$) for Na^+, but does not influence the same parameters for K^+ (Table 1). Addition of ATP, a substrate for Na^+/K^+-exchanging ATPase, into the preincubation medium blocks the PD_1-promoted effect on the cooperativity for Na^+, but does not change the slight increasing effect on the affinity for Na^+ (Table 1).

It is well known that the temperature dependence of enzyme activity (the break in the Arrhenius plot) indicates the phase transitions in the lipid bilayer. Therefore we studied the temperature dependence of Na^+/K^+-exchanging ATPase activity, incubated with

Table 1. Influence of PD_1 on rat frontal cortical Na^+/K^+-exchanging ATPase.

	Control	$[PD_1]/10^{-4}$ M
Short-term preincubation[a]		
Activity of Na,K-ATPase [b]	61(4)	65(5)
n_H for sodium	1.4(1)	0.9(1)[c]
$K_{0.5}$/mM for sodium	5.1(7)	4.1(6)
n_H for potassium	1.3(1)	1.3(1)
$K_{0.5}$/mM for potassium	1.4(2)	1.4(3)
n_H for sodium in the presence of 4 mM ATP	1.4(1)	1.3(1)
$K_{0.5}$/mM for sodium in the presence of 4 mM ATP	4.6(7)	3.3(7)
Break area (T/°C) on the Arrhenius plot	19.5(2)	21.4(2)[c]
Long-term preincubation[d]		
Activity of Na^+/K^+-exchanging ATPase in sediment[d]	59	0

[a]The enzyme preparation was preincubated with 10^{-4} M PD_1 at 25 °C for 10–25 min and the parameters of the Na^+/K^+-exchanging ATPase assayed as described in the materials and methods section. Values are given as mean ± S.E.M. of 4–7 measurements. [b](μmol P_i/mg protein per hour). [c]Significant change versus control ($p<0.05$). [d]Prolonged preincubation of the enzyme preparation (25 °C for 1–2 h) with PD_1 (4.5×10^{-4}–0.7×10^{-2} M) was followed by centrifugation of the mixture at 53 000*g* for 90 min and the activity of Na^+/K^+-exchanging ATPase both in sediment and supernatant was assayed.

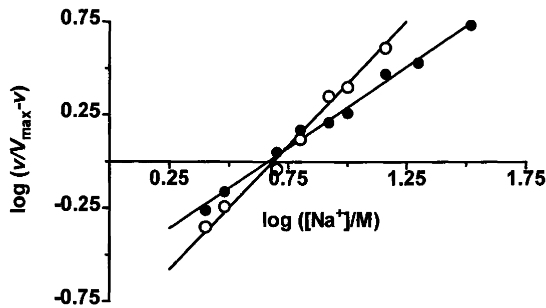

Fig. 1. Hill plots showing the effect of PD_1 on the cooperativity for Na^+ with Na^+/K^+-exchanging ATPase. The Hill coefficient (n_H) was obtained from the slope. The enzyme preparations were preincubated at 25 °C for 10–25 min in absence (○) and presence (●) of 10^{-4} M PD_1 and the Na^+/K^+-exchanging ATPase activity was assayed as described in the materials and methods section. The results of a typical experiment are shown.

PD_1 in the range 15–37 °C. This peptide causes a shift of the typical break in the Arrhenius plot (from 19.5 ± 0.2 °C to 21.4 ± 0.3 °C, $p < 0.05$, Fig. 2).

Prolonged preincubation of the enzyme preparation at 25 °C for 1–2 h with PD_1 (4.5×10^{-4}–0.7×10^{-2} M) followed by centrifugation of the mixture at 53 000 g for 90 min resulted in the loss of activity of Na^+/K^+-exchanging ATPase (Table 1) in the sediment but not in the supernatant; the total protein content in both the supernatant and the sediment, remained unchanged. After short preincubation (for 10 min at 25 °C) with PD_1 (1×10^{-6} M) followed by centrifugation, the full activity of Na^+/K^+-exchanging ATPase in sediment was re-established and the protein content in both the supernatant and sediment remained unchanged.

Discussion

The first peptitergent, PD_1, was recently designed, synthesized and crystallized.[5] PD_1 is an amphipathic peptide with a 'flat' hydrophobic surface that probably interacts with the transmembrane protein by packing around the protein in rigid, well-ordered, parallel α-helical arrangements.[5] It has been demonstrated that the PD_1 is able to solubilize and stabilize in solution such membrane proteins as bacteriorhodopsin and rhodopsin.

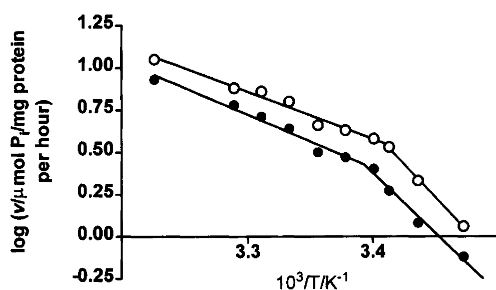

Fig. 2. Arrhenius plot of rat cortical Na^+/K^+-exchanging ATPase. The enzyme preparations were preincubated at 25 °C for 10–25 min in absence (○) and presence (●) of 10^{-4} M PD_1 and the Na^+/K^+-exchanging ATPase activity was assayed as described in the materials and methods section. The pH value of the incubation mixture was 7.4 at each given temperature. The break points are at 19.5(2) °C and 21.4(3) °C for the control and influenced enzyme, respectively ($p < 0.05$ vs. control).

It is of interest to find a possible alternative to small-molecular detergents for the solubilization in the active state of integral membrane proteins, like Na^+/K^+-exchanging ATPase, that are known to be sensitive to changes in lipid environment. We have synthesized the 24-amino-acid-long peptide PD_1 and studied its effects on rat cerebral cortical Na^+/K^+-exchanging ATPase. It is known that the Na^+ and K^+ ions are essential regulators of Na^+/K^+-exchanging ATPase, an enzyme composed of two subunits (α and β) in an equimolar ratio, and that the αβ complex (protomer) is able to hydrolyse ATP. On the other hand, both the normal and allosterically regulated transport of Na^+ and K^+ needs a higher order of organisation of the αβ complex, presumably the diprotomer $(\alpha\beta)_2$. This suggests that for cooperativity of such essential allosteric regulators of Na^+/K^+-exchanging ATPase, as Na^+ and K^+, interactions between the protomers are probably necessary. Consequently, after the desensibilization of the oligomeric Na^+/K^+-exchanging ATPase enzyme system with factors disturbing its oligomeric structure, changes in the allosteric nature of cation binding to the enzyme should occur, whereas the specific activity should remain practically the same. The demonstrated effect of the PD_1 on the activity of the enzyme suggests that this amphiphilic peptide completely eliminates the cooperativity for Na^+ and reveals only an insignificant increase on the specific activity of rat cerebral cortical Na^+/K^+-exchanging ATPase and the affinity for Na^+. Addition of a substrate (ATP, 4 mM) to the preincubation medium blocks the effect caused by PD_1 on the cooperativity for sodium, but does not change the slight increase in the affinity for sodium (Table 1). It is known that ATP stabilizes the protomeric structure of the Na^+/K^+-exchanging ATPase.[11,12] All these data suggest that that PD_1 works as a desensibilizing factor in the case of Na^+/K^+-exchanging ATPase and may be characterized as a mild detergent. Such an assumption is also supported by following facts: (i) PD_1 does not influence the parameters for potassium, the other essential cation (Table 1) and it is known that the Na-form of Na^+/K^+-exchanging ATPase is usually more labile than the K-form;[4,13] (ii) there was no change in the tertiary structure of retinal-membrane-containing proteins in the presence of PD_1;[5] (iii) different detergents, such as digitonin (non-ionic detergent), SDS (ionic detergent) and CHAPS (zwitterionic detergent), alter the cooperative binding of sodium to Na^+/K^+-exchanging ATPase in different ways but they do not show any remarkable influence on the specific activity of this enzyme;[5] (iv) digitonin and CHAPS, altering the cooperative binding of Na^+, do not show such an effect in the case of K^+.[4]

Despite these facts, we assert that the effects of PD_1

on several proteins (including Na^+/K^+-exchanging ATPase) are quite different from classical detergents (such as DOC, SDS) and its practical use as a detergent is restricted. Such a suggestion is supported by the following: firstly, it is suggested[5] that not all membrane proteins are solubilized by this particular peptitergent; secondly, the prolonged preincubation (for 1–2 h at 25 °C) of the enzyme preparation with PD_1 (4.5×10^{-4}–0.7×10^{-2} M) followed by centrifugation of mixture at 53 000*g* for 90 min results in total loss of activity of Na^+/K^+-exchanging ATPase, whereas the total protein content in both supernatant and sediment, remains unchanged. Additionally, the prolonged preincubation with another detergent, CHAPS (5×10^{-2}–2.4×10^{-3} M) yields a significant increase in the protein content in the supernatant and a decrease in the sediment, whereas at 2.4×10^{-3} M, specific activity of Na^+/K^+-exchanging ATPase and K-pNPPase (40% and 37% as compared to control, respectively) in the sediment was registered. Thirdly, PD_1 causes a shift (from 19.5 °C to 21.4 °C, $p < 0.05$) of the typical break on the Arrhenius plot (Fig. 2), but CHAPS does not.[4] It should be emphasized that the PD_1 is an amphipathic peptide with a 'flat' hydrophobic surface that could interact with transmembrane proteins by arrangement around the protein in a rigid, well-ordered, parallel α-helical structure.[5] Obviously, PD_1, owing to its hydrophobic/lipophilic nature, induces certain alterations of the lipid surroundings of Na^+/K^+-exchanging ATPase (the phasic transitions, the changes of the hydrophobic volume) during short-time preincubation, resulting in interference with cooperative binding of Na^+. It is also known that the effects of membrane lipid on Na^+/K^+-exchanging ATPase are realized mainly via the changes in membrane microviscosity, a feature of the phase transitions. The observed shift of the break point (at 19–20 °C) in the Arrhenius plot, in the presence of PD_1, indicates the phase transitions in the lipid bilayer.[14] In addition, for the interaction between subunits in the protomer of the Na^+/K^+-exchanging ATPase, the hydrophobic amino acids in an amphipathic *C*-terminal β-strand of the β-subunit play the most pronounced role.[15] The lack of influence of PD_1 at high concentration of ATP on the lipids confirms the evidence presented above.

In the case of the long-term influence of PD_1, its functional groups may destroy hydrogen bonds and cause severe structural changes (interference with protein–protein interactions, both within the protomer and between the protomers) of transmembrane Na^+/K^+-exchanging ATPase, resulting in loss of enzyme activity. The latter assumption is supported by our preliminary experiments which indicate a significant decrease in the effect of PD_1 on the activity of such transmembrane protein as Na^+/H^+-exchanger (Soomets *et al.*, unpublished data).

In summary, our data reveal the complicated nature of the influence of the new peptide, PD_1 on the solubilization of rat cerebral cortical transmembrane Na^+/K^+-exchanging ATPase. Despite the probably restricted areas of biochemical application of PD_1 as a detergent, the features of PD_1 described are of interest and remain to be studied.

Acknowledgements. This work was supported by grants from the Swedish Research Council for Engineering Sciences, Royal Swedish Academy, and TEMPUS No. S_JEP-09270–95.

References

1. Skou, J. C. *FEBS Lett. 268* (1990) 314.
2. Lingrel, J. B. and Kuntzweiler, T. *J. Biol. Chem. 249* (1994) 19659.
3. Schoner, W., Thönges, D., Hamer, E., Antolovic, R., Buxbaum, E. and Willeke, M. In: Bamberg, E. and Schoner, W., Eds., *The Sodium Pump: Structure Mechanism, Hormonal Control and its Role in Desease*, Steinkopf, Darmstadt; Springer, New York 1994, p. 332.
4. Salum, T., Zilmer, M., Kullisaar, T., Vihalemm, T. and Tähepõld, L. *Ukr. Biochem. J. 60* (1988) 47.
5. Schafmeister, C. E., Miercke, L. J. W. and Stroud R. M. *Science 262* (1993) 734.
6. Langel, Ü., Land, T. and Bartfai, T. *Int. J. Peptide Protein Res. 39* (1992) 516.
7. Karelson, E., Zilmer, M. and Tähepõld, L. *Quest. Med. Chem. (Russ.) 31* (1985) 84.
8. Kairane, C., Zilmer, M., Mutt, V. and Sillard, R. *FEBS Lett. 345* (1994) 1.
9. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem. 193* (1951) 265.
10. Robinson, J. D. *Methods Enzymol. 156* (1988) 236.
11. Jørgensen, P. L. and Andersen, J. P. *Biochemistry 25* (1986) 2889.
12. Jørgensen, P. L. *Conformational Changes in the α-Subunit, and Cationic Transport by Na^+, K^+-ATPase*, Pitman, London 1983, p. 458.
13. Boldyrev, A. A. and Svinukhova, I. A. *7th Joint Symposium of the Biochemical Societies of the GDR and USSR*, Dresden 1983, p. 94.
14. Beggah, A. T., Beguin, P., Jaunin, P., Peitsch, M. C. and Geering, K. *Biochemistry 32* (1993) 14117.
15. Boldyrev, A. A. In: Krans, E. I., Ed. (Russ.), *Biomembranes and Transport of Ions*, Moscow University 1985, p. 207.

Received July 4, 1996.