

cDNA CLONING AND SEQUENCE DETERMINATION OF PIG GASTRIC
(H⁺ + K⁺)-ATPase

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SUMMARY --- Complementary DNA to pig gastric mRNA encoding (H⁺ + K⁺)-ATPase was cloned, and its amino acid sequence was deduced from the nucleotide sequence. The enzyme contained 1034 amino acid residues (Mr. 114,285) including the initiation methionine. The sequence of pig (H⁺ + K⁺)-ATPase was highly homologous with that of the corresponding enzyme from rat, but had high degree of synonymous codon changes. Potential sites of phosphorylation by cAMP-dependent protein kinase and N-linked glycosylation sites were identified. The amino terminal region contained a lysine-rich sequence similar to that of the α subunit of (Na⁺ + K⁺)-ATPase, although a cluster of glycine residues was inserted into the sequence of the (H⁺ + K⁺)-ATPase. As the pig enzyme is advantageous for biochemical studies, the information of the primary structure is useful for further detailed studies. © 1988 Academic Press, Inc.

INTRODUCTION --- The (H⁺ + K⁺)-ATPase of gastric parietal cell is located in its secretory canaliculi and catalyzes electroneutral exchange of internal H⁺ and external K⁺ coupled with ATP hydrolysis, maintaining a large pH difference of more than 5 between the cytoplasm and gastric lumen (1). Membrane vesicles enriched in this enzyme can be prepared from pig stomach easily and in large quantity (2,3), and the kinetic properties of the enzyme have been examined extensively (4-6). As the pig enzyme is advantageous for biochemical studies on coupled ion transport and its regulation, the information of the primary structure is prerequisite for further detailed studies. In this study, we cloned and sequenced cDNA coding for pig gastric (H⁺ + K⁺)-ATPase. From the deduced amino acid sequence, significance of the amino terminal region was discussed.

MATERIALS AND METHODS

Poly(A)⁺ mRNA was prepared from pig gastric mucosa. Double stranded cDNA was synthesized according to instructions in the manual of the cloning kit (Amersham) except that T₄ DNA polymerase treatment was omitted. The two 3'-ends of the double stranded cDNA were tailed with deoxycytidine, and PstI and SstI sites of pUC18 were tailed with deoxyguanosine using terminal deoxynucleotidyl transferase. They were then annealed and introduced into an *E. coli* strain C600 (7). A 36-base probe (⁵ TCCAAGTTCCGGATCTCATCATAGACAAAGATGAG³) corresponding to the sense strand of the 3'-terminal coding region of rat (H⁺ + K⁺)-ATPase (amino acid residues 1007-1018) (8) was 5'-end-labeled with T₄ polynucleotide kinase and used for colony hybridization (7). A hybridization-positive clone (pHK-16) encoding the carboxyl terminal half of pig gastric (H⁺ + K⁺)-ATPase was obtained from among 10⁴ clones. We found a sequence that was similar to that of the 36-base probe except for changes in two bases without change in the coded amino acid residues.

To obtain a cDNA clone carrying the amino-terminal region of the enzyme, cDNA was synthesized using a 30-base synthetic primer (⁵ CTGTTCCGGCACTTGAGCACGGCATCAGGAA³) corresponding to the sense strand of the 5'-terminal region (amino acid residues 609-619) of pHK-16. The second library (10⁴ clones) was probed with random-primed DNA using an equimolar mixture of 60- and 62-base synthetic oligonucleotides, (⁵ CAGTGCCTCATGTGGGTGGCTGCAGCCATCTGCCTCATTGCCTTTGC-GATTCAGGCCAGC³) and (⁵ AATGAGTGCCAACGCCAGGTACAAATTGTCATCAGTGGTCAG-GTCTCCCTCGCTGGCTGAA³) corresponding to the antisense and sense strands of the amino terminal region of rat (H⁺ + K⁺)-ATPase (amino acid residues 110 - 129 and 126 - 146, respectively) (8) as templates, and a positive clone (pHK-M10) carrying the amino terminal half of the enzyme was obtained. No differences were found in the nucleotides in the overlapping regions of the two clones. A plasmid was prepared (7) and most of both strands of cDNA (94.9 %) were sequenced by the chain-termination method (9) following the sequence strategy shown in Fig.1.

Terminal deoxynucleotidyl transferase and random-primed DNA labeling kit were obtained from International Biotechnologies Inc. and Boehringer, respectively. Other enzymes were purchased from Takara Shuzo Co., Kyoto, Japan. γ (³²P)-ATP (5000 Ci/mmol) and α (³²P)-dCTP (400 Ci/mmol and 3000 Ci/mmol) were products of the Radiochemical Centre, Amersham. All other chemicals used were of the highest grade commercially available.

RESULTS AND DISCUSSION

DNA sequencing of two hybridization-positive clones, pHK-16 and pHK-M10 demonstrated that they contained the cDNAs coding carboxyl and amino terminal regions of pig (H⁺ + K⁺)-ATPase, respectively (Fig.1). Fig.2 shows the nucleotide sequence of cDNA encoding (H⁺ + K⁺)-ATPase determined using clones pHK-16 and pHK-M10. The translational initiation site was assigned to the Met-1 codon, as the sequence around this codon (GCACCATGG) is consistent with the consensus sequence for eukaryotic initiator sites (10). A termination codon (TAG) was found next to the Tyr-1034 codon. The primary translation product consists of

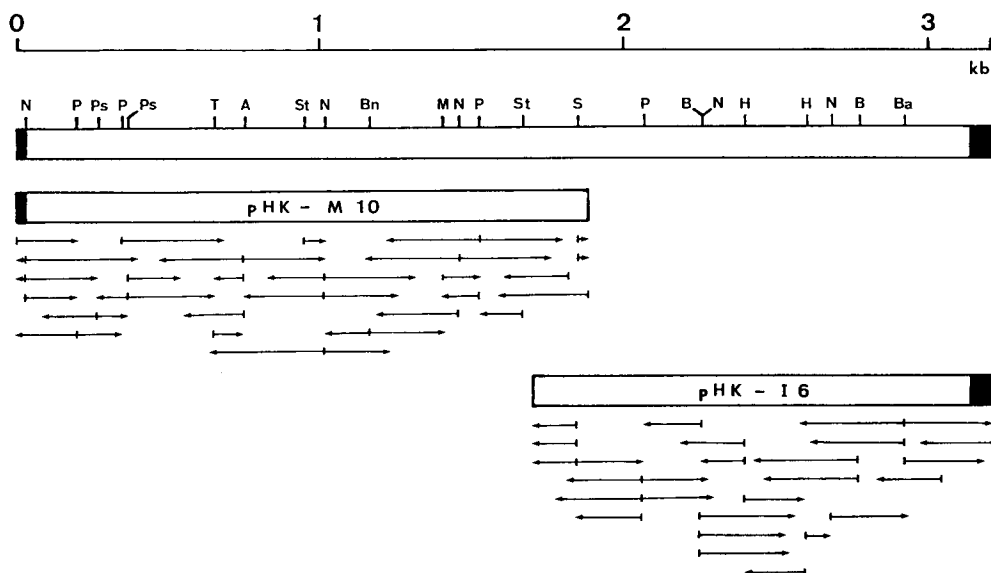


Fig.1. Restriction map and sequencing strategy for pig gastric (H⁺ + K⁺)-ATPase clones. The restriction map is a composite of those of the cDNAs whose sequences were determined. The restriction sites relevant to the sequencing strategy are shown: A, ApaLI; B, BalI; Ba, BamHI; Bn, BanII; H, HinCI; M, MluI; N, NcoI; P, PvuII; Ps, PstI; S, SmaI; St, StuI; T, TaqI. Open areas represent the reading frames, and darkened areas represent untranslated regions. The directions and extents of sequencing are shown by arrows.

1034 amino acids and the molecular weight of the protein, including the initiator methionine, was calculated to be 114,285. We identified the sequences of peptides reported previously: the amino terminal sequence at positions 3 to 18 (11), the region around the phosphorylation site at positions 384 to 387 (CSDK) (12) and the region around the fluorescein isothiocyanate-binding site at positions 515 to 522 (LVMKGAPE) (13).

Alignment of the nucleotide sequences of pig and rat (H⁺ + K⁺)-ATPases indicated 89.1 % homology in the coding region, 60.0 % homology in the 5'-noncoding region and 44.3 % homology in the 3'-noncoding region. The amino acid sequence homology between the two enzymes was 97.6 % (identical residues) with 28.9 % synonymous codon changes for amino acid residues. The 4th amino acid residue (alanine) from the initiator methionine is not present in the rat enzyme. In addition, 24 other residues of the two enzymes are different. Of these, 16 residues are scored as favored substitutions (14). Substitutions are concentrated

-33
TCTCAGCAAAGGAGGGTGGCTGCACTGGGCAAC

-1

10 20 30 40 50 60 70 80 90 100 110 120
ATGGGGAAGCGGAGAAATTATGAGCTGTACCAAGTAGAGCTGGTCTGCTAGTGGGGACATGGCAGCCAAGATGAGCAAGAAGGCGGCGGTGGTGGGGCAAGAGGAAGGAG
M G K A E N Y E L Y Q V E L G P G P S G D M A A K N S K K K A G R G G G K R K E

130 140 150 160 170 180 190 200 210 220 230 240
AAGCTGGAGAATCAAGCAAGGAGATGGAGATTAATGACCACCAAGCTGCTGGCAGAGCTGGAACAGAAATACCAGAGCAGTGCACCAAGGCGCTGTCTGCCAGCCTGGCTGCAGAG
K L E N M K K E M E I N D H Q L S V A E L E Q K Y Q T S A T K G L S A S L A A E

250 260 270 280 290 300 310 320 330 340 350 360
CTGCTGCTCGGGGATGGGCGCAAGCGGTGAGGCGCCACGGGGCACCCTGAGTACGCTCAAGTTCGCAAGGCAGCTGGGCGGCGTCTGCAGTGCCTCATGTGGGTACGAGCGGCATC
L L L R D G P N A L R P P R G T P E Y V K F A R Q L A G G L Q C L M W V A A A I

370 380 390 400 410 420 430 440 450 460 470 480
TGCCTCATTTGCCCTTTGGCATCCAGCGCCAGCGGCGACCTTACCAGCGACCAATCTATACCTGGCCTGCGCTCATCGCTGTGGTGTGGTACGAGCGGCATT
C L I A F A I Q A S E G D L T T D D N L Y L A L A L I A V V V Y T G C F G Y Y Q

490 500 510 520 530 540 550 560 570 580 590 600
GAGTTAAGACCAACATCATCGCCGCTTCAAGAACCTTGCTGCCAGCAAGCGCTGTATCGATGGGACAAGTTCAGATCAATCGACGCTCGTGGTACGAGCGGCATT
E F K S T N I I A S F K N L V P Q Q A T V I R D G D K F Q I N A D Q L V Y G D L

610 620 630 640 650 660 670 680 690 700 710 720
GTGGAGATGAAAGCGGGGATCGAGTGGCAGCGACATCCGCTCCTCAGGCGCCAGGGCGCAAGGTGGACAACCTCTCGCTGACCGGAGAGTGGAGCGCGAGACCGGCTCACCGGAG
V E M K G G D R V P A D I R I L Q A Q G R K V D N S S L T G E S E P Q T R S P E

730 740 750 760 770 780 790 800 810 820 830 840
TGCACACGAGAGCGCGGTGGAGCGCAACATCGCCTTCTTCCACCATGTGCTCGAGGGCGACCGGCGGCTGGTGGTGAACACCGGCGCGCACCATCATCGGCGGCATT
C T H E S P L E T R N I A F F S T M C L E G T A Q G L V V N T G D R T I I G R I 2

850 860 870 880 890 900 910 920 930 940 950 960
CGCTCCCTGGCGGTGAGAAACGAGAGCGGCCATCGCTATCGAAATCGAACACTTTGTGGACATCATCGAGCGCTGGCCATCTCTTTGGTGGCCAGCTTTTCATTGTGGCC
A S L A S G Y E N E K T P I A I E I E H F V D I A G L A I L F G A T F F I Y A 31

970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
ATGTGCATCGGTACACCTTCTCGCGGCGCATGGTCTTCTTCATGGCCATCGTGGTAGCCTATGTGCTGAGGGGCTGCTGGCCAGGTCACGGTGTGCTGTCCCTGACAGCGCAAGCGG
M C I G Y T F L R A M V F F M A I V V A Y V P E G L L A T Y T V C L S L T A K R 34

1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
CTGGCCAGCAAGAACTGTGTGTGAAGAAGCTGGAAGCGGTGGAGACACTGGGCTCCAGCTGATCTGCTGTGACAAGACGGGAGCCCTCACTCAGAATCGCATGCTGTGCCAC
L A S K N C V V K N L E A V E T L G S T S V I C S D K T G T L T Q N R M T V S H 40

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
CTGTGGTTCGACAACCACTCCGCTCGCTGACACTACAGAAGACCAAGTCCAGGGCAGACATTTGACCAAGTCTCAGAGACGTGGCGGGGGCTGTGCCGCTGTCCCTTCGCAACCGG
L W F D N H I H S A D T T E D Q S G Q T F D Q S S E T W R A L C R V Y L T L C N R 44

1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
GCTGCTTCAAGTGGGCGAGGACGGGTGCTGTGGCAAGCGCATCGTGTATCGGACACCGCTCCGAGACGGCGCTCAAGTCTCGAGCTGACGCTGGGCAATGCCATGGGCTAC
A A F K S G Q D A V P V P K R I V I G D A S E T A L L K F S E L T L G N A M G Y 48

1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
CGTGAGCGCTTCCCAAGTCTGCGAGATCCCTTCACTCCACCAACAGTTCAGCTGTCCATCCACACTGGAGGACCTCCGGACCCGAGGACGCTGTGTGTGATGAAGGGCGCC
R E R F P K V C E I P F N S T N K F Q L S I H T L E D P R D P R H V L V M K G A 52

1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
CCCGAGCGCTCTGGAGCGCTGAGCTCCATCTCATCAAGGGCCAGGAGCTGCCACTGGATGAGCAATGGCGGAGGCTTCCAGACTGCCTACCTCAGCCTGGAGGCGCTCGGAGAG
P E R V L E R C S S I L I K G Q E L P L D E Q W R E A F Q T A Y L S L G G L G E 56

1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
CGGGTCTGGGCTTCTGCCAGCTCTACCTGAGTGAAGGACTACCGGCTGGCTATGCTTCGACGTGGAGGCGCATGAACCTTCCAACAGTGGCGCTGTGCTGCGGAGCTCGTATCC
R V L G F C Q L Y L S E K D Y P P G Y A F D V E A M N F P T S G L S F A G L V S 60

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
ATGATAGACCCACCCCGGCGCCCGTTCTGATGCGGTGCTCAAGTGGCGAAGCAGCGCATCGGGGTGATCATGGTGACAGGTGACACCCCATGACAGCGCAAGGCCATTGCGCCAGT
M I D P P R A T V P D A V L K C R T A G I R V I M V T G D H P I T A K A I A S 64

Fig.2. Nucleotide sequence of cDNA for pig gastric (H^+ + K^+)-ATPase and its deduced amino acid sequence. The nucleotide sequence was determined using clones pHK-M10 (amino acid residues 1-617) and pHK-16 (residues 557-1034). Nucleotides are numbered above the DNA sequence starting from the first letter of the initiation methionine, and numbers on the right of each line are those of amino acid residues starting from Met-1. Asp-386 (phosphorylated during the catalytic cycle) and Lys-518 (fluorescein isothiocyanate-binding site) are indicated by arrow heads. The consensus sequences of the N-glycosylation site and phosphorylation site by cAMP-dependent protein kinase are indicated by and , respectively. The amino acid sequences determined by protein chemical analysis are underlined. The lines above the nucleotide sequence correspond to the sequence of synthetic oligo-nucleotides described in the Materials and Methods.

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1930      1940      1950      1960      1970      1980      1990      2000      2010      2020      2030      2040
GGCATCATCTCGGAAGGCAGGACAGTGGAGGACATTCAGACGCCCGCTCGGTGTGGCCGTGGACAGGTTAATCGGAAGGATGCCCGAGCGTGGTGATCAACGGCATGCAGCTG
G I I S E G S E T V E D I A A R L R V P V D Q V N R K D A R A C Y I N G M Q L 580

2050      2060      2070      2080      2090      2100      2110      2120      2130      2140      2150      2160
GACATGGACCCATCAGAGCTGGTGAAGCCCTGCGTACCCACCTGAGATGGTCTTTGCTCGCACCGATCCCCAGCAGAAGCTGGTGATTGTGGAGAGCTGCCAGCGACTGGGTGCA
D N D P S E L V E A L R T H P E M V F A R T S P Q Q K L Y I V E S C Q R L G A 720

2170      2180      2190      2200      2210      2220      2230      2240      2250      2260      2270      2280
EGTGGCTGTGACAGGGGATGGTGTGAATGACTCCCCAGCCCTGAAGAAGGCAGACATCGGTGTGGCCATGGGTATTGCTGGCTCGGATGCTGCCAAAATGCAGCCGACATGATCCTG
V A V T G D G V N D S P A L K K A D I G V A M G I A G S D A A K N A A D M I L 760

2290      2300      2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
GATGACAACCTTCGCTCCATTGTGACTGGCGTGGAGCAGGCGCGACTGATCTTTGACAACTTGAAAAAGTCCATCGCTACACGTTGACCAAGAACATCCCTGAATTGACGCCCTAC
D D N F A S I V T G V E Q G R L I F D N L K K S I A Y T L T K N I P E L T P Y 800

2410      2420      2430      2440      2450      2460      2470      2480      2490      2500      2510      2520
ATTACATCACCGTCAGCGTGGCTGCGCTGCGCTGGGTGCATCACCATCCTCTTCATTGAACCTGCGACTGACATCTTTCCATCTGTGTCCCTGGCATATGAGAAGCCGAGAGTGAC
I Y I T V S V P L P L G C I T I L F I E L C T D I F P S V S L A Y E K A E S D 840

2530      2540      2550      2560      2570      2580      2590      2600      2610      2620      2630      2640
ATCACCTGCGCGCGCGGAATCCAAAGCGTGACCGTTGGTCAACGAGCCCTGGCTGCTACTCTCTACTCCAGATCGGTGCCATCCAGTCATTTCGCTGGGTTCAGTGACTACTTC
M H L R P R N P X R D R L V N E P L A A Y S Y F Q I G A I Q S F A G F T D Y F 880

2650      2660      2670      2680      2690      2700      2710      2720      2730      2740      2750      2760
GCCATGGCCAGGAGGGCTGGTTCCTGCTGTGTGGGCTGCGGCCACAGTGGGAGAACCACACCTACAAGACCTGCAGGACAGCTATGGCCAGGAGTGGACGTTTGGGCGAG
A M A Q E G W F P L L C V G L R P Q W E N H H L Q D L Q D S Y G Q E W T F G Q 920

2770      2780      2790      2800      2810      2820      2830      2840      2850      2860      2870      2880
CTGTACCAAGCAGTACACCTGCTACACTGTGTTCTTCATCAGCATCGAGATGTGGCAGATTGCTGACGTCTCTATCCGCAAGACCGCCGCTCTCAGCCTTCAGCAGGGGCTTCTTC
L Y Q Q Y T C Y T V F F I S I E M C Q I A D V L I R K T R R L S A F Q Q G F F 960

2890      2900      2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
AACAGGATCCTGGTGATCGCCATCGTTCCAGGTCTGCATCGGCTGCTTCCTGTGCTACTGCCCGGGATGCCAACATCTTCAACTTCATGCCCAITTCGGTTCAGTGGTGGCTG
N R I L V I A I V F Q V C I G C F L C Y C P G M P N I F N F M P I R F Q W W L 1000

3010      3020      3030      3040      3050      3060      3070      3080      3090      3100      3110      3120
CCCATGCCCTTTGGCTCCTGCTATGCTCTATGATGAGATCAGGAACTTGGAGTTTCGCTGTGCCCCAGGGAGCTGGTGGGACCAGGAACCTACTATTAGAGGGACCATTGCTG
P M P F G L L I F V Y D E I R K L G V R C C P G S W W D Q E L Y Y * 1034

3130      3140      3150      3160      3170
GCCATCCTTGCATACACCACAGTGGGGGGCAGGGACACACGGAACCCCTGGG

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Fig. 2 - Continued.

in two regions (positions 1 to 75 and positions 570 to 600), in which 9 and 4 residues, respectively, are changed. In the α subunit of the closely related $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, 63.0 % of the amino acid residues are identical with those of pig $(\text{H}^+ + \text{K}^+)\text{-ATPase}$ (15).

In the deduced amino acid sequence, consensus sequences for sites phosphorylated by cAMP-dependent protein kinase (KRXXS and RRXS) (16) were found at residues 359 to 363 (KRLAS) and residues 950 to 953 (RRLS), respectively. As histamine activates cAMP-dependent protein kinase, which is involved in the control of acid secretion from parietal cell (17), it will be interesting to know whether the enzyme activity is modulated by phosphorylation. Three Asn residues (Asn-225, 493, and 730) similar to known N-glycosylation sites (NX(S/T)) (18) were also found.

Gastric ($H^+ + K^+$)-ATPase is classified into cation transporting ATPases, such as ($Na^+ + K^+$)-, Ca^{2+} -, K^+ - and H^+ -ATPases (19). The primary structures of these enzymes are highly homologous, and conserved regions may be essential for common catalytic steps, such as ATP-binding, phosphorylation and dephosphorylation of conserved aspartic acid residue (Asp-386), and transmission of energy to cation transport. On the other hand, the cation selectivity may be determined by less homologous regions. ($H^+ + K^+$)-ATPases have a lysine-rich consensus sequence similar to that of the α subunits of ($Na^+ + K^+$)-ATPase in their amino terminal region (15, 20-25), $K_{1-2}X_{0-2}K_{1-3}X(GXGGG)(K/R)_{1-3}X(K/R)_{1-2}X_{1-4}Z_2XK_2E$ where Z is E, D or N, although a cluster of glycine residues (GXGGG) is inserted in the middle of the sequence of ($H^+ + K^+$)-ATPase (Fig.3). Since this lysine-rich sequence is not found in other related cation transporting ATPases (19), it may have a common regulatory role(s) in cation binding and its occlusion by ($H^+ + K^+$)- and ($Na^+ + K^+$)-ATPases. The cluster of glycine residues found only in ($H^+ + K^+$)-ATPase may determine the enzyme specificity for H^+ .

		1	10	20	30	40	50		
H ⁺ /K ⁺	Pig	MG	KAENY	ELYQVE	LGPGPSGDM	AAK	MSKKKAGRGGGKKRKEK	LENMKKEME	
	Rat	MG	K ENY	ELYSVE	LGTGPGGDM	AAK	MSKKKAGGGGKKRKEK	LENMKKEME	
Na ⁺ /K ⁺	Pig	MG	KGVGR	DKY	EPAAVSEHGD	K	KKA	KKER	DMDELKKEVS
	Sheep	MG	KGVGR	DKY	EPAAVSEHGD	K	KKA	KKER	DMDELKKEVS
	Chicken	MG	KGAGR	DKY	EPTATSEHGT	K	KKKA	KER	DMDELKKEIS
	HeLa	MG	KGVGR	DKY	EPAAVSEQGD	KK	G KKG	KKDR	DMDELKKEVS
	Rat α	MG	KGVGR	DKY	EPAAVSEHGD	KK	S KKA	KKER	DMDELKKEVS
	α (+)	MG	RGAGR	E Y	SPAATTAENG	G K	KKQ	KEK	ELDELKKEVA
	α III	MGDK	KDDK	SSP		KK	S KA	KERR	DLDDLKKEVA
Torpedo	MG	KGAAS	EKY	QPAATSEN	AK	NS KKS	KSK	TTDLDELKKEVS	
Artemia					AK	KKQ	KKGK	DLNELKKELD	

Fig.3. Comparison of the N-terminal amino acid sequences of ($H^+ + K^+$)-ATPases from pig and rat and those of ($Na^+ + K^+$)-ATPase α -subunits from various species. The sequences of ($H^+ + K^+$)-ATPases shown are for pig (this study) and rat (8), and those of ($Na^+ + K^+$)-ATPase are for pig kidney α (15), sheep kidney α (20), chicken α (21), HeLa cells α (22), rat α , $\alpha(+)$, αIII (23), *Torpedo californica* electric organ α (24), and the brine shrimp *Artemia salina* (25). The residue numbers are based on the sequence for pig ($H^+ + K^+$)-ATPase. The first five amino acids of rat, sheep, and pig α and rat $\alpha(+)$ subunits are known to be removed by post-translational processing (15,20,23). Basic amino acid residues are shown in boldface. The line was drawn above the lysine-rich sequence.

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REFERENCES

1. Faller, L., Jackson, R., Malinowska, D., Mukidjam, E., Rabon, E., Saccomani, G., Sachs, G. and Smolka, A. (1982) *Ann. N. Y. Acad. Sci.* **402**, 146-163.
2. Sachs, G., Chang, H. H., Rabon, E., Schackman, R., Lewin, M. and Saccomani, G. (1976) *J. Biol. Chem.* **251**, 7690-7698.
3. Maeda, M., Tagaya, M. and Futai, M. (1988) *J. Biol. Chem.* **263**, 3652-3656.
4. Wallmark, B., Stewart, H. B., Rabon, E., Saccomani, G. and Sachs, G. (1980) *J. Biol. Chem.* **255**, 5313-5319.
5. Stewart, B., Wallmark, B. and Sachs, G. (1981) *J. Biol. Chem.* **256**, 2682-2690.
6. Rabon, E. C., McFall, T. L. and Sachs, G. (1982) *J. Biol. Chem.* **257**, 6296-6299.
7. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
8. Shull, G. E. and Lingrel, J. B. (1986) *J. Biol. Chem.* **261**, 16788-16791.
9. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. and Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161-178.
10. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857-872.
11. Lane, L. K., Kirley, T. L. and Ball, W. J. Jr. (1986) *Biochem. Biophys. Res. Commun.* **138**, 185-192.
12. Walderhaug, M. O., Post, R. L., Saccomani, G., Leonard, R. T. and Briskin, D. P. (1985) *J. Biol. Chem.* **260**, 3852-3859.
13. Farley, R. A. and Faller, L. D. (1985) *J. Biol. Chem.* **260**, 3899-3901.
14. Dayhoff, M. O., Schwartz, R. M. and Orcutt, B. C. (1978) *Atlas of Protein Sequence and Structure* (Dayhoff, M. O. ed.) Vol.5, Suppl.3, pp.345-352, (National Biomedical Research Foundation, Silver Spring, Md.).
15. Ovchinnikov, Y. A., Modyanov, N. N., Broude, N. E., Petrukhin, K. E., Grishin, A. V., Arzamazova, N. M., Aldanova, N. A., Monastyrskaya, G. S. and Sverdlov, E. D. (1986) *FEBS Lett.* **201**, 237-245.
16. Krebs, E. G. and Beavo, J. A. (1979) *Ann. Rev. Biochem.* **48**, 923-959.
17. Chew, C. S. (1985) *J. Biol. Chem.* **260**, 7540-7550.
18. Hubbard, S. C. and Ivatt, R. J. (1981) *Ann. Rev. Biochem.* **50**, 555-583.
19. Serrano, R. (1988) *Biochim. Biophys. Acta* **947**, 1-28.
20. Shull, G. E., Schwartz, A. and Lingrel, J. B. (1985) *Nature* **316**, 691-695.
21. Takeyasu, K., Tamkun, M. M., Renaud, K. J. and Fambrough, D. M. (1988) *J. Biol. Chem.* **263**, 4347-4354.
22. Kawakami, K., Ohta, T., Nojima, H. and Nagano, K. (1986) *J. Biochem. (Tokyo)* **100**, 389-397.
23. Shull, G. E., Greeb, J. and Lingrel, J. B. (1986) *Biochemistry* **25**, 8125-8132.
24. Kawakami, K., Noguchi, S., Noda, M., Takahashi, H., Ohta, T., Kawamura, M., Nojima, H., Nagano, K., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. and Numa, S. (1985) *Nature* **316**, 733-736.
25. Morohashi, M. and Kawamura, M. (1984) *J. Biol. Chem.* **259**, 14928-14934.