When the gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase was solubilized by n-dodecyl β-maltoside and electrophoresed in blue native-polyacrylamide gels (BN-PAGE), one major band at about 360 kDa was observed. Since this band was recognized by both monoclonal antibodies 1218 (anti-α) and wheat germ agglutinin (anti-β), the H\textsuperscript{+},K\textsuperscript{+}-ATPase in its native state exists in a dimeric (αβ)\textsubscript{2} form. The site of interaction between the heterodimers was determined using Cu\textsuperscript{2+}-phenanthroline cross-linking. The Cu\textsuperscript{2+}-phenanthroline reagent reacted with the H\textsuperscript{+},K\textsuperscript{+}-ATPase to produce an αβ-dimer with inhibited H\textsuperscript{+},K\textsuperscript{+}-ATPase activity. This cross-linking and enzyme inhibition were prevented by ATP. Cross-linking followed by N-ethylmaleimide blockade of maleimide-reactive SH groups, then reduction and fluorescein 5-maleimide labeling, defined a single fluorescent tryptic peptide of about 6.5 kDa that had been cross-linked. Since its N-terminal amino acid is Val\textsuperscript{561}, the peptide probably ends at Arg\textsuperscript{566} or Arg\textsuperscript{621} and/or Cys\textsuperscript{565} and/or Cys\textsuperscript{615} are probably within the region of closest contact between the two α-subunits.

The gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase is a member of the ion-motive, phosphorylating P-type ATPases which include, in mammals, the Na\textsuperscript{+},K\textsuperscript{+},Ca\textsuperscript{2+}, and H\textsuperscript{+},K\textsuperscript{+}-ATPases. This gastric enzyme catalyzes an electroneutral exchange of extracytoplasmic K\textsuperscript{+} for cytoplasmic H\textsubscript{2}O\textsuperscript{−} (1). The gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase consists of two subunits, a catalytic α-subunit and a heavily glycosylated β-subunit (2). The catalytic α-subunit, a 114-kDa protein (3–5), contains the sites of nucleotide binding and phosphorylation (6) and runs at a relative molecular mass of 94–100 kDa in reducing SDS-PAGE.\textsuperscript{1} The β-subunit (7, 8), which has a relative molecular mass of about 60–80 kDa in SDS-PAGE, has a core protein of 34 kDa (9). Using lectin-affinity chromatography, the solubilized H\textsuperscript{+},K\textsuperscript{+}-ATPase α-subunit co-purified with the β-subunit, and the luminal loop between the membrane-spanning segments TM7 and TM8 of the H\textsuperscript{+},K\textsuperscript{+}-ATPase α-subunit was shown to be a site of strong association with the β-subunit (10–12).

The radiation inactivation target size of the gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase was 444 ± 10 kDa in the absence and 371 ± 9 kDa in the presence of Mg\textsuperscript{2+}, whereas H\textsuperscript{+} transport in reconstituted proteoliposomes was shown to have a mean target size of 388 ± 48 kDa (13–15). Two-dimensional crystallization of membrane-bound H\textsuperscript{+},K\textsuperscript{+}-ATPase showed that the unit cell dimension was 115.1 Å with p4 projection symmetry, suggesting strong protein-protein interaction between (αβ)\textsubscript{2} protomers (16). These studies suggest the H\textsuperscript{+},K\textsuperscript{+}-ATPase is likely to exist as a dimeric (αβ)\textsubscript{2}-heterodimer in the original membrane.

To obtain specific information about the organization of the ATPase, blue native (BN)-gel electrophoresis was used to identify its oligomeric nature. Disulfide cross-linking using Cu\textsuperscript{2+}-phenanthroline was followed by identification of the regions of cross-linking by fluorescein maleimide (F-MI) labeling following NEM protection of maleimide-accessible cysteines.

**EXPERIMENTAL PROCEDURES**

Enzyme Preparation—The H\textsuperscript{+},K\textsuperscript{+}-ATPase was derived from hog gastric mucosa by previously published methods, which involve differential and density gradient centrifugation (9). The vesicles obtained are composed of about 85% ATPase protein and are 90% cytoplasmic side out. The ion impermeability of the vesicles was determined by the increase in K\textsuperscript{+} stimulation of ATPase activity in the presence of KCl due to the ionophore, nigericin. The specific activity in the presence of nigericin was 120 μmol of ATP hydrolyzed mg\textsuperscript{−1} protein h\textsuperscript{−1}, and in the absence of nigericin, 10 μmol mg\textsuperscript{−1} h\textsuperscript{−1}. P\textsubscript{i} release was measured by the method of Yoda and Hokkin (17) and protein concentration by the Lowry (18) method. BN-polyacrylamide gel electrophoresis (PAGE) of solubilized enzyme BN-PAGE was carried out according to the method of Schägger and von Jagow (19) with slight modifications. 1 mg of gastric vesicles containing the H\textsuperscript{+},K\textsuperscript{+}-ATPase were resuspended in 0.3 ml of a solution composed of 1% n-dodecyl β-maltoside, 50 mM Tris/HCl, pH 7.0, and kept on ice for 10 min. The suspension was centrifuged at 110,000 × g for 15 min at 4 °C. About 70% of the ATPas was solubilized by this procedure. 60 μl of sample buffer (5% Coomassie Blue G-250, 0.5 M 6-aminon-capric acid) and 150 μl of a buffer solution (1.5 M 6-aminon-ca ric acid, 0.15 M Bis-Tris/HCl, pH 7.0) were added to the supernatant (300 μl) containing 0.7 mg of H\textsuperscript{+},K\textsuperscript{+}-ATPase protein. A 70-μl sample of this solution was placed on top of a 6% (34:1 acrylamide:dimethylacrylamide) gel from 13% (34:1 acrylamide:methylene bisacrylamide) 1.5-mm thick gradient slab gel, using BN-polyacrylamide gel containing 0.01% n-dodecyl β-maltoside, prepared by the method of Schägger and von Jagow (19). The cathode buffer consisted of 50 mM Tricine, 15 mM Bis-Tris, pH 7.0, and 0.02% Coomassie Blue G-250, and the anode buffer consisted of 50 mM Bis-Tris/HCl, pH 7.0. The gel was run for 2 h at 100 V constant voltage and then at 200 V constant voltage for 6 h. Two lanes were run for standard molecular mass markers (one lane for jack bean urease to give 545- and 272-kDa molecular mass markers, the other lane for bovine serum albumin giving 132- and 66-kDa molecular mass markers). The gel was destained in a solution composed of 30% methanol and 10% acetic acid. Prior to transfer to PVDF, the gel was washed for 2 h in a buffer composed of 0.1 M boric acid/NaOH, pH 8.8, 0.12% SDS with several changes of buffer. Proteins of this Coomassie Blue-stained gel were transferred to a PVDF membrane using a solution of 10% methanol, 180 mM glycine, 25 mM Tris buffer.

Western Analysis—Western immunoblotting was carried out by pre-

---

*This work was supported in part by United States Veterans Administration Senior Medical Investigator Award and National Institutes of Health Grants DK40615 and 41301. Sequencing of the peptides was supported by National Institutes of Health BRS Shared Instrumentation Grant 1S00RR0555-01 to UCLA. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: CURE, Bldg. 113, Rm. 324, 11301 Wilshire Blvd., Los Angeles, CA 90073. Tel.: 310-268-4672; Fax: 310-321-9478.

1The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; BN, blue native; WGA, wheat germ agglutinin; Cu-phenanthroline complex; NEM, N-ethylmaleimide; F-MI, fluorescein 5-maleimide; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol; Tricine, N-2-hydroxy-1,1-bis-(hydroxyethyl)glycine; PVDF, poly(vinylidene fluoride); DTT, dithiothreitol; mAb, monoclonal antibody; CDTA, trans-1,2-diamino-cyclohexane-N,N,N′,N′-tetraacetic acid.
Cu$^{2+}$ Cross-linking of the H$^+\cdot$K$^+$-ATPase

Previously described methods (9, 20). A biotinylated WGA was used for detecting the $\beta$-subunit of the H$^+\cdot$K$^+$-ATPase (9), and mAb 1218 was used for detecting the $\alpha$-subunit of the H$^+\cdot$K$^+$-ATPase (20).

Effects of Ligands—For examining ligand effects on functional organization of the H$^+\cdot$K$^+$-ATPase, the H$^+\cdot$K$^+$-ATPase was treated with 3 mM ATP, ADP, or MgCl$_2$, or 0.3 mM EDTA, solubilized and then separated on BN-PAGE as described above.

Reaction of Cu$^{2+}$-Phanenthroline with the Gastric H$^+\cdot$K$^+$-ATPase—A solution of Cu$^{2+}$-phenanthroline was prepared freshly before use. 0.1 ml of an aqueous solution of 20 mM CuSO$_4$ was added to an equal volume of 40 mM 1,10-phenanthroline in N,N-dimethylformamide to provide 10 mM of Cu$^{2+}$-phenanthroline complex (0.2 ml), which was kept on ice. The solution of H$^+\cdot$K$^+$-ATPase was suspended in 50 mM Tris/HCl, pH 7.4, at a protein concentration of 1 mg/ml. A 200-$\mu$l aliquot of the gastric H$^+\cdot$K$^+$-ATPase was treated with 2 $\mu$l of 10 mM Cu-phenanthroline complex and kept for 30 min on ice. The reaction was stopped by addition of 2 $\mu$l of 0.5 M NEM and 1 $\mu$l of 0.2 M EDTA on ice. The suspension was centrifuged at 110,000 $\times$ g for 45 min to remove excess Cu-phenanthroline complex and other reagents. The pellet was resuspended in 50 mM Tris/HCl, pH 7.0, at a protein concentration of 1 mg/ml. The effects of ligands on the reaction were examined by addition of the cross-linking reagent to enzyme solution containing individually 2 mM ATP, ADP, Mg$^{2+}$, 200 mM KCl, 10 mM EDTA, or 1 mM HVO$_2^-$. The gastric H$^+\cdot$K$^+$-ATPase was also solubilized with Nonidet P-40 as described previously (10, 12) and cross-linked. 1 $\mu$l of the enzyme was solubilized with 0.5 ml of 0.2% Nonidet P-40, 50 mM Tris/HCl, pH 7.4, and centrifuged at 110,000 $\times$ g for 15 min. The supernatant was reacted with 0.1 ml Mg-phenanthroline for 30 min on ice. Reaction with Cu-phenanthroline was stopped by adding 10 mM NEM. The reaction mixture was run on 5% Tricine gel after adding 40% sample buffer.

Inhibition of ATPase—An aliquot of gastric H$^+\cdot$K$^+$-ATPase at 1 mg/ml was treated with Cu-phenanthroline at concentrations ranging from 0.1 to 100 $\mu$M on ice for 30 min. The reaction was stopped by centrifugation without NEM treatment, since NEM can inhibit the ATPase activity. After the reaction suspension was centrifuged, the pellet was resuspended in 50 mM Tris/HCl, pH 7.0, at a protein concentration of 1 mg/ml, and ATPase activity was measured by the method of Yonetani and Tomlin (37). In a typical experiment, 10 $\mu$l of the H$^+\cdot$K$^+$-ATPase were pre-reacted with 100 $\mu$M Cu-phenanthroline on ice, as described above, and then incubated at 37 $\degree$C for 10 min in 1 ml of buffer composed of 2 mM Mg-ATP, 2$\mu$g of nigericin, and 50 mM Tris/HCl (pH 7.0) with or without 0.1 mM KCl, and the K$^+$-stimulated activity was determined. In other experiments, various ligands such as 2 mM ATP, ADP, or Mg$^{2+}$, or 0.3 mM EDTA were present during the cross-linking reaction.

SDS-Gel Electrophoresis—The SDS-solubilized protein or peptide fragments were combined with 20% volume of sample buffer (0.3 ml Tris/HCl, pH 7.4, 10% SDS, 5% glycerol, and 0.25% bromphenol blue) and the solution placed on top of a 10% (34:1 acetamide/methylene bisacrylamide) to 21% (17:1 acetamide/methylene bisacrylamide) gradient gel. After polymerization using the Tricine buffer method of Schägger and von Jagow (21). The gel was run for 20-24 h at 48 mA constant current, along with a lane for prestained molecular mass standards (Bio-Rad, 106–16 kDa) and CNBr fragments of horse myoglobin (Sigma, 17-2.5 kDa).

Alternatively the proteins after reaction with the Cu-phenanthroline complex were dissolved in 0.2% SDS, 1 mM NEM, 50 mM Tris/HCl, pH 7.0, at a protein concentration of 1 mg/ml, and combined with a 30% volume of sample buffer (0.3 ml Tris/HCl, pH 7.4, 10% SDS, 50% sucrose, and 0.025% bromphenol blue). The samples were loaded on top of a 5-15% gradient SDS-gel prepared as described above and run at 45 mA constant until the dye reached the bottom of the gel. We obtained a migration pattern of the proteins such as the $\alpha$-$\alpha$-dimer at about 190 kDa, the $\alpha$-subunit at about 95 kDa, and the $\beta$-subunit at about 60–95 kDa in the 5-15% gradient SDS gel. For clearer separation of the $\alpha$-$\alpha$-dimer from monomer, we used a 5% Tricine gel. In this 5% homogeneous Tricine gel, we observed that the $\beta$-subunit migrates more slowly than the $\alpha$-subunit, while the dimer migrates at 190 kDa as expected.

The gel slices were stained by 0.1% Coomassie Blue in 45% methanol and 10% acetic acid or electrotransferred to PVDF membranes as described previously (12).

F-MI Identification of Site of Cross-linking—The gastric H$^+\cdot$K$^+$-ATPase that had been cross-linked with Cu-phenanthroline (1 mg/ml protein in 50 mM Tris/HCl, pH 7.0) was treated with 10 mM NEM at room temperature for 20 min for extensive blocking of free thiol groups and centrifuged. The pellet was resuspended in 0.1 M Tris/HCl, pH 8.2, 0.1 M DTT, at a protein concentration of 1 mg/ml and incubated at room temperature for 30 min. The suspension was centrifuged at 110,000 $\times$ g for 45 min to remove excess DTT. The pellet was resuspended in 50 mM Tris/HCl, pH 7.0, buffer at a protein concentration of 1 mg/ml, and centrifuged at 110,000 $\times$ g for 45 min again. The pellet was resuspended in 50 mM Tris/HCl, pH 7.0, and 0.1 mM F-MI. The suspension was incubated at room temperature for 10 min, centrifuged, and then resuspended in 50 mM Tris/HCl, pH 8.2, at a protein concentration of 1 mg/ml. If no reducing agent was added, labeling by F-MI of the NEM-reacted cross-linked enzyme was minimal, showing that N-ethylmaleimide was able to react with essentially all the cysteines that could react with fluorescein maleimide.

Trypsin Digestion of F-MI-labeled Gastric H$^+\cdot$K$^+$-ATPase—The gastric H$^+\cdot$K$^+$-ATPase labeled with F-MI was digested at 37 $\degree$C for 1 h with trypsin (1/50 ratio of protein to trypsin). The suspension was centrifuged at 110,000 $\times$ g for 45 min. The supernatant containing the cytoplasmic fragments was run on Tricine gradient SDS-PAGE. The pellet was resuspended in 1% SDS, 50 mM Tris/HCl, pH 7.0, and ice and boiled for 2 min. The pellet portion was also run on Tricine gradient SDS-PAGE.

Sequencing—Peptide bands were sequenced with a gas phase sequencer at the UCLA Protein Microsequencing facility using the Applied Biosystems 475A system composed of a 470A sequencer, a 120A quencer at the UCLA Protein Microsequencing facility using the Applied Biosystems 475A system composed of a 470A sequencer, a 120A data module. For each peptide it was possible to follow the sequence for 15 amino acids or more, allowing unambiguous assignment of sequence. In the present study, the numbering of positions in the H$^+\cdot$K$^+$-ATPase is based on the known N-terminal amino acid sequence in the case of the $\alpha$-subunit of hog enzyme and hence is one less than the cDNA-derived sequence (22).

Materials—The materials were of the highest grade purity available. Cupric sulfate pentahydrate, 1,10-phenanthroline monohydrate, trypsin type XIII, CDTA, ATP, n-dodecyl $\beta$-d-maltoside, octaethylene glycol monododecyl ether, Nonidet P-40, 6-aminohexanoic acid, and Brij-Triton X-100 were obtained from Sigma. Biotinylated WGA, avidin-horseradish peroxidase, horseradish peroxidase color development reagent, and 4-chloronaphtol were from Bio-Rad. Reagents for Western analysis were from Promega. PVDF membranes were from Millipore. Coomassie G-250 and R-250 were from Sigma.

RESULTS

BN-PAGE of the n-Dodecyl $\beta$-D-Maltoside-solubilized Gastric H$^+\cdot$K$^+$-ATPase—After solubilization of the gastric vesicles on ice with 1% n-dodecyl $\beta$-d-maltoside buffer and electrophoresis, a protein band was seen at about 360 kDa with an additional weaker band at 720 kDa. Fig. 1 presents Coomassie Blue staining (Panel A) and Western analysis using mAb 1218 and WGA (Panel B). These bands were stained by both mAb 1218 (Fig. 1, lane 3) which shows the presence of the $\alpha$-subunit of the H$^+\cdot$K$^+$-ATPase (20) and by WGA (Fig. 1, lane 4), which shows the presence of the $\beta$-subunit at these positions (9). These protein bands also were stained by mAb 146, which recognizes mainly the $\alpha$-subunit in hog enzyme (20). The band at about 360 kDa therefore probably represents an ($\alpha \beta$)$_2$-dimer. When
Cu\textsuperscript{2+} Cross-linking of the H\textsuperscript{+},K\textsuperscript{+}-ATPase

**A**

![Image](http://www.jbc.org/Downloaded.png)

**B**

![Image](http://www.jbc.org/Downloaded.png)

**Fig. 2.** SDS-PAGE of the membrane-bound H\textsuperscript{+},K\textsuperscript{+}-ATPase treated with Cu\textsuperscript{2+}-phenanthroline complex. Cu-phenanthroline reaction with the H\textsuperscript{+},K\textsuperscript{+}-ATPase and 5-15% Tricine gradient gel electrophoresis of reacted H\textsuperscript{+},K\textsuperscript{+}-ATPase was carried out as described under "Experimental Procedures." Panel A shows the Coomassie-stained gel and Panel B represents mAb 1218 staining (lanes 1a and 2a) and WGA staining (lanes 1b and 2b). Lane 1 represents unreacted H\textsuperscript{+},K\textsuperscript{+}-ATPase. Lane 2 represents an electrophoretic pattern of enzyme stained with 0.1 mM Cu-phenanthroline. Lanes 3, 4, 5, 6, and 7 represent Cu-phenanthroline (0.1 mM) reaction with enzyme pretreated with 0.2 M KCl, 2 mM MgCl\textsubscript{2}, 2 mM ATP, 2 mM Mg\textsubscript{2+}, and 10 mM EDTA, respectively. Lane 8 represents the DTT reduced product of the reacted enzyme that is shown in lane 2.

1% Nonidet P-40 was used as a nondenaturing detergent instead of n-dodecyl \(\beta\)\(\delta\)-maltoside, similar results were observed. The weaker staining band at about 720 kDa probably represents an (\(\alpha\beta\))\(_{4}\) tetramer. Given the different intensities of staining of the 360-kDa band and the 720-kDa band, the most frequent oligomer is the (\(\alpha\beta\))\(_{2}\)-dimer (\(\approx 95\%\)). The presence of the (\(\alpha\beta\))\(_{2}\)-dimer was ligand-independent since this dimer was still present even if the gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase was treated with ligands such as ATP, ADP, Mg\textsubscript{2+}, ATP-CDTA, and Mg\textsuperscript{2+} (data not shown).

The Cu-phenanthroline reaction with the membrane-bound gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase provided a 190 kDa band with an additional minor band near the top of gel, which was also recognized by mAb 1218. The yield of the 190-kDa band was about 30% based on optical scanning of the Coomassie Blue stain. Fig. 2 illustrates the Coomassie Blue-stained gel (Panel A) and the Western immunoblot using mAb 1218 and WGA (Panel B). As shown in this figure, the band at 190 kDa (Fig. 2, Panel A, lanes 2, 3, and 4) contains only the \(\alpha\)-subunit, recognized by mAb 1218 (Panel B, lane 2a). The \(\beta\)-subunit was shown to be same in unreacted enzyme and Cu-phenanthroline-reacted enzyme, which indicates that the \(\beta\)-subunit had not reacted with Cu-phenanthroline (Panel B, lanes 1b and 2b). When enzyme was reacted with Cu-phenanthroline in the presence of ATP or Mg\textsubscript{2+}, the \(\alpha\)-\(\alpha\)-dimer was not formed (Fig. 2, lanes 5 and 6). Vanadate was also relatively effective in inhibiting dimerization (data not shown). Other ligands such as K\textsuperscript{+} and Mg\textsuperscript{2+} did not inhibit the formation of dimers (Fig. 2, lanes 3 and 4). When the cross-linking reaction was carried out in the presence of chelating agents such as EDTA, the formation of the \(\alpha\)-\(\alpha\)-dimer was inhibited as shown in Fig. 2, lane 7. These chelating agents are able to remove cupric ion from Cu-phenanthroline and prevent Cu\textsuperscript{2+} complex formation (28).

As now shown in Fig. 2, lane 8, the \(\alpha\)-\(\alpha\)-dimer induced by Cu-phenanthroline reaction was reduced by DTT, showing that cross-linking was due to disulfide formation and/or Cu-protein complex (28).

**Fig. 3.** Inhibition of ATPase activity by Cu-phenanthroline. The gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase was reacted with Cu-phenanthroline (0.1-100 \(\mu\)M) and centrifuged to remove unreacted Cu-phenanthroline. ATPase activity of the washed enzyme was measured as described under "Experimental Procedures." Panel A shows that the inhibition of the gastric H\textsuperscript{+},K\textsuperscript{+}-ATPase is dependent on the concentration of Cu-phenanthroline. Panel B shows that ATP pretreated enzyme was not affected by Cu-phenanthroline.

Inhibition of ATPase Activity by Cu-Phenanthroline—As shown in Fig. 3, Panel A, Cu-phenanthroline inhibited K\textsuperscript{+}-stimulated ATPase activity in a concentration dependent manner with an IC\textsubscript{50} of about 12 \(\mu\)M. As shown in Fig. 3, Panel B, the presence of 2 \(\mu\)M ATP prevented this inhibition. The ATP treated H\textsuperscript{+},K\textsuperscript{+}-ATPase retained 99 \(\pm\) 0.5% of control activity. Other ligands such as Mg\textsuperscript{2+} or K\textsuperscript{+} were without effect.

When the H\textsuperscript{+},K\textsuperscript{+}-ATPase that had been treated with Cu-phenanthroline was exposed to either 0.1 mM DTT or 0.2 mM \(\beta\)-mercaptoethanol, the \(\alpha\)-\(\alpha\)-dimer was reduced to provide the monomer as shown in Fig. 2, lane 8. Treatment with DTT alone inhibited enzyme activity by about 80% and treatment of the cross-linked enzyme restored 20% of control activity showing probable reversal of the inhibition induced by cross-linking (data not shown).
would start at Val561 and end probably at Arg616 or Arg621. If a single N-terminus sequence beginning VLGFXQLYL. Based on only in the cytoplasmic domain and was localized to a single not present in the membrane domain (resulted in inhibition of the H⁺,K⁺-ATPase activity and formation of a stable αα-dimer. Dimer formation was not affected by K⁺ or Mg²⁺ but was prevented by ATP even in the presence of CDTA and partially by HVO₂⁻. These data suggest that ATP binding may alter the distance between the cysteines via conformational changes or mask the cysteines available for Cu-phenanthroline reaction. Cu-phenanthroline reaction also produces αα-dimers with the Na⁺,K⁺-ATPase (23–26). In contrast to the gastric ATPase, in this closely homologous enzyme dimer formation was induced by the addition of ATP (24) and ADP and K⁺ inhibited αα-dimer formation (25).

The C-terminal 64-kDa peptide of the Na⁺,K⁺-ATPase had been shown to be essential for dimerization (26). Recently, using various chimeras, a cytoplasmic region (no. 554–785) in the α-subunit of the Na⁺,K⁺-ATPase was reported to be necessary for specific αα association (30). When the site of cross-linking in the H⁺,K⁺-ATPase was identified as detailed above, it was found to be within a 6.5-kDa fragment beginning at Val601 and ending at Arg630 or Arg631, based on molecular size. This suggests that Cys605 and/or Cys615 are the cross-linked residues in the gastric ATPase.

The N-terminal 55 amino acid sequence of this domain in the Na⁺,K⁺-ATPase (no. 554–609) is very similar to the fragment of the H⁺,K⁺-ATPase defined biochemically as the major site of cross-linking in this study. In particular, when the sequences of the H⁺,K⁺-ATPase around Cys605 and Cys615 are compared with the sequences of the Na⁺,K⁺-ATPase around Cys611 and Cys606, very high homology is seen. Thus, the sequence, VLGFC, containing the first of these cysteines is identical in both the H⁺,K⁺ and Na⁺,K⁺-ATPase and the C-terminal 25-amino acid sequence of the H⁺,K⁺-ATPase tryptic fragment (no. 592–617) containing the second of these cysteines shows very high homology with the corresponding region (no. 583–607) of the Na⁺,K⁺-ATPase. The high homology of these regions suggests that the Cu-phenanthroline dependent dimerization probably occurs in this domain of both enzymes via cross-linking of the corresponding cysteines. This region of close association is within the nucleotide binding domain of the ATPases which is consistent with the effects of nucleotides on cross-linking. Other regions of the enzyme could also be in close contact, but not revealed by this particular reagent.

Acknowledgments—We thank Dr. Audree Fowler for performing the peptide sequencing, and Charlie Hong for his excellent technical assistance. Dr. Adam Smolka generously supplied the mAb 1218 and Dr. Paul Mangeat the mAb 146.

REFERENCES

1. Sachs, G., Chang, H. H., Rabon, E., Schackman, R., Lewin, M., and Saccomani, G. (1976) J. Biol. Chem. 251, 7690–7698
2. Hall, K., Perez, G., Sachs, G., and Rabon, E. (1991) Biochim. Biophys. Acta 1077, 173–179
3. Maeda, M., Ishizaki, J., and Futai, M. (1988) Biochem. Biophys. Res. Commun. 157, 203–209
4. Shull, G. E., and Lingrel, J. B. (1986) J. Biol. Chem. 261, 16788–16791
5. Bamberg, K., Mercier, F., Reuben, M. A., Kobayashi, Y., Munson, K., and Sachs, G. (1992) Biochim. Biophys. Acta 1131, 69–77
6. Saccomani, G., Dalley, D. W., and Sachs, G. (1979) J. Biol. Chem. 254, 2821–2827
7. Reuben, M. A., Lasater, L. S., and Sachs, G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6767–6771
8. Toh, B.-H., Gleeson, P. A., Simpson, R. J., Moritz, R. L., Callaghan, J. M., Goldkorn, I., Jones, C. M., Martinelli, T. M., Mu, F. T., Humphris, D. C., Pettitt, J. M., Mori, Y., Masuda, T., Sobieszcuk, P., Weinstock, J., Mantamadiotis, T., and Baldwin, G. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6418–6422
9. Hall, K. P., Perez, G., Anderson, D., Gutierrez, C., Munson, K., Hersey, S. J., Kaplan, J. H., and Sachs, G. (1990) Biochemistry 29, 701–706
10. Okamoto, C. T., Karpilow, J. M., Smolka, A., and Forte, J. G. (1990) Biochim. Biophys. Acta 1037, 360–372
11. Callaghan, J. M., Toh, B.-H., Pettitt, J. M., Humphris, D. C., and Gleeson, P. A. (1990) J. Cell Sci. 95, 563–575
12. Shin, J. M., and Sachs, G. (1994) J. Biol. Chem. 269, 8642–8646
13. Rabon, E. C., Gunther, R. D., Bassiliana, S., and Kempter, E. S. (1988) J. Biol. Chem. 263, 16189–16194
14. Saccomani, G., Sachs, G., Cappoletti, J., and Jung, C. Y. (1981) J. Biol. Chem. 256, 7727–7729
15. Schrijen, J. J., Van Groningen-Luyben, W. A. H. M., Nauta, H., De Pont, J. J., H. H. M., and Bonting, S. L. (1983) Biochim. Biophys. Acta 731, 329–337
16. Hebert, H., Xian, Y., Hacksell, I., and Mårdh, S. (1992) FEBS Lett. 299, 159–162
17. Yoda, A., and Hokin, L. E. (1970) Biochim. Biophys. Res. Commun. 40, 880–884
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
19. Schläger, H., and von Jagow, G. (1991) Anal. Biochem. 199, 223–231
20. Mercier, F., Bayle, D., Besancon, M., Joye, T., Shin, J. M., Lewin, M. J. M., Prinz, C., Reuben, A. M., Soumarnon, A., Wong, H., Walsh, J. H., and Sachs, G. (1993) Biochim. Biophys. Acta 1149, 151–165
21. Schläger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
22. Lane, L. K., Kirley, T., and Ball, W. J., Jr. (1986) Biochem. Biophys. Res. Commun. 138, 185–192
23. Periyasamy, S. M., Huang, W.-H., and Askari, A. (1987) J. Biol. Chem. 258, 9878–9885
24. Huang, W.-H., and Askari, A. (1981) Biochim. Biophys. Acta 645, 54–58
25. Askari, A., Huang, W.-H., and Antier, J. M. (1980) Biochemistry 19, 1132–1140
26. Ganjeizadeh, M., Zolotarjova, N., Huang, W.-H., and Askari, A. (1995) J. Biol. Chem. 270, 15707–15710
27. Martin, D. W., and Sachs, J. R. (1992) J. Biol. Chem. 267, 23922–23929
28. J. T. H. (1983) Methods Enzymol. 91, 380–609
29. Schläger, H., Cramer, W. A., and von Jagow, G. (1994) Anal. Biochem. 217, 220–230
30. Kosier, J. C., Blanco, G., and Mercer, R. W. (1995) J. Biol. Chem. 270, 14332–14339
Dimerization of the Gastric H,K-ATPase
Jai Moo Shin and George Sachs

J. Biol. Chem. 1996, 271:1904-1908.
doi: 10.1074/jbc.271.4.1904

Access the most updated version of this article at http://www.jbc.org/content/271/4/1904

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 14 of which can be accessed free at
http://www.jbc.org/content/271/4/1904.full.html#ref-list-1