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MINI-REVIEW

Functional Domains of the Gastric HK ATPase

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Abstract

The gastric $H^+ + K^+$ ATPase is a member of the phosphorylating class of transport ATPase. Based on sequence homologies and CHO content, there may be a *b* subunit associated with the catalytic subunit of the $H^+ + K^+$ ATPase. Its function, if present, is unknown. The pump catalyzes a stoichiometric exchange of H^+ for K^+, but is also able to transport Na^+ in the forward direction. This suggests that the transport step involves hydronium rather than protons. The initial binding site is likely to contain a histidine residue to account for the high affinity of the cellular site. The extracellular site probably lacks this histidine, so that a low affinity for hydronium allows release into a solution of pH 0.8. Labelling with positively charge, luminally reactive reagents that block ATPase and pump activity has shown that a region containing H5 and H6 and the intervening luminal loop is involved in necessary conformational changes for normal pump activity. The calculated structure of this loop shows the presence of an *a* helical, *b* turn, and *b* strand sector, with negative charges close to the membrane domain. This sector provides a possible site of interaction of drugs with the $H^+ + K^+$ ATPase, and may be part of the K^+ pathway in the enzyme.

Key Words: Gastric H^+ transport; ATPase; omeprazole; K^+ site.

Introduction

There are three known classes of proton-transporting ATPase in mammals. The first, the F_1F_0 of mitochondria, functions as an ATP synthetase rather than a "proton pump" but is closely related to what have been called

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vacuolar pumps as occur in various intracellular organelles. These are the second class of ATP-driven proton pumps. They are characterized by being of multi-subunit construction and by hydrolyzing ATP without the formation of a covalent intermediate. Moreover, it appears, as for F_1F_0, that the subunits are arranged into two complexes, a membranal complex and an extramembranal complex. The latter appears to be charged with the hydrolysis of ATP, the former with the transmembrane transport of protons. There have been several recent reviews of this type of pump (Alawqati, 1986; Forgac and Cantley, 1984). These vacuolar pumps are electrogenic, and some but not all appear to have a specific requirement for Cl^- ions for full transport activity.

The gastric $H^+ + K^+$ ATPase is, in contrast, a member of the phosphorylating type of ATP-driven ion pump, and even though there may be hints at a wider distribution for this type of enzyme, its only proven location is in the parietal cell of the gastric mucosa. There are other proton pumps in the E-P class, such as in *Neurospora, saccharomyces*, and *S. fecalis* (Brooker and Slayman, 1983; Serrano *et al.*, 1986; Solioz *et al.*, 1987; Pederson, 1983). In contrast to the first two of these, the gastric enzyme is an antiport pump, exchanging cytosolic protons for extracellular K^+ (Ganser and Forte, 1973; Sachs *et al.*, 1976). This has allowed easier access to the kinetic steps involved in ATP turnover and therefore correlation between transport and hydrolysis. In fact, it is more closely related to another antiport pump, the $N^+ + K^+$ ATPase, than to the fungal proton ATPase. Development of knowledge of the kinetics and conformational changes of $H^+ + K^+$ ATPase has been much facilitated by the voluminous literature on the $Na^+ + K^+$ and Ca^{++} ATPase (Jorgensen and Andersen, 1988).

The gastric proton pump has also been investigated as a target for therapeutically effective anti-ulcer drugs. This has allowed the development of inhibitors of its functions that are also interesting investigative tools of its structure (Wallmark *et al.*, 1987; Sachs *et al.*, 1988).

This review will focus on the nature of the ions that can be transported and a possible approach to defining the location of inhibitory sites in the extracellular domain of the proteins comprising the $H^+ + K^+$ ATPase.

Structure of the Catalytic Subunit

The catalytic subunit from both rat and hog has been sequenced. The *c*DNA sequence shows about 60% homology with the catalytic subunit of the NaK ATPase (Shull and Lingrel, 1986; Maeda *et al.*, 1988a). The major regions of homology appear to be in what has been considered to be the catalytic region of the NaK ATPase, and comprises the phosphorylation site,

the FITC binding site, and the FSBA binding region. In contrast, there is less homology in the first membrane-spanning sequence, the third membrane-spanning sequence, and from the sixth membrane-spanning sequence until the end of the protein. Presumably these regions are concerned with the specialized properties of the $H^+ + K^+$ ATPase.

The hydropathy profile of the catalytic subunit of the $H^+ + K^+$ ATPas is similar to that of the $N^+ + K^+$ ATPase. There are four predicted membrane-spanning sequences prior to the phosphorylation site at asp385. The predicted cytosolic loop between hydrophobic sequences 4 and 5 (H4, H5) contains the phosphorylation site, the FITC binding site, the FSBA binding site, and also pyridoxal phosphate binding (Maeda *et al.*, 1988b). It is therefore generally accepted that this major sector of the enzyme is in the cytosolic domain. Beyond membrane-spanning sequence H5 it is not as easy to determine the number or location of membrane-spanning loops. There appears to be good reason to postulate a fairly long, charged extracellular loop between H5 and H6. The postulated H6 sector of the $H^+ + K^+$ ATPase is considerably less hydrophobic than the H6 sector of the $Na^+ + K^+$ ATPase, and is predicted more by analogy than by hydrophobicity. In the case of the Na^+ pump, there is controversy as to whether the C terminal sequence is cytosolic or extracellular (Shull and Lingrel, 1986; Ovchinnikov, 1987). There may be an even or add number of membrane-spanning sequences in this catalytic subunit, and the same reservation must apply to the secondary structure of the catalytic subunit of the $H^+ + K^+$ ATPase. The relative positions of some of the membrane-spanning sequences can be deduced from the hydropathy profile. The short extracellular sequences between H1 and H2 and H3 and H4 suggest close apposition between these pairs of membrane-spanning sequences. The longer loop between H5 and H6, and the uncertainty as to whether there are seven, nine, or eight total membrane-spanning sequences, makes the structure of this second region of the membrane domain of the protein even more uncertain. Figure 1 shows the hydropathy profile of the hog $H^+ + K^+$ ATPase.

There are three potential glycosylation sites in the catalytic subunit. One, however, has the sequence NDS and is less likely to be glycosylated than the other two. These are all present in what is thought to be the cytosolic domain of the protein. It is thought that N-glycosylation takes place in the rear on the extracellular face of membrane-inserted proteins. There appears to be evidence that asn 493 is glycosylated in both rat and pig enzyme based on staining of tryptic digests with con A and peptide sequencing (Tai *et al.*, 1989; Hall *et al.*, 1989a). Con A binding in vesicles occurs mainly from the extracellular face, and hence if indeed asn 493 is glycosylated, this is from the extracellular face. It has not been established as yet that the glycosylation consensus sequences are valid for this enzyme, but, on the other hand, all the

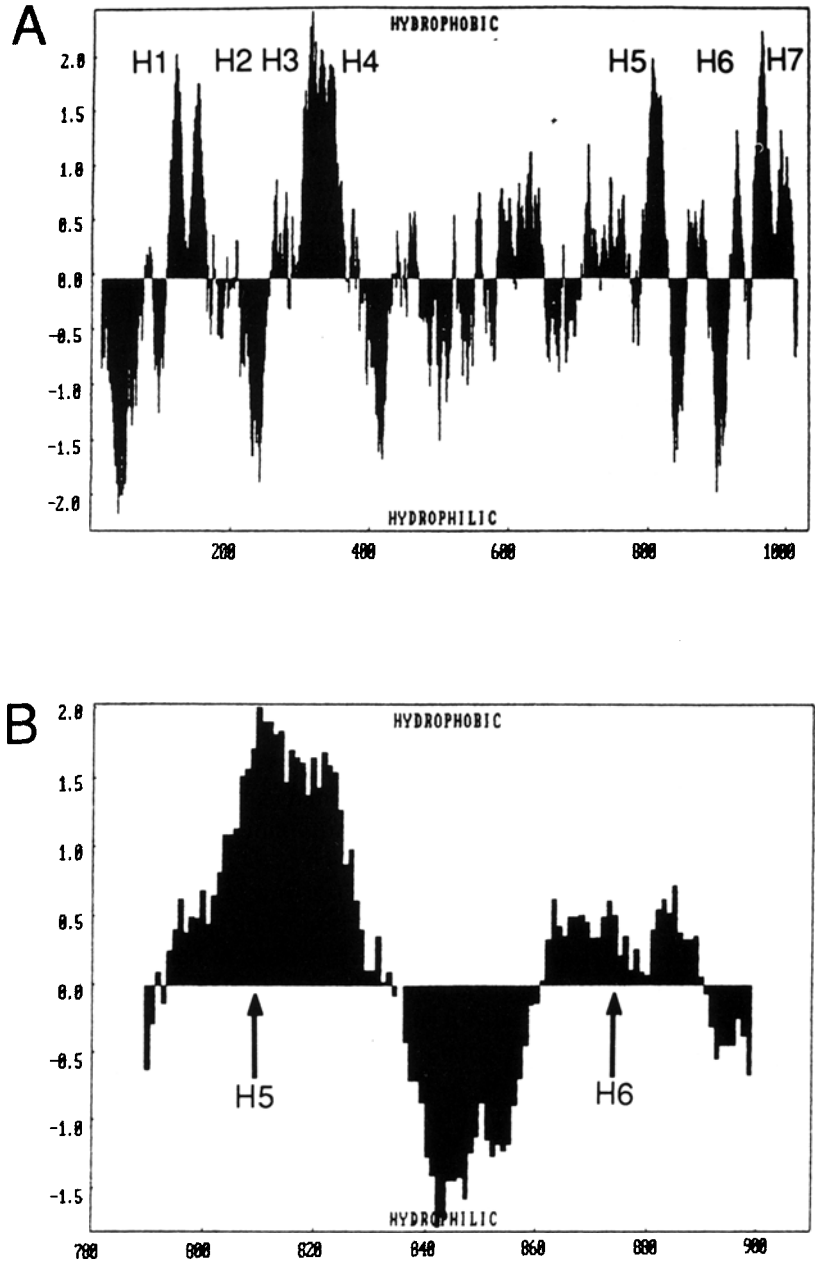

Fig. 1. (A) Calculated hydropathy of the catalytic subunit of the hog HK ATPase, using a window of 21 amino acids. (B) An expanded plot of the postulated H5 and H6 sectors with the intervening hydrophilic extracellular loop that may be involved in omeprazole and K competitive antagonist binding.

con A binding is removed by glycopeptidase F (N-glycanase). If indeed the consensus sequence starting at asn 493 is N-glycosylated from the extracellular face, perhaps there are regions of the putative cytosolic domain of the pump protein that are membrane inserted. The membrane insertion would be between the peptide chains, rather than in the phospholipid of the bilayer, which would not allow prediction based on hydrophobicity.

On the other hand, although it is unlikely that N-glycosylation occurs from the cytosolic face, it is not certain, following glycosylation, that this orientation is maintained in the mature protein, nor that all the protein is indeed glycosylated.

The Presence of Other Subunits

It is known that the $Na^+ + K^+$ ATPase has not only an *a* or catalytic subunit but also a *b* subunit present in stoichiometric amounts. This glycosylated 33-kD subunit has been sequenced and has been shown to have a small cytosolic domain, a single membrane-inserted domain, and the majority of the protein is then extracellular with three glycosylation consensus sequences that are glycosylated (Brown *et al.*, 1987). The function of the *b* subunit is not known. It is sufficiently tightly associated with the *a* subunit that ionic detergents are necessary for separation, with consequent inactivation of the enzyme. Target analysis of the activation of the enzyme show that an *ab* protomer appears necessary for most of the functions of the ATPase (Karlish and Kempner, 1984). Expression of functional pump requires message for both subunits, and it has been suggested that the *b* subunit is required for correct membrane insertion (Geering *et al.*, 1985). Labeling with a photoaffinity derivative of ouabain, NAP-ouabain, labelled the *a* and also, to a minor extent, the *b* subunit (Hall and Ruohoi, 1980). The interaction of ouabain with the $Na^+ + K^+$ ATPase is partially competitive with K^+. It has also been suggested that yet a third subunit, the *y* subunit, a peptide of about 1000 MWt, is part of the $Na^+ + K^+$ ATPase complex (Collins and Leszyk, 1987).

Until recently, there was rather little attention paid to the possibility that other subunits were required for $H^+ + K^+$ ATPase activity. For example, it has been possible to express the protein of the $H^+ + K^+$ ATPase using vectors containing only a catalytic subunit *c*DNA, and it appears the protein is inserted into the plasma membrane (Caplan *et al.*, 1989). However, it is not known whether this protein is functional.

Three sets of observations suggest that possibly there are additional subunits associated with the catalytic subunit of the $H^+ + K^+$ ATPase. First, target MWt analysis showed that the primary unit had a MWt of about 150 kD, clearly larger than the catalytic subunit and clearly smaller than a

dimer thereof (Rabon *et al.*, 1988). Second, there is a large amount of carbohydrate associated with purified pump vesicles not accounted for by glycosylation of the catalytic subunit. This carbohydrate binds wheat germ agglutinin rather than con A and is also located on the extracellular surface (Hall *et al.*, 1989a; Okamoto *et al.*, 1989). The WGA staining is diffusely distributed between 60 and 80kD. A peptide of about 35 kD is produced following glycopeptidase F treatment. Evidence for extracellular glycosylation derives from labelling with ^{3}H-UDP-galactose and galactosyl transferase, in intact and permeable gastric vesicles that are inside out, as well as WGA-immunogold staining of gastric vesicles following embedding. In fact, both Coomassie staining and galactose labelling suggest the presence of 2 MWt regions of CHO-containing protein, at 65 and 80 kD. N-glycanase treatment of gastric vesicles provides a protein of 35 kD, and partial sequence shows homology with the β subunits of the $Na^+ + K^+$ ATPase. Finally, as shown below, sequencing of a labelled region of the enzyme showed the presence of a sequence 30 or 46% homologous to the *b*1 or *b*2 sequence of the $Na^+ + K^+$ ATPase, respectively.

In analysis of the target MWt of the $H^+ + K^+$ ATPase, it was shown that either phosphorylation or size of the peptide had an apparent MWt of 146 kD, ATPase and pNPPase of 260 kD, both sizes too large either for only a monomer or dimer of the catalytic subunit alone. Cross-linking studies have shown the presence of a possible heterodimer, since the MWt of 168 kD is probably too small to be a homodimer of the catalytic subunit (Rabon and Bassilian, 1989).

Catalytic Cycle of the $H^+ + K^+$ ATPase

In general, ion transporting ATPases must convert the energy derived from the scalar hydrolysis of ATP to vectorial transport of ions. It is thought that this is brought about by conformational changes in the transport protein so that the ion binding sites change sidedness and affinity for the ion. The conformation where the ion binding sites face the cytosol is called E_1 and that where the ion binding sites face the extracellular surface is called E_2. In the phosphorylating class of ATPases, such as the H^+ ATPase of *Saccaromyces* and *Neurospora*, the Ca^{++} ATPase of sr and plasma membrane, and the $Na^+ + K^+$ ATPase, the chemical cycle is divided experimentally by the formation and breakdown of an aspartyl phosphate, the phosphorylated intermediate. In the case of the $H^+ + K^+$ ATPase, the intermediate is formed in the presence of Mg^{++} and ATP, and in the absence of K^+ breaks down very slowly. This hydrolysis is rate limiting in the absence of extracellular K^+. Extracellular K^+ is required for the rapid breakdown of the

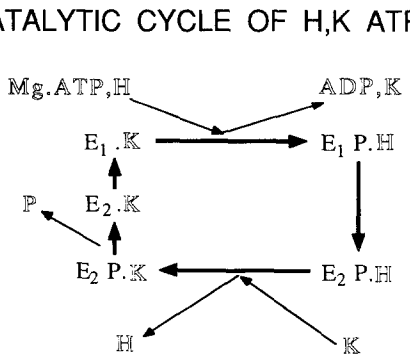

Fig. 2. A condensed scheme for the catalytic cycle of the Hk ATPase, showing the E1 and E1-P conformations where the ion binding sites face the cytosol, and the E2-P and E2 conformations where the ion binding sites face the extracellular solution. The hydrolysis of ATP is thought to proceed sequentially through these conformations, the E1-P, E2-P, E2, and then E1.

phosphoenzyme, and the rate-limiting step now appears to be the translocation of K following the breakdown of E-P. The scheme that is shown (Fig. 2) postulates and E_1 conformation, which is phosphorylated by ATP in the presence of Mg^{+6} and presumably binds the H^+ ion from the cytosolic surface to form an E_1-P.H^+ complex. This converts spontaneously to an E_2-P conformer which releases protons to the extracellular solution, binds K^+, and dephosphorylates to form eventually the $E_1.K^+$ complex from which K^+ is released to the cytosolic solution. The $H^+ + K^+$ ATPase, as the $Na^+ + K^+$ ATPase, is therefore a countertransport E-P pump (Wallmark *et al.*, 1980; Reenstra *et al.*, 1988).

Transport by the $H^+ + K^+$ ATPase

The most interesting aspect of in-translocating ATPases, and perhaps the most difficult to study, is the nature of the ion binding sites on the cellular and extracellular face. It is reasonable to feel that these sites are three-dimensional constructs made of several chains of the ATPases. Not until adequately ordered crystals are available will there by definitive evidence as to the residues that make up the binding site, but various approaches may give useful information for interpretation of crystal patterns when these are available.

The gastric $H^+ + K^+$ ATPase has a special property that makes it a particularly challenging enzyme to consider, namely that it is able to generate a pH of 0.8 at a cytosolic pH of 7.3. This is the largest ion gradient known in mammalian biology and raises the question of the nature of the site that

is able to bind and dissociate protons at the necessary pK_a's. Recently it has been suggested, for protonmotive ATPases of the mitochondrial type, that it is the hydronium ion, H_3O^+, rather than the proton, that is transported, removing the need for concepts such as proton wires in this type of pump (Boyer, 1988).

The $H^+ + K^+$ ATPase can transport Na^+ in the forward direction, detected by isotope uptake methods in isolated gastric vesicles, which are inside out (Polvani *et al.*, 1989). This suggests that there is an absence of a specialized pathway for protons, and that indeed the ion transported in the forward direction is likely to be either the hydronium ion, or the monohydrated Na^+ ion as a hydronium surrogate. If it is the hydronium ion that is transported, it is the decrease of affinity for this ion in the E2 conformer of the enzyme that determines the pH gradient that can be formed, rather than a group of extraordinarily low pK_a.

The binding site of the cytosolic face must be of high affinity. Thus if there is a histidine group of pK_a about 7 in the E_1 form able to bind hydronium, it will exist as a protonated imidazolium, with one molecular of water associated. If in the E_2 form this histidine group moves away from the ion binding site, into a hydrophobic pocket, the hydronium can now dissociate as the pK_a will be lowered. The ion binding site now would consist of carbonyl oxygens, with no readily protonatable group, and the hydronium ion is now bound. If the dissociation constant for the hydronium ion is about 150 mM, as for the Na in the E_2 conformation of the $Na^+ + K^+$ ATPase, the $H^+ + K^+$ ATPase will be able to pump down to a pH of 0.8. K^+ will bind to this site from the extracellular face with relatively high affinity in the E_2 conformation, and as the E_1 conformation is reestablished with approach of the histidine group, the K^+ affinity will decrease and K^+ will release into the cytosolic medium. This concept is illustrated in Fig. 3. There may in fact be more than one histidine involved. For example there is a pair of histidines (Nos. 903, 904) on the predicted cytosolic face on the C terminal side of membrane-spanning sequence H6, unique to the $H^+ + K^+$ ATPase and absent from the $Na^+ + K^+$ ATPase (Shull and Lingrel, 1986; Maeda *et al.*, 1988a). It is known that the stoichiometry of transport at pH 7.4–6.1 appears to be two H^+ for two K^+. For development of a pH gradient of more than six units and a tenfold gradient for K^+, the maximum thermodynamic stoichiometry cannot exceed one H^+ for one K^+. This perhaps can be explained by two protonatable groups within the site and a switch from proton to hydronium transport as the pH gradient increases and from unhydrated to hydrated K ion countertransport.

By using the histidine derivatizing agent diethyl pyrocarbonate, it has been possible to show the essentiality of histidine groups in the enzyme, and

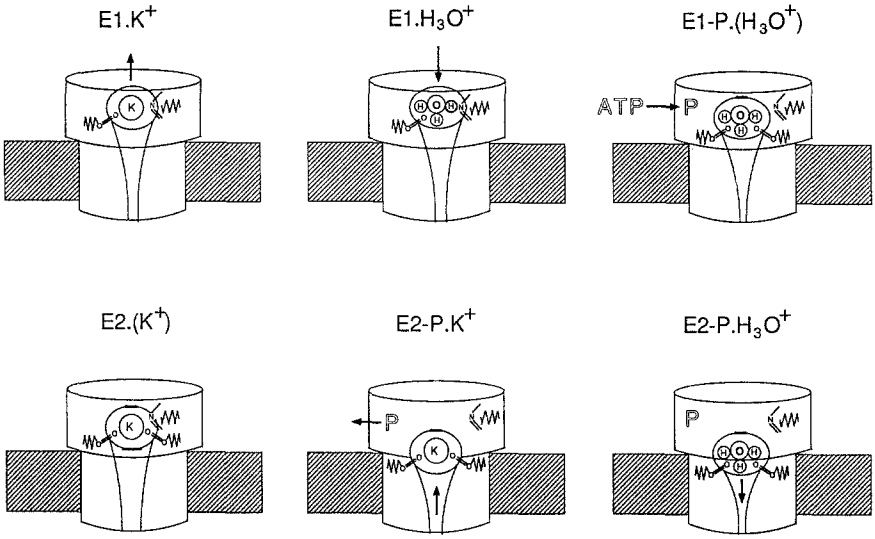

Fig. 3. A concept for the structure of the ion transport site of the HK ATPase. In the upper half of the figure, the change from E1.K to E1-P$(H_3O)^+$ is illustrated as binding of a proton to a histidine from the cytosolic face, along with one molecule of water. The histidine moves into a hydrophobic pocket, and a barrier to retrograde movement of the hydronium is formed (thick line). The ion binding site moves toward the membrane domain. In the site the proton is now present as hydronium ion in a neutral binding site. This site is of low affinity and is larger than the initial E1 conformation. This site now preferentially binds K as shown in the lower half of the diagram and, with the splitting of E_2-P, forms a partially occluded K conformation (barriers shown as heavier lines). This converts rapidly in the HK ATPase, in contrast to the NaK ATPase, to the E1.K conformation, where the site now contains the histidine residue. K is therefore released into the cytosolic solution as the affinity for K is decreased by the reinsertion of the histidine into the ion binding domain. The binding of ATP accelerates the loss of K form the E1 conformation. In this model the ion binding sites are shown as moving with respect to the plane of the membrane, with changes in internal structure to account for affinity changes and, along with movement, changes in barriers on either side of the site in the E1 and E2 configurations to provide vectorial movement of the ions.

a plot of pK_m against pH has shown the presence of a group of pK_a 7 to be involved in catalysis (Saccomain *et al.*, 1980).

On the extracellular face, various cations can act as K surrogates, in the sequence $Tl^+ > K^+ > Rb^+ > NH_4^+ > Cs^+ \gg Na^+, Li^+$ for both ATPase activity and acid transport. The suggestion is that the cation binding site from the extracellular face is larger in dimension than the site on the cytosolic face. This would certainly decrease the affinity for the hydronium ion, allowing its release. It does not seem likely that only a shape change of the binding site is sufficient to account for the large change in affinity for the hydrogen ion, but removal of a histidine group into a hydrophobic pocket and release of protons in the form of hydroniums would be an adequate explanation. Hence, part of the conformational change at the ion binding sites from the

E_1 to the E_2 conformation would involve a change in the dimension of the cation binding site to provide part of the change in affinity of the site, part the loss of a histidine group at the binding site. The change in position of the histidine residue would also generate the barrier necessary to prevent backward transport and allow forward transport. Following release of hydronium, K^+ now binds to the site with high affinity. Reorientation of the histidine lowers the barrier to cytosolic-directed transport, decreases K^+ affinity, and allows binding of the proton to reinitiate the cycle. Thus there are changes of affinity and sidedness of ion access.

The motion of the protein that contributes to the conformational change is detected by changes in extrinsic or intrinsic fluorescence. Labelling of these pumps by fluorescein isothiocyanate has provided a particularly useful means of showing conformational changes in the H^+K^+ ATPase, the E_1 state giving a higher quantum yield than the E_2 state. The FITC labelled site, ASN 517, is therefore in a more hydrophobic environment in the E_1 state. Whether this site is close to the membrane or peripheral to the cytosolic sector of the protein is not known.

There are several possible pump models that are analogous to mechanical pumps. For example, a reciprocating pump or a peristaltic pump are obvious possibilities. In the case of a reciprocating pump, the motion of the protein perpendicular to the axis of the membrane pushes and pulls the site across a barrier. A peristaltic pump would be modelled as a rocking motion of peptide chains laterally to the plane of the membrane. In either case, the motion of the ion binding sites could be small or large in order to change either sidedness or affinity. The magnitude of change of fluorescence of extrinsic probes argues for quite significant changes in shape of the protein. As discussed below, reagents that react with the extracellular face of the enzyme prevent transphosphorylation from ATP, thus affecting the conformation across the membrane, suggesting conformational changes transmitted across large distances.

The charge carried by the pump during its transport cycle can give information on the nature of the sites. Although the overall reaction of the $H^+ + K^+$ ATPase is electroneutral, both the forward and reverse reactions have been shown to be charge carrying (Fendler *et al.*, 1988; Lorentzon *et al.*, 1988). In contrast, in the case of the $Na^+ + K^+$ ATPase, only the Na^+ step has been shown to be charge carrying, one charge moving per three Na^+ ions transported (Goldshlegger *et al.*, 1987). For the latter pump, this has led to the postulate that two carboxylic acids are involved in cation binding. In the case of the $H^+ + K^+$ ATPase, the unoccupied site must be electroneutral, and the occupied site charged (e.g., histidine in E_1, hydronium in E_2), to allow for charge transfer in the forward and the reverse direction.

Functional Regions of the $H^+ + K^+$ ATPase

The exploration of functional regions of the transport ATPases has involved the use of group-selective reagents, especially those which respond in terms of protection by ligands such as ATP and ions. Most have been defined by the protection by ATP against inhibition, and thus are thought to react with amino acids at or close to the catalytic center of the enzymes (Saccomani *et al.*, 1980, 1981). With cloning expression of these ATPases, a more detailed understanding is likely to emerge, since it is now possible to locate the reacted amino acids within the primary sequence of the enzyme. A molecular description of these enzymes must await sufficiently detailed crystallographic analysis.

In the case of the $H^+ + K^+$ ATPase, there are two classes of reagent that provide some insight into topology and function. Both react exclusively from the luminal face. One is a SH derivatizing reagent based on its chemical properties, the other a reversible K^+ competitive reagent.

Omeprazole is a reagent that, due to its weak base properties, accumulates in acid spaces. There it undergoes an acid-catalyzed conversion to a cationic sulfenamide that reacts with two cysteine residues per mole enzyme phosphate to provide irreversible inhibition of the $H^+ + K^+$ ATPase. This reagent reacts from the luminal surface of the enzyme (Lorentzon *et al.*, 1987). The chemistry of this reagent is illustrated in Fig. 4. As a result, the inhibited enzyme is unable to catalyze either ATPase activity, pNPPase activity, or phosphorylation of the aspartyl group (Lorentzon *et al.*, 1985).

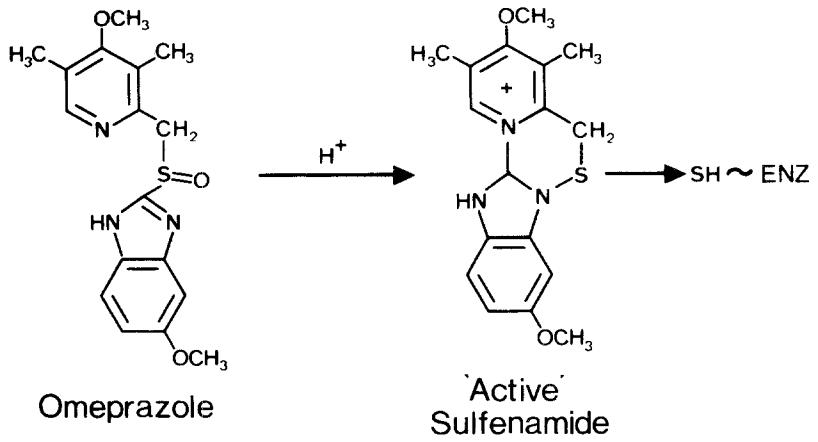

Fig. 4. The reaction sequence for omeprazole following acid activation. This results in the formation of the cationic sulfenamide which then reacts with luminally accessible SH groups on the HK ATPase.

The conformation of the inhibited enzyme is likely to be an E_2 conformer, but without the possibility of effective interaction with K^+, since the pNPPase is blocked.

Separation of labelled peptides on HPLC following digestion with trypsin produced various peptides. One of those gave a sequence asn-ile-pro-glu as the four N-terminal amino acids (Sachs *et al.*, 1989). This sequence is present at the predicted cytosolic/membrane interface immediately prior to hydrophobic sequence 5. The length of the peptide corresponded to about 30 amino acids. There are only two cysteine residues within this peptide, one in the predicted membrane-spanning sequence and one in the postulated extracellular loop between membrane-spanning sequences 5 and 6. One or both of these cysteines are labelled by omeprazole. Modelling this loop by minimization techniques places the extracellular cysteine external to the N-terminal *a* helix in the extracytosolic loop, hence it is less likely to be responsible for the inhibition than the binding of omeprazole to the cysteine in the intramembranal domain of H5. Since this reagent is luminal, it places H5 as membrane spanning and the loop between H5 and H6 as extracellular. This particular peptide accounts for 30% of the omeprazole incorporated. It may be that the other peptides also contain this sequence and reflect variations in tryptic hydrolysis, or they may be in other regions of the enzyme. However, evidence from labelling with a K competitive reagent suggests a significant functional role for this region of the enzyme.

SCH28080, a pyridyl 1, 2*a*-imidazole, is a K^+ competitive reagent that has been shown to inhibit from the luminal face of the enzyme (Keeling *et al.*, 1989a, b). This reagent reacts with either the E_2-P or the E_2 form of the enzyme, and induces and E_2 form when reacted with FITC derivatized $H^+ + K^+$ ATPase. To provide a K^+ competitive photoaffinity derivative, the tritiated methylated quaternary compound with an azido group, Me-DAZIP$^+$, was synthesized. The formula is shown in Fig. 5. This reagent reacted competitively in the dark, and in a K^+ protectible manner during photolysis. The reaction was also saturating as a function of Me-DAZIP$^+$ concentration, showing that it was the compound bound to the enzyme that was photolytically active Munson and Sachs, 1988).

Tryptic cleavage and separation on Tricine SDS gels allowed sequencing of the only labelled region. The sequence that corresponded to the known sequence of the catalytic protein of the $H^+ + K^+$ ATPase had for its N-terminal sequence leu-val-asn-glu. This corresponds to the postulated extracellular face of hydrophobic sequence 6. In terms of predicted structure and MWt of the peptide, the sequence cannot extend into another membrane-spanning loop (Munson and Sachs, in preparation).

Therefore, labelling with this reagent confirms the extracellular nature of the sequence leu-val-asn-glu and the membrane-spanning nature of H6.

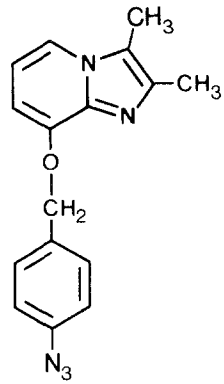

Fig. 5. The formula for the photoaffinity analog, Me-DAZIP, of the K competitive antagonist, SCH28080.

It has been shown that the presence of SCH28080 prevents inhibition by omeprazole, independent of the pH gradient inhibition due to SCH28080. This conclusion was based on the finding that an equivalent degree of acid gradient inhibition by SCN did not affect omeprazole inhibition (Hersey *et al.*, 1988). The finding that omeprazole and Me-DAZIP$^+$ label peptides in the H5, H6 region also suggests close interaction between the sites that react with these compounds. This in turn leads to the conclusion that omeprazole reaction with this region is of functional significance. It may be noted that since Me-DAZIP$^+$ is K^+ competitive, binding of Me-DAZIP$^+$ excludes K^+, and vice versa, from this region of the enzyme. Omeprazole is not K^+ competitive. It is tempting to speculate that this sector of the enzyme is either involved in inducing the K^+ conformation of the enzyme or contains part of the K^+ pathway of the $H^+ + K^+$ ATPase. It is not possible to conclude that this region contains the K^+ site of the molecule. Modelling of this extracellular loop of the $H^+ + K^+$ ATPase predicts that the N-terminal region is an amphipathic helix. This is followed by a *b* turn and a *b* strand moving back into the membrane. Close to the membrane there are two carboxylic acid residues in the *a* helical portion and one carboxylic acid in the *b* strand. This negatively charged region may be involved in targetting either of the inhibitory cations, SCH28080, or the omeprazole sulfenamide. The model is illustrated in Fig. 6.

It appears that covalent reaction in this region, an extracellular or close to extracellular domain, results in changes in conformation that prevent reorientation of the cytosolic sector to an E_1 conformation, since phosphorylation is inhibited. Accordingly, conformational changes in its enzyme must be transmitted across the membrane domain. Binding of bulky substituents on the extracellular face of H5 and H6 or the extracellular loop joining these two sequences prevents the conversion of the E_2 to the E_1 form.

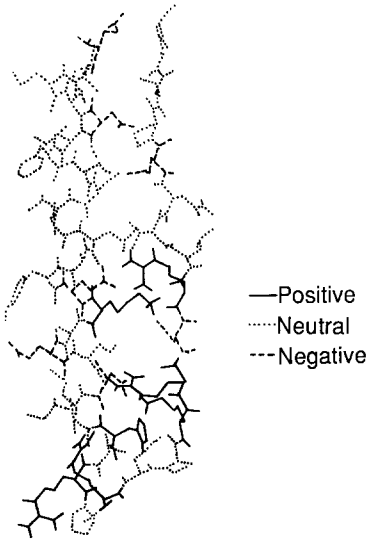

Fig. 6. Calculated structure of the luminal loop between H5 and H6 of the HKATPase, showing the presence of an *a* helix, a *b* turn, and a *b* strand. This region may be the initial site of interation of omeprazole and highly selective K competitive antagonists of the HKATPase. The N terminal region is calculated to be an amphipathic *a* helix, which is followed by a *b* turn. From there, the protein returns into the membrane region as a *b* strand. There is calculated to be strong electrostatic interaction between the *a* helix and *b* strand around the region of the *b* turn. Toward the membrane there are three carboxylic acids perhaps involved in initial binding of either omeprazole sulkenamide or SCH28080. Interaction of the hydrophobic face of the *a* helix could be with the C terminal region, if this is extracytosolic, or with a putative *b* subunit.

When sequencing the MeDAZIP$^+$-labelled region, a second sequence was discovered. This sequence showed, starting at amino acid No. 263 of the *b* subunit of the $Na^+ + K^+$ ATPase, about 30% homology to the *b*1 sequence and 46% homology to the *b*2 sequence of the $Na^+ + K^+$ ATPase.

It has also been shown that there is considerable carbohydrate content of hog gastric vesicles (Spenney *et al.*, 1974). A small amount of carbohydrate is associated with the catalytic subunit as detected by con A binding (Tai *et al.*, 1989), and a larger amount with a broad region of 60–100 kD detected by WGA binding and galactose acceptor activity (Okamoto *et al.*, 1989; Hall *et al.*, 1989b). In both instances it appears that the carbohydrate is N-linked based on removal by glycopeptidase F. The sidedness of the WGA staining region is luminal since UPD-gal transferase activity is increased about 20-fold upon permeabilization of intact inside-out vesicles and WGA immunogold binds to the luminal (extracellular) face of the vesicles. The con A labelling also appear to be on the luminal face based on post-embedding staining with con A immunogold (Hall *et al.*, 1989b).

It is known that the *b* subunit of the $Na^+ + K^+$ ATPase contains a considerable amount of carbohydrate, and when the CHO content of $H^+ + K^+$ ATPase vesicles and sidedness combined with sequence homology are taken together, it appears that a *b* subunit may be associated with the $H^+ + K^+$ ATPase. If this subunit is also labelled by Me-DAZIP$^+$, it seems likely that there is a close association between the two subunits of the $H^+ + K^+$ ATPase. This may also be the case with the $Na^+ + K^+$ ATPase since NAP ouabain labelled both subunits.

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