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# *Review article: the control of gastric acid and* Helicobacter pylori *eradication*

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## SUMMARY

This review focuses on the gastric acid pump as a therapeutic target for the control of acid secretion in peptic ulcer and gastro-oesophageal reflux disease. The mechanism of the proton pump inhibitors is discussed as well as their clinical use. The biology of *Helicobacter pylori* as a gastric denizen is then discussed, with special regard to its mechanisms of acid resistance. Here the properties of the products of the urease gene clusters, ureA, B and ureI, E, F, G and H are explored in order to explain the unique location of this pathogen. The dominant requirement for acid resistance is the presence of a proton gated urea transporter, UreI, which increases access of gastric juice urea to the intrabacterial urease 300-fold. This enables rapid and continuous buffering of the bacterial periplasm to ≈pH 6.0, allowing acid resistance and growth at acidic pH in the presence of 1 mM urea. A hypothesis for the basis of combination therapy for eradication is also presented.

## INTRODUCTION

In the last 25 years of the 20th century, a series of revolutions occurred in our understanding of the biology of gastric acid secretion and of acid related disease, particularly with respect to the role of *Helicobacter pylori*. Whereas 'no acid, no ulcer' remains as true as it were in 1910, a new aphorism is required to state our current therapeutic strategy. At the beginning of the 21st century, the treatment and outcome of acid related disease have changed radically. Peculiarly, these changes, although remarkable, have passed largely unheralded by the general public. On the other hand, we have become much more aware of the long-term consequences of gastro-oesophageal reflux disease (GERD) and of *H. pylori* infection, consequences that will again change our method of treatment.

The introduction of histamine-2 receptor antagonists allowed, for the first time, successful, relatively routine, medical management of peptic ulcer disease.[1] However, it then became clear that duodenal ulcer treatment required maintenance therapy with these drugs, raising the question as to the reasons for recurrence.[2] Additionally, the effectiveness of these antagonists against not only histamine-, but also gastrin-stimulated secretion, placed the release of histamine as the pivotal event in the regulation of acid release from the parietal cell.[1] This, in turn, led to the recognition of the enterochromaffin-like cell rather than the mast cell, as the master neuro-endocrine cell regulating parietal cell function.[3]

After their introduction, it was realized that the clinical effectiveness of these receptor antagonist drugs was somewhat limited, even at high doses. Whereas acute treatment and maintenance of duodenal ulcer disease was satisfactory, certainly compared to before their

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introduction, the treatment of gastro-oesophageal erosion was less so. However, at the same time as the first of these receptor antagonists was introduced, the acid pump of the stomach (the gastric H, K ATPase) was described and its potential as a better therapeutic target was recognized.[4–6]

The drugs that were introduced as an improvement in therapy, particularly of GERD, were targeted at the acid pump. Now, truly reliable treatment of all types of acid related diseases became available in the form of proton pump inhibitors.[7] However, the problem of recurrence of peptic ulcers was still present in spite of superior inhibition of acid secretion during initial therapy.

At the same time that the first proton pump inhibitor (omeprazole) was patented (1979), a pathologist in Western Australia, Robyn Warren, was intrigued by the observation that bacteria were present at the sites of ulcer disease. He managed to convince a young resident, Barry Marshall, that this might provide an explanation for ulcer recurrence and the door to *Campylobacter* now *Helicobacter pylori* was opened.[8] Today it is recognized that infection by this organism is the major culprit in peptic ulcer disease and eradication is now commonly performed by a combination of a proton pump inhibitor and amoxycillin and clarithromycin.[9] This review will discuss the last two of these advances, the proton pump inhibitors and their mechanisms and profiles and the peculiarities of an organism that has chosen the stomach as its favoured habitat.

## THERAPEUTIC ASPECTS OF THE GASTRIC H, K ATPASE, THE GASTRIC ACID PUMP

### *Ion transport cycle of the acid pump*

The gastric H, K ATPase is an ion-motive ATPase, belonging to a family of these, called the P type ATPases. These ATPases transport ions as a function of a cycle of phosphorylation and dephosphorylation of the transport protein.[10] A subfamily of these P type ATPases are the $P_2$ ATPases, which transport small cations such as $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and $H^+$. In this $P_2$ group are the closely related Na, K and H, K ATPases distinguished by the presence of two, rather than one, sub-units, a catalytic, ion transporting sub-unit (the alpha sub-unit), and a stabilizing sub-unit (the beta sub-unit).[11] The Na, K and H, K ATPase are about 65% homologous. However, digoxin is absolutely selective for the Na, K ATPase whereas the proton pump inhibitors, owing to their chemical properties, are absolutely selective for the gastric H, K ATPase. These ATPases have six membrane inserted segments which are regarded as their core structure containing the ion transport pathway and either two such segments preceding the core structure to give eight membrane segments in the transition metal pumps ($P_1$ ATPases) or four segments following the core structure to provide 10 segments in the $P_2$ ATPases. The beta sub-unit has a single trans-membrane segment.

The gastric pump is present in high concentrations in the parietal cell and at much lower concentrations in the collecting duct of the kidney. Whereas maximal acidity in the parietal cell canaliculus is $< 1.0$, that in the kidney is never less than 4.0. Whatever the pathway of stimulation of the parietal cell, the common event is recruitment of the pump, present in cytoplasmic tubules into the membrane of the secretory canaliculus, to form microvilli lining this space, and activation of a KCl pathway to allow $K^+$ to access the external surface of the ATPase and secretion of $Cl^-$.

The coupling between the cycle of phosphorylation and dephosphorylation and ion transport is best described as due to a sequential set of conformational changes. In the absence of MgATP the pump is in a conformation able to bind MgATP and the outward ion ($H_3O^+$), the $E_1$ conformation. With the binding of MgATP and the ion, the protein is phosphorylated in a sequence common to all the P type ATPases and the ion moves into the membrane domain of the catalytic sub-unit ($E_1$-P). Within the membrane domain, the transport ion is enclosed so that it loses communication with the cytoplasmic side and has not yet opened to the outside face, the occluded conformation. The outside face now opens forming the $E_2$-P conformation. At this stage the outward ion is extruded and the inward ion binds. With this, the pump dephosphorylates ($E_2$ conformation), followed by the inward ion, become occluded. From this state, the ion-binding site opens to the interior and the inward ion is released as MgATP rebinds. Figure 1 illustrates these steps for the gastric H, K ATPase.

The pump is thought to pump hydronium ions ($H_3O^+$) rather than protons, since at high pH values it is able to pump $Na^+$. It is able to release $H_3O^+$ at a concentration of 160 mM, therefore generating an external pH of 0.8. This corresponds to a 4 million-fold gradient with respect to the inside of the parietal cell. It is able to

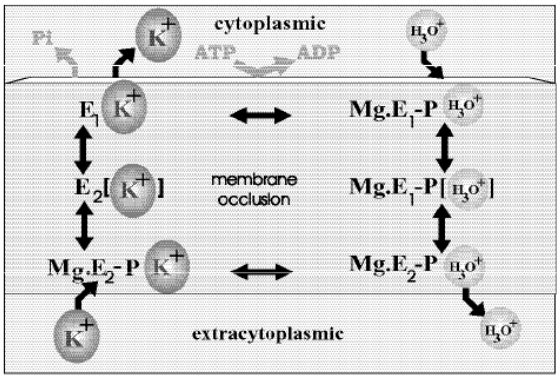


Figure 1. The reaction cycle of the gastric $H^+$, $K^+$ ATPase showing the $E_1$ form of the enzyme that is phosphorylated by ATP as the hydronium ion, $H_3O^+$, is bound. This form converts to $E_2$-P as the hydronium ion is released after passing through the trapped or occluded form. With binding of $K^+$, the cycle progresses by dephosphorylation, occlusion and release of $K^+$ to the cytoplasm from the $E_1$ form of the enzyme.

reabsorb $K^+$, generating an inward gradient of about tenfold. As such, this is the most powerful pump known in mammalian systems and generates an acidic space within the secretory canaliculus of the parietal cell at least a thousand-fold greater than anywhere else in the body. In the absence of $K^+$ on its external surface, the pump locks into the $E_2$-P conformation and does not turnover. Essential for activation of acid secretion is the presentation of $K^+$ to the outside surface of the pump. This occurs as the pump moves into the microvilli of the secretory canaliculus due to activation of a $K^+Cl^-$ pathway.

### *Structure of the ATPase*

The three-dimensional structure of these pumps is becoming clearer so that these conformational changes can be described in molecular terms. There are four distinct domains of the catalytic sub-unit: a large cytoplasmic domain, a stalk domain which connects the cytoplasmic to the membrane domain, and an extracytoplasmic domain. The cytoplasmic domain undergoes large conformational changes as the ion is transported outwards, changing from a loose structure to a more compact one as the ion moves to the external surface ($E_1$ to $E_2$ transition). The ion passes through the stalk and into the membrane domain, which also changes conformation as the ion binding site changes orientation from facing inward to facing outward. The cytoplasmic domain in the ATP and inward ion binding conformation ($E_1$) is a relatively open structure with at least two large separate lobes, corresponding to the loops between M2 and M3 and M4 and M5. The latter contains the phosphorylation site and MgATP binding region. In the ion site facing outward ($E_2$-P) conformation, the cytoplasmic domain is more compact and the separation between the lobes less evident.

The stalk contains the extensions of the membrane segments into the cytoplasmic region and forms a link for the passage of ions into the membrane. The membrane domain of the catalytic sub-unit of the H, K ATPase contains 10 inserted segments. The arrangement of these segments and the relative mobility of these is vital for the transport of the ions. The beta sub-unit has a single transmembrane segment with most of the protein on the outside surface of the pump, closely associated with the beginning of M8 in the catalytic sub-unit. It is N-glycosylated at seven sites. Its function appears to be stabilization of the membrane region of the alpha sub-unit, perhaps to lock TM8 in place so that $K^+$ can bind for inward transport. The external domain contains the loops connecting the membrane segments of the catalytic sub-unit and most of the beta sub-unit, with six or seven N-linked glycosylation sites. The largest loop between the membrane segments of the alpha sub-unit is between M7 and M8.

Different biochemical and mutagenesis experiments have provided evidence that there is a close association between M4 and M6, M5 and M7 and M6 and M9. Recent data on 2D and three dimensional crystals of the sarcoplasmic reticulum (sr) $Ca^{2+}$ ATPase have allowed placement of the membrane segments relative to each other and soon we should be able to place the side-chains of the membrane-inserted segments in their appropriate positions to reveal the ion pathways through the membrane. Prior to crystallization a series of site directed mutations have been made, which implicate M4, M5 and M6 as being intimately involved in the pathway transporting $H_3O^+$ outward and $K^+$ inward.

### *Activation of the H, K ATPase*

In the absence of stimulation, the vast majority of the pump molecules present in the parietal cell are in cytoplasmic membrane structures called tubulovesicles. Upon stimulation, these tubulovesicles transform into

the microvilli of an infolding of the parietal cell, termed the secretory canaliculus. Along with this transformation, the canalicular membrane acquires the property of a KCl conductance, thus supplying $K^+$ to the outside surface of the enzyme. This enables the enzyme to cycle, exporting $H^+$ and reabsorbing $K^+$, leaving the $Cl^-$ to accompany the secreted $H^+$. Various proteins, such as ezrin and Rab, generally involved in membrane recycling, have been identified in this activation mechanism. In humans, there is continuous recycling of the pump between the tubulovesicles and the secretory canaliculus, to give a basal acid secretion. Upon stimulation by food, there is a rapid recruitment of the pump into the canalicular membrane.

*Towards a three-dimensional map of the H, K ATPase*

Recently, three dimensional reconstructions have been published of the $H^+$ ATPase of Neurospora and the $Ca^{2+}$ ATPase of the sarcoplasmic reticulum.[12, 13] These confirm the presence of 10 membrane segments in the catalytic sub-unit of the $P_2$ type ATPases and show the likely presence of a vestibule at the luminal face of the enzymes. They both show a large cytoplasmic sector, a stalk and a membrane sector with a small external sector. The advent of three dimensional crystals of the sr $Ca^{2+}$ ATPase at 3.7 Å resolution (Toyoshima, C) will do much to advance the field. The model shown in Figure 2 illustrates the different associations by other methods that have been described in the Na, K, Ca and H, K ATPases, where M1, M2 and M3, M4 are juxtaposed and M4, M5, M6 and M8 are close to the transport pathway of the pump and M9, M10 and the beta sub-unit are peripheral to the transport sector.

*Putative transport mechanism*

The rate of ion transport by a P2 type ATPase is about $10^4$ times slower than through a channel and $10^2$ times

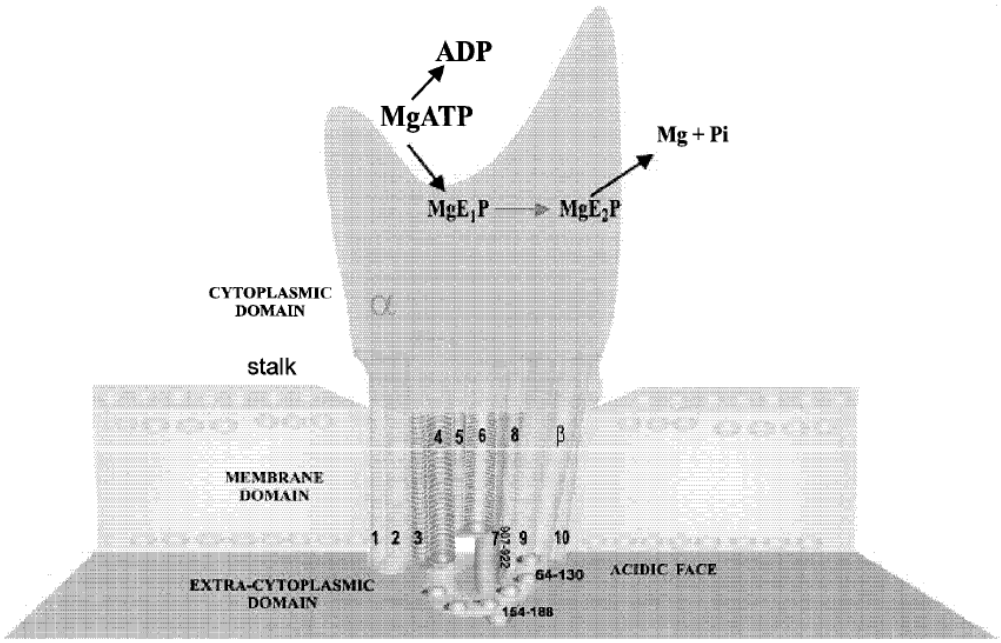


Figure 2. The probable arrangement of the membrane segments of the alpha and beta sub-units of the $H^+$, $K^+$ ATPase with interactions of M8 and beta residue regions numbered and M6 to M9 and M5 to M7 numbered at the end of M8 in the figure. The transport region of the membrane domain is considered to involve particularly M5 and M6 along with M4 and perhaps M8, as illustrated schematically. The cytoplasmic domain is visualized with a groove-admitting ATP and ion. The groove closes as the enzyme goes from $E_1$ to $E_2$. The majority of the beta sub-unit is on the outside surface and in the case of hog enzyme has 6 N-linked glycosylation sites. A vestibule is present bounded by M4, M5, M5–M6 loop M6 and M7, M8.

slower than through a carrier. This is due to the larger conformational changes necessary in a pump, compared to these other types of transporters. Several approaches have shown that these ATPases undergo conformational changes as a function of ion binding. For example, the fluorescence of FITC labelled H, K ATPase is rapidly quenched with the addition of $K^+$ as is the Na, K ATPase.[14, 15] The rate of exchange of tritium or deuterium is markedly altered as the $E_1$ conformation changes to $E_2$.[16, 17] Electron diffraction images of the pumps in the $E_1$ or $E_2$ state show large changes in shape of the cytoplasmic domain.[18, 19]

The location of ion transport is through the membrane domain. In the case of the Na, K, Ca and H, K ATPases, the ion transport pathway becomes occluded during the transport cycle, that is to say the ions are trapped within the membrane domain as the pump transits from $E_1$ to $E_2$ and vice versa, as if a gate on either side of the ion is closed during ion movement across the membrane domain. Site directed mutagenesis has identified several residues within the membrane domain as relevant to occlusion and transport. In particular, hydrophilic amino acids such as aspartic acid, glutamic acid, serine and threonine in M4, M5, M6 and perhaps M8 have been identified by this approach as related to ion flux through the membrane. M5 and M6 have also been identified as probably mobile within the membrane domain, as a function of loss of K binding after trypsinization.[20, 21]

The crystal structure of other ATPases of the same family suggests the presence of a luminal vestibule in the pump. By analogy to the structure of the K channel of bacteria that has been resolved, the vestibule contains binding sites for $K^+$ that remove the water of hydration from the ion as it moves through an inverted filter funnel and then moves through the transport site as the naked ion. In the K channel, this was recognized even before crystallization since hydrophobic amines blocked K movement. In the case of the H, K ATPase a series of hydrophobic amines, such as imidazopyridines, quinazolines or aza-indoles are all $K^+$ competitive inhibitors binding at the luminal surface.[22–24] Site directed mutations have identified residues in M3/M4 and M5/M6 as involved in binding an imidazo-pyridine, SCH28080. These may then be considered to form part of the vestibule admitting $K^+$.[25, 26]

If so, then the region containing M5 and M6 and the connecting loop between these membrane segments is a target for hydrophobic drugs able to penetrate the luminal vestibule of the pump. An example of this class of drug is the proton pump inhibitors.

## THE PROTON PUMP INHIBITORS

### *Mechanism of the proton pump inhibitors*

All the proton pump inhibitors have a similar core structure, a substituted pyridyl methylsulfinyl benzimidazole. The pyridine moiety gives them the property of being a protonatable weak base with $pK_a$ in the range of 4.0–5.0. In the un-protonated form they are membrane permeable prodrugs, so that they will concentrate in spaces more acidic than pH 4.0. In people, the only space where the pH can go lower than 4.0 is the canaliculus of the active gastric parietal cell. They are prodrugs since these compounds are subject to a $H^+$ catalysed conversion where they undergo a rearrangement to sulfenic acids and thence to sulfenamides.[27]

The latter are the product in acidic solutions but the former may be responsible for inhibition of the ATPase under acid secreting conditions. As a class, these drugs are therefore acid-unstable, requiring protection against gastric acidity for oral formulation and requiring reconstitution for i.v. administration. The four compounds available differ in terms of acid stability. Pantoprazole is the most stable, followed by omeprazole and lansoprazole and then rabeprazole. The first three have a pKa of $\approx 4.0$ whereas the pKa of rabeprazole is close to 5.0. The higher pKa of rabeprazole accounts for its relative acid instability but this drug will also concentrate 10-fold relative to the other compounds, hence it will concentrate 10-fold in an acidic space of pH 4.0, compared to no accumulation for the other drugs. At the pH of a fully activated parietal cell canaliculus, accumulation is theoretically 1000-fold for omeprazole, lansoprazole and pantoprazole and 10 000-fold for rabeprazole.

The acid space accumulation is a key feature of these drugs, giving them a large therapeutic index, since they are highly concentrated at their target, the site of acid secretion. Following this, their conversion to a reactive sulfenic acid or sulfenamide (which form disulphides with one or more of the accessible cysteines in the pump) provides an ability to covalently inhibit the gastric ATPase, providing acid inhibition of long duration even after the drug has disappeared from the blood. Both the sulfenic acid and the sulfenamide are permanent cations, and are therefore relatively

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