J. Membrane Biol. 61, 55–60

The Journal of
Membrane Biology

H^+/ATP Stoichiometry for the Gastric ($K^+ + H^+$)-ATPase

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Summary. The initial rate of ATP-dependent proton uptake by hog gastric vesicles was measured at pH's between 6.1 and 6.9 by measuring the loss of protons from the external space with a glass electrode. The apparent rates of proton loss were corrected for scalar proton production due to ATP hydrolysis. For vesicles in 150 mM KCl and pH 6.1, corrected rates of proton uptake and ATP hydrolysis were 639 ± 84 and 619 ± 65 nmol/min × mg protein, respectively, giving an H^+/ATP ratio of $1.03 \pm .07$. Furthermore, at all pH's tested the ratio of the rate of proton uptake to the rate of ATP hydrolysis was not significantly different than 1.0. No proton uptake (< 10 nmol/min × mg protein) was exhibited by vesicles in 150 mM NaCl at pH 6.1 despite ATP hydrolysis of 187 ± 46 nmol/min × mg (nonproductive hydrolysis). Comparison of the rates of proton transport and ATP hydrolysis in various mixture of KCl and NaCl showed that the H^+/ATP stoichiometries were not significantly different than 1.0 at all concentrations of K^+ greater than 10 mM. This fact suggests that the nonproductive rate is vanishingly small at these concentrations, implying that the measured H^+/ATP stoichiometry is equal to the enzymatic stoichiometry. This result shows that the isolated gastric ($K^+ + H^+$)-ATPase is thermodynamically capable of forming the observed proton gradient of the stomach.

Kay words: ($K^+ + H^+$)-ATPase, H^+/ATP stoichiometry, gastric microsomes, proton transport, HCl secretion, membrane transport.

Microsomes isolated from the fundic gastric mucosa have been shown to possess a K^+-stimulated, Mg^{2+}-dependent ATPase [7, 8, 9, 12]. Immunochemical and other studies have shown that these membranes are largely derived from oxyntic cells [3, 19]. Furthermore, the isolated membranes exist in a vesicular form and can transport (accumulate) protons in the presence of K^+, Mg^{2+} and ATP [15, 18, 21, 24, 26]. The possible relationship between this system, the ($K^+ + H^+$)-ATPase, and gastric HCl secretion is of great interest. Isolated gastric vesicles have been shown to form pH gradients of no more than 4.5 pH units [10, 17, 21], while intact oxyntic cells with an internal pH of approximately 7 must form and maintain a pH gradient of at least 6 pH units. Thus, some question has been raised as to the ability of the isolated ($K^+ + H^+$)-ATPase to establish the *in vivo* proton gradient [11, 14]. Since the magnitude of the pH gradient formed *in vitro* will be conspicuously limited by the permeability of the isolated vesicles to protons [15, 16], alternative methods for assessing the capability of the ($K^+ + H^+$)-ATPase to serve as the gastric H^+ pump are desired.

The maximal electrochemical gradient, $\Delta\bar{\mu}_i$, that can be formed by an ion-translocating ATPase is a function of the free energy of hydrolysis of ATP, ΔG_{ATP}, and the stoichiometry of ions transported to ATP hydrolyzed, N_i, as shown by the relationship

$$\Delta G_{ATP} \geqq N_i \Delta\bar{\mu}_i. \quad (1)$$

Conversely, knowledge of the free energy of hydrolysis of ATP and the electrochemical gradients formed allows a limit to be set on the molecular stoichiometry of the pump. By comparing the rate of proton uptake to the rate of ATP hydrolysis the H^+/ATP stoichiometry of the ($K^+ + H^+$)-ATPase can be determined. Provided the measurements are made under initial rate conditions, i.e., zero gradient, the measured rate of proton uptake should not be affected by the permeability of the membrane to protons. Thus, the experimentally determined H^+/ATP stoichiometry of the isolated ($K^+ + H^+$)-ATPase (N_i in Eq. (1)) can be used along with the *in vivo* values for ΔG_{ATP} and the chemical potentials of K^+ and H^+ in order to

0022-2631/81/0061-0055 $01.20

assess the competency of the isolated $(K^+ + H^+)$-ATPase.

Based on the measured H^+/ATP ratio of 1.0, the isolated $(K^+ + H^+)$-ATPase appears to be capable of forming the pH gradient observed *in vivo*. The results reported here differ with those of Sachs and coworkers [21, 23, 24, 26] and possible reasons for these discrepancies are discussed.

Materials and Methods

Preparation of Vesicles

Gastric vesicles were prepared from hog stomach as previously described [8, 17]. Vesicles banding in 20% sucrose (layer I) were frozen and stored at −20 °C until use. Prior to use, vesicles (~4 mg protein/ml) were thawed and diluted 60-fold into 20 mM glycylglycine-2 mM $MgSO_4$ buffer containing 150 mM KCl (unless stated otherwise) and incubated at 25 °C for 2 hr. Vesicles were then harvested at 145,000 × *g* for 55 min and resuspended to ~1.5/ml in the incubation buffer.

Proton Uptake Procedure

Temperature equilibrated vesicles (1.0 ml) were diluted with an equal volume of buffer into a water-jacketed beaker at 25 °C and the pH adjusted to the designated initial value with 10 mM NaOH. Specific conditions (pH, ionophores, etc.) are reported for individual experiments. The experiment was initiated by the addition of 20 µl of 100 mM Mg-ATP, whose pH was adjusted so as not to change the pH of the reaction mixture. Proton loss from the external solution was measured with a Radiometer PHM 84 meter and a C type electrode. Changes in the external pH were recorded on a Varian Recorder at 0.002 pH units/cm. Proton loss was calibrated by the addition of aliquots of 10 mM NaOH. Control experiments showed that the half-time for electrode response/mixing was less than 0.3 sec.

ATPase Assay

Vesicles were assayed under the identical conditions as the proton uptake experiments. After the addition of ATP, 0.3-ml aliquots were serially removed from the reaction mixture and quenched with 0.33 ml of ice cold 28% Cl_3COOH (wt/vol). Protein was removed by centrifugation, and 0.6 ml of the supernatant was taken for analysis of inorganic phosphate using the method of Sanui [25]. Calibration curves were constructed by assaying standard solutions of inorganic phosphate under identical conditions. Protein concentration was measured by the method of Lowry et al. [20] using bovine serum albumin as a standard.

Results

The addition of MgATP to a suspension of hog gastric vesicles, equilibrated with 150 mM KCl at pH 6.1, caused a rapid alkalinization of the external medium as shown in Fig. 1. Previous studies have used this alkalinization as evidence for vectorial proton transport and pH gradient formation [18, 21, 24, 26]; we will refer to this process as ATP-induced proton uptake. The response was not observed with vesicles equilibrated in 150 mM NaCl (Table 1) or for KCl-equilibrated vesicles in the presence of 5 µM nigericin (Fig. 1) or 4 mM zinc sulfate, an inhibitor of the microsomal ATPase [26]. Moreover, after ATP-induced proton uptake reached its maximum, addition of 5 µM nigericin caused a rapid acidification of the medium (Fig. 1). The apparent maximum alkalinization of the medium was not due to ATP depletion (*see* below), and we have interpreted it as the approach to a steady state as predicted by a pump-leak system [15].

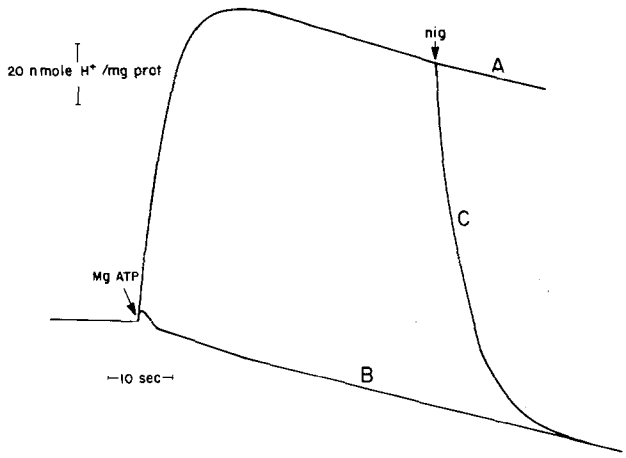

Fig. 1. ATP-dependent proton uptake by hog gastric vesicles. Vesicles (2 ml at 0.75 mg protein/ml) were equilibrated with 150 mM KCl, 2 mM $MgSO_4$ and 20 mM glycylglycine at pH 6.1 and 25 °C. At the indicated time 2 µmol of MgATP was added. Alkalinization of the external medium (H^+ uptake) is shown by an upward deflection. Proton uptake was calibrated by the addition of aliquots of 10 mM NaOH. Traces are shown for (*A*) untreated vesicles, (*B*) vesicles treated with 5 µM nigericin prior to adding ATP and (*C*) vesicles treated with 5µM nigericin at the indicated time (*nig*)

ATP-induced proton uptake was seen for KCl-equilibrated vesicles at all pH-s between 6.1 and 6.9 (Fig. 2 and Table 2). However, at the higher pH values the observed alkalinization was smaller than at pH 6.1 and was followed by a more pronounced acidification (Fig. 2). This acidification was shown to be caused by the scalar production of protons in the external medium as a result of ATP hydrolysis by the following observations: (i) the ratio of acid produced to ATP hydrolyzed (f_H) varied with pH (Table 2), being greater at more alkaline pH, in the same manner as the production of protons by ATP hydrolysis [1]; (ii) acid production was seen in the presence of nigericin where no pH gradient was formed; (iii) the addition of 5 µM nigericin to vesicles that had previously formed a pH gradient released the same amount of acid irrespective of the time of addition, showing that measurable dissipation of the pH gradient did not occur.

Table 1. Extent of proton uptake by gastric vesicles[a]

Internal buffer			External buffer			Valinomycin	Maximum extent of proton uptake (nmol/mg memb. prot.)
(mM)		(pH)	(mM)		(pH)		
150	KCl	6.1	150	KCl	6.1	−	103±22(12)
150	KCl	6.1	150	KCl	6.1	+	110±18(4)
150	KCl	6.5	150	KCl	6.5	−	72± 8(4)
150	KCl	6.5	150	KCl	6.5	+	75± 7(2)
150	KCl	6.9	150	KCl	6.9	−	57± 5(3)
150	KCl	6.9	150	KCl	6.9	+	70(1)
150	NaCl	6.1	150	NaCl	6.1	−	<2(5)
150	NaCl	6.1	150	NaCl	6.1	+	<6(1)
150	NaCl	6.1	75	NaCl		−	42± 2(3)
			75	KCl	6.1		
150	NaCl	6.1	75	NaCl		+	81± 2(2)
			75	KCl	6.1		

[a] Vesicles at 750 mg protein/ml were incubated at 25 °C with 2 mM $MgSO_4$, 20 mM glycylglycine and the indicated internal buffer. Vesicles were then transferred to the reaction chamber, where the external buffer conditions were sometimes altered as indicated in the external buffer column. Valinomycin was included at 1 μM where indicated. Proton uptake was initiated by the addition of 1 mM MgATP. Maximum extent of proton uptake refers to the measured peak value after correction for scalar proton production as described in the text. Values are shown as the mean±SE for the number of experiments shown in parentheses.

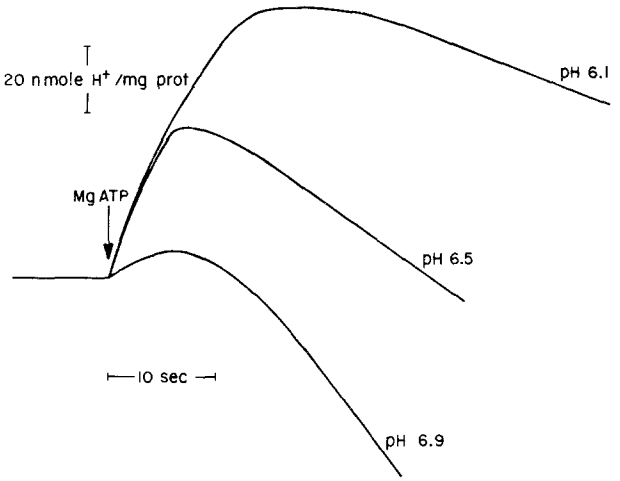

Fig. 2. The pH dependence of ATP-dependent proton uptake by hog gastric vesicles. Vesicles were equilibrated with 150 mM KCl, 2 mM $MgSO_4$ and 20 mM glycylglycine at 25 °C at the indicated pH. Proton loss from the external medium was measured as described in Fig. 1

Exemplary experiments showing the time course of inorganic phosphate production (ATPase) by gastric vesicles equilibrated with either KCl or NaCl are presented in Fig. 3. The rate of ATP hydrolysis was constant for at least the initial 40 sec during which the rates were determined. The mean rates of ATP hydrolysis, measured under the same conditions as proton uptake, are summarized in Table 2.

Since the rate of ATP hydrolyis was constant during the establishment and maintenance of the pH gradient (*cf.* Figs. 1 and 3), the rate of scalar proton production from ATP hydrolysis was assumed to be constant over the same time course. Thus, the observed rate of acid production after the establishment of a pH gradient was used to correct the observed rate of proton uptake. At pH 6.1, near the isoprotonic pH for ATP hydrolysis, this correction was less than 5% of the measured initial rate of proton uptake. However, the correction at higher pH was more severe. The corrected initial rates of proton uptake are reported in Table 2. Also shown in Table 2 are the H^+/ATP stoichiometries (rate of proton uptake/rate of ATP hydrolysis) for vesicles equilibrated under various conditions. In no case was the calculated H^+/ATP stoichiometry significantly greater than 1.0.

Since the H^+/ATP ratios shown in Table 2 were calculated using the total rate of ATP hydrolysis, the implied molecular stoichiometry would be an underestimation if a significant ATPase activity existed that was not coupled to proton transport. In previous studies the nonproductive ATPase rate in the absence of KCl was taken as a "basal rate" and subtracted from the rate at high KCl in order to obtain the

Table 2. Stoichiometry of proton uptake to ATP hydrolysis[a]

Reaction conditions	H^+ uptake[b]	ATPase	H^+ uptake[b]/ATPase	f_H[c]
	nmol/(mg prot × min)			
KCl, pH. 6.1	639± 84(14)	619± 65(4)	1.03±0.07(9)	0.05
KCl, pH 6.1+val	655± 77(3)	653± 80(3)	1.00±0.04(2)	0.05
NaCl, pH 6.1	<10(3)	187± 46(5)	<0.06(3)	0.04
KCl, pH 6.5	604±107(4)	576±114(2)	1.05±0.04(4)	0.24
KCl, pH 6.9	501± 14(4)	533(1)	0.94±0.03(4)	0.57

[a] All vesicles were equilibrated with 2 mM $MgSO_4$, 20 mM glycylglycine and 150 mM salt and pH as indicated. Proton uptake and ATPase rates were measured as described in the text.
[b] H^+ uptake was corrected for scalar proton production as described in the text.
[c] Ratio of the rate of scalar proton production to the rate of ATP hydrolysis.

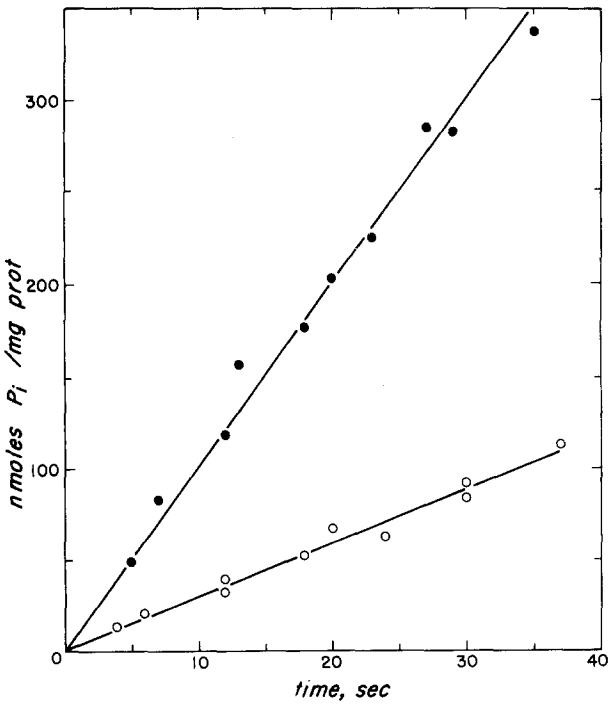

Fig. 3. The production of inorganic phosphate from ATP by hog gastric vesicles in KCl or NaCl. Vesicles (2.0 ml at 0.75 mg protein/ml) were equilibrated with 2 mM $MgSO_4$ and 20 mM glycylglycine, at pH 6.1 and 25 °C, containing either 150 mM KCl (●) or 150 mM NaCl (○). At the indicated times 0.3-ml aliquots of the reaction mixture were removed and quenched by the addition of 0.33 ml of ice cold 28 % trichloroacetic acid. Inorganic phosphate was measured as described in the Methods

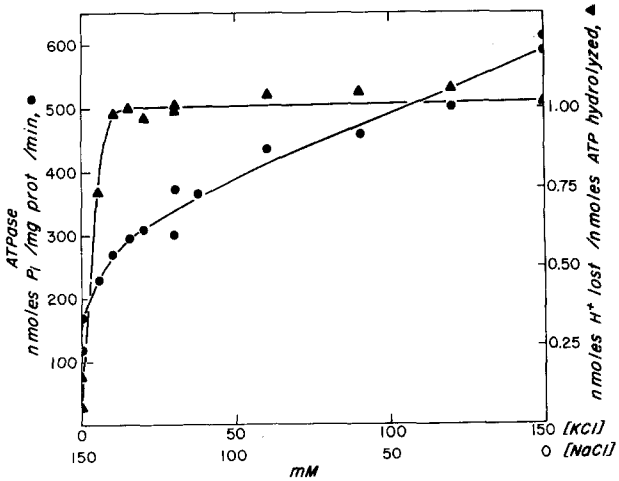

Fig. 4. Rate of ATP hydrolysis and H^+/ATP stoichiometry as functions of the ratio of NaCl to KCl. Vesicles (0.5 mg protein/ml) were equilibrated with the stated concentrations of NaCl and KCl, 2.0 mM $MgSO_4$ and 20 mM glycylglycine at pH 6.1 and 25 °C. The initial rates of ATP hydrolysis were measured from the formation of inorganic phosphate as described in Fig. 3. The initial rates of proton uptake were calculated from pH electrode measurements as described in the text. The H^+/ATP stoichiometry is equal to the rate of proton uptake divided by the rate of ATP hydrolysis

reported stoichiometry [24, 26]. Using the rate of ATP hydrolysis in 150 mM NaCl as a basal rate, the data of Table 1 give a molecular stoichiometry of no more than 1.5. In order to evaluate such a procedure, we measured the rates of ATP hydrolysis and proton transport for vesicles equilibrated in various mixtures of KCl and NaCl. As shown in Fig. 4, the rate of ATP hydrolysis increased continuously as the $[K^+]$ was increased. If a constant, K^+-independent, ATPase activity existed, the measured H^+/ATP stoichiometry based on the uncorrected rates should decrease continuously as the $[K^+]$ is increased. Figure 4 shows that this is clearly not the case as the H^+/ATP stoichiometry is 1.0 at 10 mM K^+ and greater.

In addition to measuring the H^+/ATP stoichiometry, the maximum extent of proton uptake was also measured. As with the rate of proton uptake, the extent was corrected for scalar proton production due to ATP hydrolysis. The results in Table 1 show that, in 150 mM KCl at pH 6.1, 103 nmol of protons are taken up per mg of protein. The extent of proton uptake was smaller at higher pH; however, this may be due in part to inaccuracies caused by the increased rate of scalar proton production at higher pH. It should be noted that while vesicles in 150 mM NaCl showed virtually no proton uptake, the addition of 75 mM KCl to the external buffer caused significant uptake of protons which was increased twofold upon the addition of the potassium ionophore valinomycin (Table 1).

Discussion

The secretion of a relatively voluminous flow of gastric acid requires the expenditure of considerable cellular energy. While the precise intracellular conditions are not known, combining reasonable values of pH 7 and 120 mM K^+ with the measured pH (1.0) and K^+ concentration (10 mM) of the gastric juice [4] gives concentration gradients across the secretory membrane of 10^6 and 12 for H^+ and K^+, respectively. The sum of the chemical potentials of these ion gradients is about −10 kcal/mol. The free energy of hydrolysis of ATP calculated from $\Delta G'_o$ (13) and the measured intracellular concentrations of ATP, ADP and P_i [5, 6] is about −13 kcal/mol. Therefore, as predicted by Eq. (1) for an electroneutral K^+/H^+ exchange pump where $N_{H^+}=N_{K^+}$, the ratio of H^+ transported to ATP hydrolyzed must be approximately 1 and cannot be as large as 2. If the gastric H^+ pump were an electrogenic proton translocating ATPase, a stoichiometry of 2 H^+/ATP would require that $\Delta\bar{\mu}_{H^+}$ across the secretory membrane be less negative than −6.5 kcal/mol (280 mV); i.e., a 10^6-fold concentration gradient of protons (−8.4 kcal/mol,

360 mV) could be established only if the membrane potential, $\Delta\psi$, across the secretory membrane were at least 80 mV (mucosal side negative). While there has been some disagreement on the polarity of the potential across the secretory surface [2, 27], none of the reported values even approach 80 mV. Thus, we would conclude that an electrogenic gastric H^+ pump would also require a H^+/ATP stoichiometry of less than 2.

As shown in Table 2 for vesicles equilibrated in KCl, the ratio of the rate of proton translocation to the rate of ATP hydrolysis is 1.0. This result is seriously discrepant with the value of 3.1 ± 0.4 reported by Sachs and coworkers [24, 26]. While the exact cause of the large discrepany is unknown, we point out that in the results of Schackmann et al. [26], the rates of ATP hydrolysis were approximately 10-fold lower and the maximum uptake of protons was 2.5 times less than in the present study. We therefore suggest that the use of a relatively inactive vesicle preparation may have given rise to some difficulties in calculating stoichiometries.

In the evaluation of the H^+/ATP stoichiometry it is important to establish whether, and to what extent, there are nonproductive components to the ATPase activity, i.e., ATPase activity not associated with H^+ transport. One approach has been to subtract ATPase activity in the absence of K^+, as a constant nonproductive rate of hydrolysis, from that in the presence of K^+. When this was done by Sachs et al. a H^+/ATP stoichiometry of 4.1 was obtained [24]. If we use our ATPase in 150 mM NaCl as the basal rate this correction gives a stoichiometry of 1.5 in 150 mM KCl. Furthermore the value increases at the $[K^+]$ is decreased. On the other hand when the total ATPase activity is compared to the rate of proton uptake the H^+/ATP ratio remains constant when the $[K^+]$ is greater than 10 mM. This suggests (i) that the subtraction of a constant, basal, ATPase activity is inappropriate, and (ii) that any K^+ independent ATPase activity is vanishingly small at 10 mM K^+ and above. Unfortunately, the data cannot rule out the existence of a K^+-stimulated nonproductive ATPase activity. Therefore with this caveat, we conclude that the molecular H^+/ATP stoichiometry of the $(K^+ + H^+)$-ATPase is 1.0. This value, but not the previously reported values of 3 and 4, is entirely consistent with the vesicular $(K^+ + H^+)$-ATPase being the primary gastric pump.

The data of Fig. 4 are also in accord with the hypothesis that the ATPase activity seen in the absence of K^+ is catalyzed by the $(K^+ + H^+)$-ATPase and not by a second enzyme. Were there to be a second ATPase operating in 150 mM NaCl, its activity would have to be completely inhibited by the addition of 5 mM K^+ in order for the observed coupling of ATP hydrolysis and H^+ transfer to occur. A single enzyme is also favored by studies on the phosphoenzyme intermediate associated with the $(K^+ + H^+)$-ATPase [22, 28]. The phosphoenzyme is formed from ATP and its hydrolysis and turnover are accelerated by K^+ [22]. The level of phosphoenzyme is highest in the absence of K^+ and under these conditions there is still a demonstrable hydrolysis of ATP (i.e., basal rate) occurring through the phosphoenzyme [22,28]. The existence of two distinct enzymes would require that the isolated phosphoenzyme is not the $(K^+ + H^+)$-ATPase.

Any explanation for the ATPase activity in the absence of K^+ must also account for the inability of the ATPase to transport protons as well. Recent reports have proposed that proton transport into the vesicles is associated with the phosphoenzyme and that the rate acceleration caused by internal K^+ is the result of K^+ binding to the phosphoenzyme and increasing its rate of hydrolysis [28, 29]. In addition, Wallmark et al. [29] propose that concomitant with phosphoenzyme hydrolysis, K^+ is transported out of the vesicular wall space. The nonproductive ATPase activity in the absence of K^+ may reflect the ability of H^+ to bind to the internal K^+site and catalyze the dephosphorylation of the enzyme. If translocation of the bound ion from the internal space to the external space is coupled to the hydrolysis, then in the absence of K^+ a proton would be transported out of the vesicles during the dephosphorylation step and the entire cycle would lead to no net H^+ transport.

This work was supported in part by a grant from the U.S. Public Health Service, AM10141. A preliminary communication of some of these results was presented at the Conference on Hydrogen Ion Transport in Epithelia held in Frankfurt am Main, Germany, July 8–12, 1980.

References

1. Alberty, R.A. 1968. Effect of pH and metal ion concentration on the equilibrium hydrolysis of adenosine triphosphate to adenosine diphosphate. *J. Biol. Chem.* **243**:1337–1343
2. Canosa, C.A., Rehm, W. 1968. Microelectrode studies of dog's gastric mucosa. *Biophys. J.* **8**:415–430
3. Chang, H., Saccomani,G., Rabon, E., Schackmann, R., Sachs, G. 1977. Proton transport by gastric membrane vesicles. *Biochim. Biophys. Acta* **464**:313–327
4. Davenport, H. 1969. Physiology of the Digestive Tract. p. 111. Yearbook Medical Publishers, Chicago
5. Durbin, R.P., Michelangeli, F., Nickel, A. 1974. Active transport and ATP in frog gastric mucosa. *Biochim. Biophys. Acta* **367**:177–189
6. Forte, J.G., Adams, P.H., Davies, R.E. 1963. Source of the gastric mucosal potential difference. *Nature (London)* **197**:874–876
7. Forte, J.G., Forte, T.M., Saltman, P. 1967. K^+-stimulated phosphatase of microsomes from mucosa. *J. Cell Physiol.* **69**:293–304

8. Forte, J.G., Ganser, A.L., Beesley, R., Forte, T.M. 1975. Unique enzymes of purified microsomes from pig fundic mucosa. *Gastroenterology* **69**:175–189
9. Forte, J.G., Ganser, A.L., Ray, T.K. 1976. The K^+-stimulated ATPase from oxyntic glands of gastric mucosa. *In*: Gastric Hydrogen Ion Secretion. D.K. Kasbekar, G. Sachs, and W. Rehm, editors. pp. 302–330. Marcel Dekker; New York
10. Forte, J.G., Lee, H.C. 1977. Gastric adenosine triphosphatases: A review of their possible role in HCl secretion. *Gastroenterology* **73**:921–926
11. Forte, J.G., Machen, T.E., Obrink, K.J. 1980. Mechanisms of gastric H^+ and Cl^- transport. *Annu. Rev. Physiol.* **42**:111–126
12. Ganser, A.L., Forte, J.G. 1973. K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochim. Biophys. Acta* **307**:169–180
13. Guynn, R.W., Veech, R.L. 1973. The equilibrium constants of the adenosine triphosphate hydrolysis and the adenosine triphosphate-citrate lyase reactions. *J. Biol. Chem.* **248**:6966–6972
14. Kidder, G.W., III. 1980. Theories on gastric acid secretion. *Ann. N.Y. Acad. Sci.* **341**:259–273
15. Lee, H.C., Breitbart, H., Berman, M., Forte, J.G. 1979. Potassium-stimulated ATPase activity and hydrogen transport in gastric microsomal vesicles. *Biochim. Biophys. Acta* **553**:107–131
16. Lee, H.C., Breitbart, H., Forte, J.G. 1980. The functional role of K^+ ATPase in proton transport by gastric microsomal vesicles. *Ann. N.Y. Acad. Sci.* **341**:297-311
17. Lee, H.C., Forte, J.G. 1978. A study of H^+ transport in gastric microsomal vesicles using fluorescent probes. *Biochim. Biophys. Acta* **508**:339–356
18. Lee, J., Simpson, G., Scholes, P. 1974. An ATPase from dog gastric mucosa: Changes of outer pH in suspensions of membrane vesicles accompanying ATP hydrolysis. *Biochem. Biophys. Res. Commun.* **60**:825–832
19. Limlomwongse, L., Forte, J.G. 1970. Developmental changes in ATPase and K^+-stimulated phosphatase of tadpole gastric microsomes. *Am. J. Physiol.* **219**:1717–1722
20. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275
21. Rabon, E., Chang, H., Sachs, G. 1978. Quantitation of hydrogen ion and potential gradients in gastric plasma membrane vesicles. *Biochemistry* **17**:3345–3353
22. Ray, T.K., Forte, J.G. 1976. Studies on the phosphorylated intermediate of K^+-stimulated ATPase from rabbit gastric mucosa. *Biochim. Biophys. Acta* **306**:169–180
23. Saccomani, G., Stewart, H.B., Shaw, D., Lewin, M., Sachs, G. 1977. Characterization of gastric mucosal membranes. IX. Fractionation and purification of K^+-ATPase-containing vesicles by zonal centrifugation and free-flow electrophoresis technique. *Biochim. Biophys. Acta* **465**:311–330
24. Sachs, G., Chang, H.H., Rabon, E., Schackmann, R., Lewin, M., Saccomani, G. 1976. A nonelectrogenic H^+ pump in plasma membranes of hog stomach. *J. Biol. Chem.* **251**:7690–7698
25. Sanui, H. 1974. Measurement of inorganic orthophosphate in biological materials: Extraction properties of butyl acetate. *Anal. Biochem.* **60**:489–504
26. Schackmann, R., Schwartz, A., Saccomani, G., Sachs, G. 1977. Cation transport by gastric H^+:K^+ ATPase. *J. Membrane Biol.* **32**:361–381
27. Villegas, L. 1962. Cellular location of the electrical potential difference in frog gastric mucosa. *Biochim. Biophys. Acta* **64**:359–367
28. Wallmark, B., Mardh, S. 1979. Phosphorylation and dephosphorylation kinetics of potassium-stimulated ATP phosphohydrolase from hog gastric mucosa. *J. Biol. Chem.* **254**:11899–11902
29. Wallmark, B., Stewart, H.B., Rabon, E., Saccomani, G., Sachs, G. 1980. The catalytic cycle of gastric $(H^+ + K^+)$-ATPase. *J. Biol. Chem.* **255**:5313–5319

Received 11 November 1980; revised 13 January 1981