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THE POTENCY OF SUBSTITUTED BENZIMIDAZOLES SUCH AS E3810, OMEPRAZOLE, Ro 18-5364 TO INHIBIT GASTRIC H^+,K^+-ATPase IS CORRELATED WITH THE RATE OF ACID-ACTIVATION OF THE INHIBITOR

MAGOTOSHI MORII, HIDEYUKI TAKATA, HIDEAKI FUJISAKI* and NORIAKI TAKEGUCHI†
Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, 930–01 and * Tsukuba Research Laboratories, Eisai Co. Ltd, Tsukuba, 300-26, Japan

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Abstract—The half maximal inhibitory concentrations (IC_{50}) of substituted benzimidazoles for the H^+,K^+-ATPase in hog gastric vesicles were measured by using the pyruvate kinase–lactate dehydrogenase-linked system in which hydrolysis of ATP was coupled with the oxidation of NADH. The vesicles were incubated in a solution containing a high concentration of KCl, valinomycin and Mg-ATP, and the intravesicular medium was acidified. The inhibitor was activated in the acidic medium and reacted with SH groups on the luminal (intravesicular) side of the ATPase. The active compound formed in the extravesicular medium (pH 6.11) was quenched by GSH. Under these conditions, IC_{50} of new compound E3810, 2[{4-(3-methoxypropoxy)-3-methylpyridine-2-yl}methyl-sulfinyl]-1*H*-benzimidazole sodium salt, was 0.072 μM and that of omeprazole was 0.47 μM at 25°. On the other hand, the rates of formation of active compounds, tetracyclic sulfenamide derivatives, from original substituted benzimidazoles in 0.1 N HCl (k) were determined by measuring optical density at the characteristic wavelengths of the active compounds. There was a good correlation between IC_{50} and k for various substituted benzimidazoles including E3810, methoxy derivative of E3810, omeprazole, Ro 18-5364, H compound, picoprazole and timoprazole. This fact suggests that the rate of the formation of the acid-activated compound is a main factor determining the potency of the inhibitor.

Omeprazole, a substituted benzimidazole, inhibits gastric H^+,K^+-ATPase, resulting in arrest of gastric acid secretion *in vivo* and *in vitro* [1–6]. Omeprazole itself is not an active compound. Transformation of omeprazole into the active compound in acidic environments such as lumen of gastric glands and the intravesicular space of gastric vesicles is necessary to inhibit the enzyme [7–14]. The active compound, the tetracyclic planar sulfenamide derivative of omeprazole, modifies the SH-group(s) of H^+,K^+-ATPase from the luminal side of gastric glands or the intravesicular side of gastric vesicles. The half maximal inhibitory concentration IC_{50} for H^+,K^+-ATPase activity in gastric vesicles is one of well-approved indexes for the potency of inhibitor. The intravesicular space of gastric vesicles mimics the acidic lumina of gastric glands. The intravesicular space is acidified by addition of valinomycin and Mg-ATP, and inhibitors are activated in the vesicles. However, an IC_{50} of 5.8 μM for omeprazole reported in acidified gastric vesicles at 37° [15] was 12 times larger than the value of 0.46 μM in gastric glands [16].

In this study, we tried to find a more reasonable value for IC_{50} in gastric vesicles, since we did not think that substituted benzimidazoles were less effective in gastric vesicles than gastric glands. The H^+,K^+-ATPase activity in gastric vesicles has been expressed by the amount of inorganic phosphate released by hydrolysis of ATP for a predetermined period such as 10 min after addition of Mg-ATP. If the inhibitory reaction started immediately after the addition of Mg-ATP and the rate of inhibition were constant, the extent of inhibition as a function of the inhibitor concentration in the predetermined period would reflect the inhibitory power of the inhibitor. However, the inhibitory process does not start immediately after the addition of Mg-ATP in the reaction medium in the cases of substituted benzimidazoles, because of the presence of unresponsive time which reflects the time necessary for producing enough amount of the active inhibitor in the intravesicular space. The unresponsive time depends on the acidity in the intravesicular space and the concentration of the inhibitor.

In this study, we continuously measured the enzymatic activity by using the coupled enzyme method [17, 18], and determined the half maximal inhibitory concentrations (IC_{50}) of substituted benzimidazoles such as a new compound E3810 (Fig. 1), omeprazole and Ro 18-5364 [19]. We also measured the rate of transformation from an original compound to the active compound in 0.1 N HCl. We found a good correlation between the IC_{50} values and the transformation rates. The good correlation suggests that we can estimate approximate IC_{50} values of substituted benzimidazoles that are newly synthesized for evaluation of their gastric antisecretory activities without measuring the H^+,K^+-ATPase activity in gastric vesicles and without sacrificing the lives of experimental animals.

MATERIALS AND METHODS

Preparation of hog gastric vesicles. Membrane vesicles containing the H^+,K^+-ATPase in 250 mM

† To whom correspondence should be addressed.

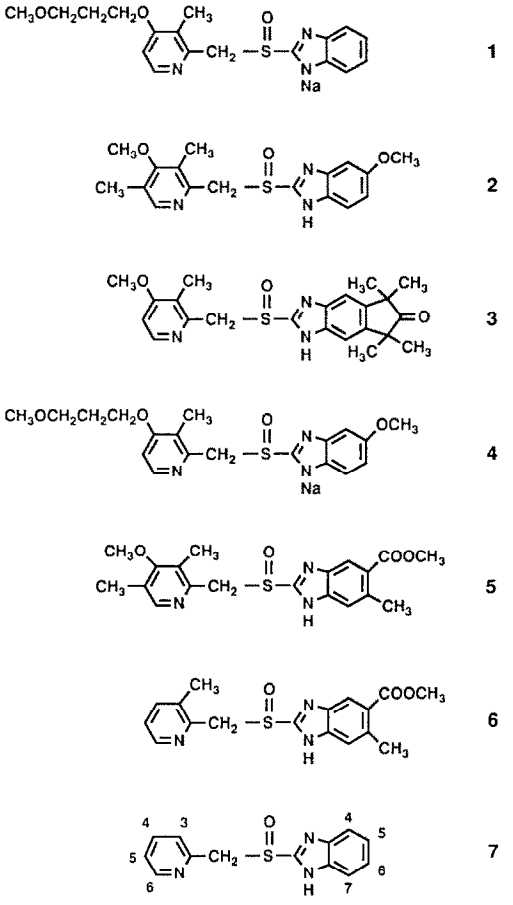

Fig. 1. Structures of substituted benzimidazoles used in this study. (1) E3810; (2) omeprazole; (3) Ro 18-5364; (4) methoxy derivative of E3810; (5) H compound; (6) picoprazole; (7) timoprazole.

sucrose were prepared from hog stomachs as described previously [20]. They were stored at −85° and used within 20 days. Protein concentration was determined by the method of Lowry *et al.* [21] with bovine serum albumin as standard. The specific activity of the K^+-ATPase measured by a conventional method in a solution containing 3 mM $MgSO_4$, 3 mM ATP, 40 mM Tris–HCl (pH 7.4), 10 mM KCl and 10 μg/mL of valinomycin was 42 ± 4 μmol of inorganic phosphate/mg of protein/hr (mean ± SE, N = 7) [22].

Activation of substituted benzimidazoles in HCl. Inhibitor was incubated in 0.1 N HCl at 25° at a final concentration of 10 μM. The time dependent change in the absorbance of the solution was measured with an Aminco DW-2C UV-VIS spectrophotometer. The optical density of acid-activated compound was measured at 335 nm for E3810, 345 nm for H compound, 370 nm for a methoxy derivative of E3810 and omeprazole, and 350 nm for picoprazole, timoprazole and Ro 18-5364. The wavelengths were determined as described later in this text.

Measurements of enzyme activity. The H^+,K^+-ATPase activity was assayed by the pyruvate kinase–lactate dehydrogenase-linked system in which hydrolysis of ATP is coupled with the oxidation of NADH [17, 18]. Final concentrations of reaction mixture were; 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)–KOH (pH 6.11), 155 mM KCl, 1 mM GSH, 0.2 mM $MgCl_2$, 6 μg/mL of valinomycin, 10 μg/mL of hog vesicles, 2 mM Mg-ATP, 27.5 IU/mL of lactate dehydrogenase, 10 IU/mL of pyruvate kinase, 0.8 mM phosphoenolpyruvate, and 0.4 mM NADH. The decrease in the amount of NADH was measured with the Aminco DW-2C UV-VIS spectrophotometer in the dual beam mode set at 340 and 500 nm at 25°. Under these conditions at pH 6.11 (not at pH 7.0 or 7.4), the activity of the coupling enzymes was enough to follow the production of ADP. In fact, addition of ADP instantly decreased the amount of NADH. When necessary, inhibitors at different concentrations were added just before the addition of Mg-ATP. The K^+ plus Mg^{2+}-ATPase activity was measured in the presence of 155 mM K^+ plus 2.2 mM Mg^{2+}. Since pyruvate kinase is a K^+-dependent enzyme, it is not possible to measure ATPase activities by omission of K^+ from the reaction mixture [18]. Therefore, we measured the apparent Mg^{2+}-ATPase activity in the presence of 5 mM K^+. That is, 10 mM MES–KOH was replaced with 10 mM MES–NaOH (pH 6.11) and 155 mM KCl with 5 mM K^+ plus 250 mM sucrose, and valinomycin was depleted from the incubation solution. Under the conditions, the contribution of the K^+-ATPase activity in tight vesicles to the apparent Mg^{2+}-ATPase activity can be neglected because of the low KCl conductance of the vesicle membrane and the low K^+ concentration gradient of 5 mM. The K^+-ATPase activity in leaky or broken vesicles, which consisted of a minor component in the vesicle preparation, contributed to the apparent Mg^{2+}-ATPase activity. However, values experimentally obtained for the apparent Mg^{2+}-ATPase activity were very small compared with those of the K^+ plus Mg^{2+}-ATPase activity as shown in Fig. 5 (curves a and b), therefore, this ambiguity of the apparent Mg^{2+}-ATPase activity did not induce significant error in evaluation of IC_{50} for the K^+-ATPase activity, which was defined as the difference between the K^+ plus Mg^{2+}-ATPase activity and the apparent Mg^{2+}-ATPase activity. The Mg^{2+}-ATPase activity was not affected by the treatment with substituted benzimidazoles [23]. Furthermore, the K^+-ATPase activity in leaky vesicles was not inhibited by inhibitors because of the presence of GSH and lack of the proton gradient across the vesicle membrane. Four determinations were averaged for each measurement.

Chemicals and drugs. Lactate dehydrogenase (550 IU/mg at 25° in 50% glycerol) and pyruvate kinase (200 IU/mg at 25° in 50% glycerol) were obtained from Boehringer (Mannheim, F.R.G.); 2-[{4-(3-methoxypropoxy)-3-methylpyridin-2-yl}-methylsulfinyl]-1*H*-benzimidazole sodium salt (E3810) and 5-methoxy-2-[{4-(3-methoxypropoxy)-3-methylpyridin-2-yl}methylsulfinyl]-1*H*-benzimidazole sodium salt (methoxy derivative of E3810) were synthesized in the Department of Organic Synthesis at Tsukuba, Eisai Co. (Tokyo, Japan); omeprazole was obtained from Fujisawa-Astra Co. (Osaka, Japan); Ro 18-5364 was obtained from F. Hoffman-La Roche Co. (Basle, Switzerland); H

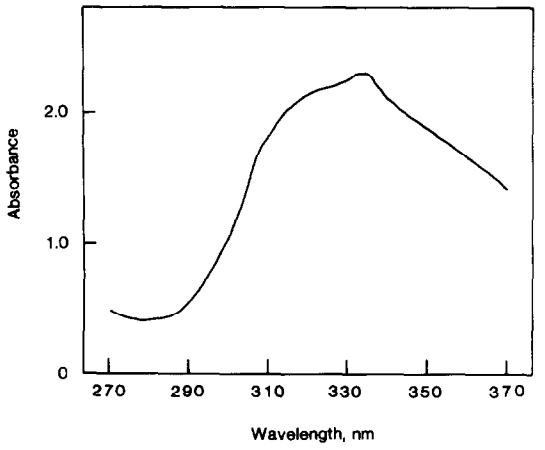

Fig. 2. The absorbance spectrum of the tetrafluoroborate salt of the acid-activated compound of E3810, a tetracyclic sulfenamide, at 25°. The compound was solubilized in dimethylsulfoxide at 20 μM.

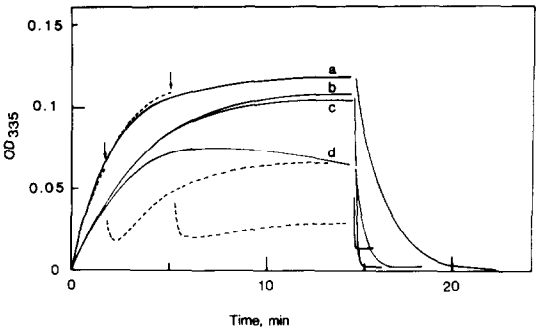

Fig. 3. Time dependent changes of absorbance of E3810 activated at 25°. At the zero time, 10 μM E3810 was added to HCl solutions. (a) 0.1 N HCl; (b) 0.01 N HCl; (c) 0.001 N HCl; (d) 0.0001 N HCl. At about 15 min, 100 μM GSH was added to these solutions. Broken lines show the changes in the absorbance when the pH of the solution was changed from 1 to 4 by addition of the necessary amount of NaOH at the times indicated by arrows.

compound, picoprazole and timoprazole were synthesized as previously described [24]. Tetrafluoroborate salts of sulfenamides of substituted benzimidazoles were synthesized as described elsewhere [9]; pepsin (activity: 800–1600 units/mg protein) was obtained from Wako Pure Chemicals (Osaka, Japan). Other chemicals used were highest purity available.

RESULTS

Transformation of substituted benzimidazoles into active compounds

The tetrafluoroborate salt of the acid-activated compound of E3810, a tetracyclic sulfenamide, was synthesized and solubilized in dimethylsulfoxide at 20 μM. Its absorption spectrum is shown in Fig. 2. The peak of absorption was observed at 335 nm. The absorption spectrum of E3810 solubilized at 10 μM in 0.1 N HCl also showed the peak at 335 nm (data not shown). The identity of both wavelengths indicates that the active compound formed in 0.1 N HCl was a cyclic sulfenamide, as previously shown for omeprazole (its peak wavelength of absorbance was 370 nm) [14]. Similarly, we found that the formation of sulfenamides from other substituted benzimidazoles such as methoxy derivative of E3810, H compound, picoprazole, timoprazole and Ro 18–3564. The formation could be followed by measuring absorbance at 370, 345, 350, 350 and 350 nm, respectively.

Figure 3 shows time dependent increases in absorbance at 335 nm of E3810 activated in 0.1, 0.01, 0.001 and 0.0001 N HCl, respectively. In 0.1–0.001 N HCl, the optical densities initially increased and then reached plateau levels about 10 min after addition of the inhibitor to the HCl solutions. The optical density in 0.0001 N HCl initially increased and then gradually decreased, indicating that the active compound of E3810 at high pH values was unstable. In the case of omeprazole, the optical density reached plateau levels at all HCl concentrations in the range from 0.1 N to 0.0001 N (data not shown). The broken lines in Fig. 3 show the time dependent changes of the optical density when the pH of the solution was increased from 1 to 4 at the arrows by addition of NaOH. These results show that the sulfenamide compound is less stable at higher pH values [8, 9]. The activating process in 0.1 N HCl is considered to be a pseudo-first order reaction because the proton concentration is much larger than the drug concentration (10 μM). In fact, the curve could be simulated with a single exponential equation. The rate constants of the formation of active compounds of E3810 and omeprazole in 0.1 N HCl were 0.0105 ± 0.0007 (mean ± SE, N = 5) and 0.0036 ± 0.0001/sec (N = 3), respectively. The rates of other substituted benzimidazoles used are shown in Table 1. The rates of transformation of E3810 and omeprazole were approximately independent of the pH values of the media in the range from pH 1 to 4, although the production of the active compound decreased as the pH increased.

Addition of 100 μM GSH quickly decreased the optical density (Fig. 3). The half time for the decrease in 0.1 N HCl was 79 sec for E3810, 72 sec for methoxy derivative of E3810, 70 sec for omeprazole and 67 sec for Ro 18–5364, indicating that substituents in pyridine and benzimidazole rings contribute to the reactivity of the sulfenamides with GSH. The reactivity of the active compound of E3810 with GSH in the experiment shown in Fig. 3. and the other four experiments was in the order of pH 4 > pH 3 > pH 2 > pH 1 without an exception.

The sulfenamide of E3810 also reacted with pepsin (Fig. 4). This finding indicates the possibility that substituted benzimidazoles such as E3810 and omeprazole [14] modulates the peptidase activity *in vivo*.

Inhibition of the K^+-ATPase activity by active compounds of substituted benzimidazoles

From the decrease in the optical density of NADH coupled with ATP regeneration, the K^+-ATPase

Table 1. IC_{50} values for inhibition of H^+,K^+-ATPase in gastric vesicles and rate constants for acid-activation in 0.1 N HCl of various substituted benzimidazoles at 25°. The rate constants of activation were measured at 10 μM of inhibitor

Inhibitors	IC_{50} (μM) in gastric vesicles	Rate constants of activation in 0.1 N HCl (/sec)
Timoprazole	15.0	8.5×10^{-4}
Picoprazole	3.3	2.3×10^{-3}
Omeprazole	0.47	3.6×10^{-3}
Methoxy E3810	0.16	4.8×10^{-3}
H compound	0.16	4.8×10^{-3}
E3810	0.072	1.05×10^{-2}
Ro 18-5364	0.056	1.2×10^{-2}

activity was measured continuously (Fig. 5). Inhibitors used in this study did not disturb the coupled enzyme system. The inhibitor was added just before the addition of Mg-ATP. The vesicles started to take up protons immediately after the addition of Mg-ATP (data not shown). However, inhibition did not start immediately after the addition of Mg-ATP. Figure 6 shows that the inhibition starts about 5 min for 1 μM E3810 and 9 min for 0.1 μM E3810 after the addition of Mg-ATP. These results indicate that there is a pH buffering action in the intravesicular medium [14] and some critical amount of the activated compound must be produced in the intravesicular space before the start of inhibition of the ATPase. In this experiment, the active compound formed in the extravesicular space (pH 6.11) was quenched by GSH (1 mM). Figure 7 shows effects of E3810 and omeprazole on the K^+-ATPase activity. The half maximal inhibitory concentration (IC_{50}) was 0.072 μM for E3810 and 0.47 μM for omeprazole. The IC_{50} values for other compounds are listed in Table 1.

The rate constants of the formation of active compounds in 0.1 N HCl are plotted against their IC_{50} values in gastric vesicles in Fig. 8. We found a good correlation between these two parameters ($r = -0.968$).

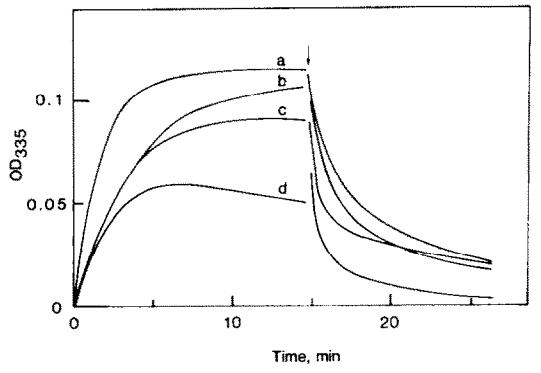

Fig. 4. Reaction of the acid-activated E3810 with pepsin. The absorbance was measured at 335 nm at 25°. At the arrow, pepsin at a final concentration of 2.5 mg/mL was added. (a) 0.1 N HCl; (b) 0.01 N HCl; (c) 0.001 N HCl; (d) 0.0001 N HCl.

DISCUSSION

Direct addition of preactivated compound of substituted benzimidazoles to a buffered vesicle solution decomposes the active compound. Therefore, the activated compound is necessary to be produced only

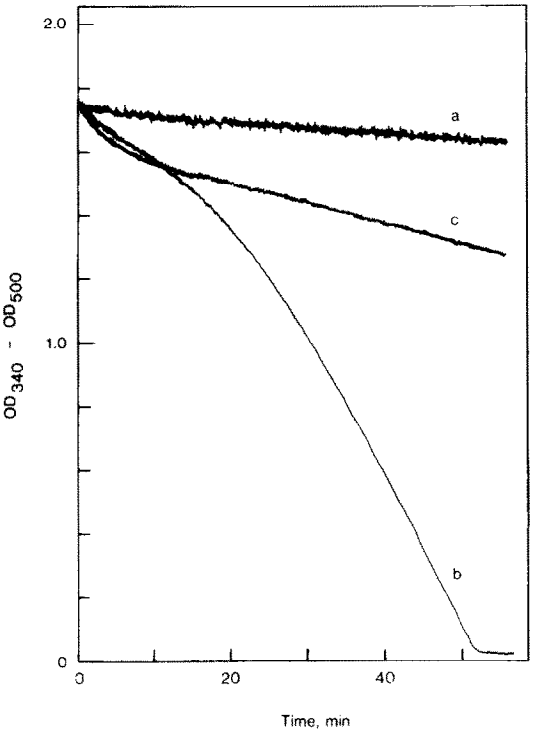

Fig. 5. Time-dependent changes of absorbance of NADH ($O.D._{340}$–$O.D._{500}$) in the vesicle solution (10 μg/mL). The composition of the solution for measurements of ATPase activities is described under Materials and Methods. Mg-ATP was added as the zero time. Curve (a) shows the change in the presence of 5 mM KCl and absence of valinomycin. Curve (b) and (c) show those in the presence of 155 mM KCl plus 6 μg/mL of valinomycin. In curve (c) 2 μM E3810 was added at the zero time. The relative residual activity (%) for the K^+-ATPase activity, for example, at 2 μM was defined as 100 × [the slope of curve (c) − the slope of curve (a)]/[the slope of curve (b) − the slope of curve (a)].

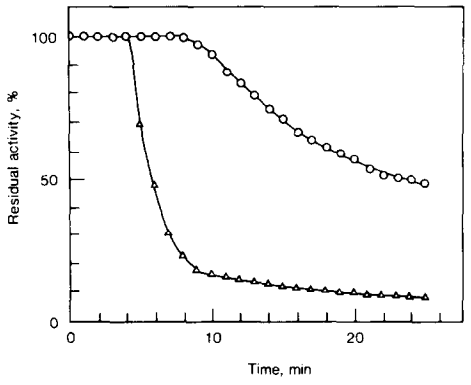

Fig. 6. Inhibition of the K^+-ATPase activity by E3810. Residual activities, defined in the legend to Fig. 5, are shown as a function of the incubation time after the addition of Mg-ATP and E3810. (○) 0.1 μM E3810; (△) 1.0 μM E3810.

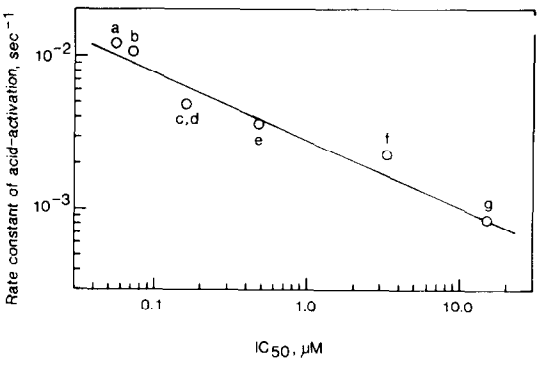

Fig. 8. The relationship between the IC_{50} values for inhibition of the K^+-ATPase in hog gastric vesicles and the rate constants of acid-activation in 0.1 N HCl of various substituted benzimidazoles at 25°. The line was drawn by the least squares method. (a) Ro 18-5364; (b) E3810; (c) methoxy derivative of E3810; (d) H compound; (e) omeprazole; (f) picoprazole; (g) timoprazole.

in the acidic intravesicular space. The conditions under which substituted benzimidazoles are transformed into activated compounds in the intravesicular space closely resembles those in the stimulated gastric mucosa. The hydrolysis of ATP by H^+,K^+-ATPase in intact vesicles resulted in the formation of a pH gradient in the presence of 2 mM Mg-ATP, 155 mM KCl and valinomycin [14, 24]. Furthermore, under the conditions, the K^+-ATPase activity in intact, ion-impermeable vesicles is stimulated by high concentrations of K^+, although that in leaky vesicles is inhibited by high K^+ [25]. IC_{50} values of several substituted benzimidazoles including a new H^+,K^+-ATPase inhibitor, E3810, in hog gastric vesicles were determined by the coupled enzyme method. In this method, the slope of the decrease of NADH absorbance coupled directly with the rate of the hydrolysis of ATP. Thus we can continuously follow the time dependent change in the enzyme activity. This method is suitable for determining the effect of inhibitors such as substituted benzimidazoles in which the inhibition did not proceed linearly with time; no inhibition occurred during the initial activating period. In the conventional method, inorganic phosphate released by hydrolysis of ATP for a predetermined time such as 10 min was determined after the termination of the ATPase reaction. Therefore, the experiments for obtaining the concentration–response curve included different unresponsive times for the predetermined incubation time, resulting in a larger IC_{50} value than the true value.

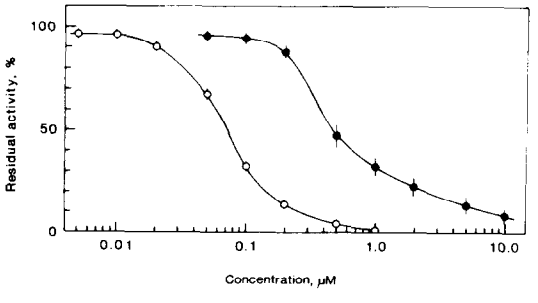

Fig. 7. Effects of E3810 and omeprazole on K^+-ATPase activity in gastric vesicles (10 μg/mL). The averaged residual activities in the 10-min period from 20 to 30 min after the addition of Mg-ATP are plotted as a function of concentration of inhibitors. Data are mean ± SE for four determinations. (○) E3810; (●) omeprazole.

When omeprazole was activated only in the intravesicular space, it has been shown that complete inhibition of H^+,K^+-ATPase required binding of about 2 moles of omeprazole per phosphorylation site [11, 12]. Complete inhibition in the absence of the intravesicular acidification (in leaky vesicles) required long-time incubation and binding of 13 moles of omeprazole per phosphorylation site [11]. In the present study we added GSH to the medium to quench the active compound formed in the medium (pH 6.11). The present IC_{50} value of omeprazole was 0.47 μM, which was 12 times smaller than the value of 5.8 μM obtained by the conventional method under the conditions when the intravesicular space was acidified [15]. Our result was comparable to a reported value of 0.48 μM for inhibition of acid secretion in isolated rabbit gastric glands measured at 37° [16]. The IC_{50} value of E3810 for the H^+,K^+-ATPase activity (0.072 μM) was 1.6 times smaller than the value of 0.12 μM for aminopyrine uptake in isolated rabbit gastric glands (data not shown). However, the present IC_{50} value of omeprazole in vesicles was still larger than that in isolated hog parietal cells ($IC_{50} = 1–5 \times 10^{-8}$ M) [26]. One possible explanation for this discrepancy is that the intravesicular pH, which was reported to be 2.4–3.9 [27], is larger than the pH in the occluded intracellular canaliculi of isolated parietal cells. In fact, leakage of protons from the vesicles has been observed at 25° [14, 28]. Another is that the IC_{50} value at 37° would be smaller than the present value measured at 25°, however, we gave up experiments at 37° because of increased leakage of protons from

the vesicles. The difference in IC_{50} values between isolated glands and isolated cells may reflect the difference in their tightness of occluded intracellular canaliculi.

A derivative of E3810 which has methoxy group at the 5-position in the benzimidazole ring was about two times less potent than E3810 (IC_{50} values in Table 1). But, omeprazole, which has a methoxy group at the 5-position in the benzimidazole ring, was two times more potent than a corresponding compound that lacks methoxy group at the 5-position (IC_{50} values of 0.5 and 1 μM for rabbit gastric glands *in vitro*, respectively) [29]. Therefore, methoxypropoxy group in the pyridine ring of E3810 gives an interesting character, which is different from that of omeprazole.

The sulfenamide derivatives can react with SH-groups of H^+,K^+-ATPase, dithiothreitol, 2-mercaptoethanol, GSH and even pepsin. The reactivity of sulfenamides with GSH was in the order of Ro 18-5364 > omeprazole > methoxy derivative of E3810 > E3810. The order is different from the order of potency of these inhibitors (Table 1), suggesting that the potency of inhibitors is not determined by the SH-reactivity of the sulfenamides. We found that there is a good correlation between the IC_{50} values for isolated hog gastric H^+,K^+-ATPase and the rate constants of the formation of active compounds of various substituted benzimidazoles in 0.1 N HCl. This is, the formation of the active compound in the acidic vesicular space is rate determining in the inhibition of H^+,K^+-ATPase in the gastric vesicle system, although other minor factors also affect the IC_{50}. The present study suggests that without measuring the enzyme activity, we can estimate the approximate half maximal inhibitory concentration of substituted benzimidazoles from the measurement of the rate of formation of sulfenamides in 0.1 N HCl.

REFERENCES

1. Lind T, Cederberg C, Ekenved G, Haglund U and Olbe L, Effect of omeprazole—a gastric proton pump inhibitor—on pentagastrin stimulated acid secretion in man. *Gut* **24**: 270–276, 1983.
2. Larsson H, Carlsson E, Junggren U, Olbe L, Sjöstränd SE, Skånberg I and Sundell G, Inhibition of gastric acid secretion by omeprazole in the dog and rat. *Gastroenterology* **85**: 900–907, 1983.
3. Sewing K-Fr, Harms P, Schulz G and Hannemann H, Effect of substituted benzimidazoles on acid secretion in isolated and enriched guinea pig parietal cells. *Gut* **24**: 557–560, 1983.
4. Cornelis BH, Lamers HW, Lind T, Moberg S, Jansen KMB and Olbe L, Omeprazole in Zollinger–Ellison syndrome. Effects of a single dose and of long-term treatment in patients resistant to histamine H_2-receptor antagonists. *New Engl J Med* **310**: 758–761, 1984.
5. Yamamoto O, Okada Y and Okabe S, Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. *Dig Dis Sci* **29**: 394–401, 1984.
6. Man WK, Thompson JN, Baron JH and Spencer J, Histamine and duodenal ulcer: effect of omeprazole on gastric histamine in patients with duodenal ulcer. *Gut* **27**: 418–422, 1986.
7. Im WB, Sih JC, Blakeman DP and McGrath JP, Omeprazole, a specific inhibitor of gastric $(H^+ + K^+)$-ATPase, is a H^+-activated oxidizing agent of sulfhydryl groups. *J Biol Chem* **260**: 4591–4597, 1985.
8. Figala V, Klemm K, Kohl B, Krüger U, Rainer G, Schaefer H, Senn-Bilfinger J and Sturm E, Acid activation of $(H^+ + K^+)$-ATPase inhibiting 2-(2-pyridylmethyl-sulphinyl)-benzimidazoles: isolation and characterization of the thiophilic 'active principle' and its reactions. *J Chem Soc Chem Commun* 125–127, 1986.
9. Lindberg P, Nordberg P, Alminger T, Brändström A and Wallmark B, The mechanism of action of the gastric acid secretion inhibitor omeprazole. *J Med Chem* **29**: 1327–1329, 1986.
10. Beil W, Hannemann H, Mädge S and Sewing K-Fr, Inhibition of gastric K^+/H^+-ATPase by acid-activated 2-((2-pyridylmethyl)sulphinyl)-benzimidazole products. *Eur J Pharmacol* **133**: 37–45, 1987.
11. Keeling DJ, Fallowfield C and Underwood A, The specificity of omeprazole as an $(H^+ + K^+)$-ATPase inhibitor depends upon the means of its activation. *Biochem Pharmacol* **36**: 339–344, 1987.
12. Lorentzon P, Jackson R, Wallmark B and Sachs G, Inhibition of $(H^+ + K^+)$-ATPase by omeprazole in isolated gastric vesicles requires proton transport. *Biochim Biophys Acta* **897**: 41–51, 1987.
13. Fryklund J, Gedda K and Wallmark B, Specific labeling of gastric H^+,K^+-ATPase by omeprazole. *Biochem Pharmacol* **37**: 2543–2549, 1988.
14. Morii M, Takata H and Takeguchi N, Acid activation of omeprazole in isolated gastric vesicles, oxyntic cells, and gastric glands. *Gastroenterology* **96**: 1453–1461, 1989.
15. Satoh H, Inatomi N, Nagaya H, Inada I, Nohara A, Nakamura N and Maki Y, Antisecretory and antiulcer activities of a novel proton pump inhibitor AG-1749 in dogs and rats. *J Pharmacol Exp Ther* **248**: 806–815, 1989.
16. Wallmark B, Jaresten BM, Larsson H, Ryberg B, Brändström A and Fellenius E, Differentiation among inhibitory actions of omeprazole, cimetidine, and SCN^- on gastric acid secretion. *Am J Physiol* **245**: G64–G71, 1983.
17. Koepsell H, Characteristics of antibody inhibition of rat kidney $(Na^+ - K^+)$-ATPase. *J Membr Biol* **44**: 85–102, 1978.
18. Scharschmidt BF, Keeffe EB, Blankenship NM and Ockner RK, Validation of a recording spectrophotometric method for measurement of membrane-associated Mg- and NaK-ATPase activity. *J Lab Clin Med* **93**: 790–799, 1979.
19. Sigrist-Nelson K, Krasso A, Muller RKM and Fischli AE, Ro 18-5364, a potent new inhibitor of the gastric $(H^+ + K^+)$-ATPase. *Eur J Biochem* **166**: 453–459, 1987.
20. Takeguchi N, Joshima R, Inoue Y, Kashiwagura T and Morii M, Effects of Cu^{2+}-*o*-phenanthroline on gastric $(H^+ + K^+)$-ATPase. Evidence for opening of a closed anion conductance by S-S cross-linking. *J Biol Chem* **258**: 3094–3098, 1983.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Asano S, Iino T, Tabuchi Y and Takeguchi N, Properties of light and heavy vesicles simultaneously prepared from hog gastric mucosae. *J Biochem (Tokyo)* **103**: 672–677, 1988.
23. Wallmark B, Larsson H and Humble L, The relationship between gastric acid secretion and gastric H^+,K^+-ATPase activity. *J Biol Chem* **260**: 13681–13684, 1985.
24. Takeguchi N and Yamazaki Y, Disulfide cross-linking of H,K-ATPase opens Cl^- conductance, triggering proton uptake in gastric vesicles. Studies with specific inhibitors. *J Biol Chem* **261**: 2560–2566, 1986.

25. Lorentzon P, Sachs G and Wallmark B, Inhibitory effects of cations on the gastric H^+,K^+-ATPase. A potential-sensitive step in the K^+ limb of the pump cycle. *J Biol Chem* **263**: 10705–10710, 1988.
26. Mårdh S, Song YH and Wallmark B, Effects of some anti-secretory drugs on acid production, intracellular free Ca^{2+}, and cyclic AMP production in isolated pig parietal cells. *Scand J Gastroenterol* **23**: 977–982, 1988.
27. Rabon E, Chang H and Sachs G, Quantitation of hydrogen ion and potential gradients in gastric plasma membrane vesicles. *Biochemistry* **17**: 3345–3353, 1978.
28. Rabon E, Takeguchi N and Sachs G, Water and salt permeability of gastric vesicles. *J Membr Biol* **53**: 109–117, 1980.
29. Brändström A, Lindberg P and Junggren U, Structure activity relationships of substituted benzimidazoles. *Scand J Gastroenterol* **20** (Suppl. 108): 15–22, 1985.