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STUDIES ON THE MECHANISM OF ACTION OF OMEPRAZOLE

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Abstract—The effects of omeprazole on preparations of pig gastric $(H^+ + K^+)$-ATPase have been studied. Omeprazole was found to inhibit the $(H^+ + K^+)$-ATPase activity in a time-dependent manner. Inhibition was more pronounced at pH 6.1 compared with pH 7.4 and decreased as the concentration of $(H^+ + K^+)$-ATPase preparation increased. The potency of omeprazole was therefore highly dependent upon the conditions used. When pre- incubated with $(H^+ + K^+)$-ATPase preparation (30 μg protein/ml) for 30 min at 37° and pH 6.1, omeprazole inhibited the $(H^+ + K^+)$-ATPase activity with an IC_{50} of 3.9 μM. This inhibition was shown to be irreversible in nature. Whilst omeprazole itself was not very potent as an inhibitor of the $(H^+ + K^+)$-ATPase activity at pH 7.4 (IC_{50} = 36 μM), transient acidification of omeprazole resulted in the formation of a compound(s) which produced marked inhibition at this pH (IC_{50} = 5.2 μM). The effects of omeprazole in the absence of acidification may have resulted from the rate-limiting formation of this compound. Radiolabelled omeprazole was shown to incorporate into the $(H^+ + K^+)$-ATPase preparation in a time-dependent and pH-dependent manner. Omeprazole, radiolabelled in three separate positions (the sulphur atom and the two adjacent carbon atoms), incorporated with equivalent time courses suggesting that the incorporation did not involve a fragmentation of the omeprazole molecule. Under conditions shown to produce a 50% inhibition of $(H^+ + K^+)$-ATPase activity, [^{14}C] omeprazole had incorporated to a level of 4–5 nmoles/mg protein. Incorporation continued beyond the point required to produce 100% inhibition of $(H^+ + K^+)$-ATPase activity and reached 30 nmoles/mg protein after 5 hr. Prior acidification of the omeprazole resulted in a more rapid initial rate of incorporation although the final level of incorporation was lower than for omeprazole. Omeprazole was also shown to interact with the $(Na^+ + K^+)$-ATPase from dog kidney. Omeprazole inhibited the $(Na^+ + K^+)$-ATPase activity (IC_{50} = 186 μM). Acid-degraded omeprazole inhibited the $(Na^+ + K^+)$-ATPase activity with greater potency (IC_{50} = 19 μM) and was also shown to incorporate into this enzyme preparation.

Some substituted benzimidazoles, such as omeprazole and picoprazole (Fig. 1) have been shown to be potent inhibitors of gastric acid secretion both *in vitro* [1, 2] and *in vivo* [3, 4]. The mechanism by which this occurs has been postulated to involve a direct inhibition of the gastric proton pump, the $(H^+ + K^+)$-ATPase [5].

Picoprazole inhibited $(H^+ + K^+)$-ATPase activity in gastric membrane preparations in a time-dependent manner [1]. Such inhibition was most readily explained by an irreversible interaction of picoprazole with the enzyme. Consistent with this, when [benzimidazole-2-^{14}C]picoprazole was incubated with the enzyme preparation, radiolabel was found to incorporate into a 100 kDa peptide [1].

Incorporation of [^{14}C]omeprazole into preparations of $(H^+ + K^+)$-ATPase resulted in a parallel (equimolar) reduction of titratable sulphydryl groups, suggesting that the inhibitory effects of omeprazole were due to its ability to react with sulphydryl groups on the enzyme essential for catalytic activity [6]. Furthermore, the observation that both the inhibition of enzyme activity and the incorporation of radiolabel with omeprazole could be reversed by mercaptoethanol, suggested that the omeprazole interacted with sulphydryl groups on the enzyme through the formation of disulphide links [6]. Beil and Sewing [7] have reported that picoprazole and omeprazole inhibited the $(H^+ + K^+)$-ATPase activity of preparations from guinea pig isolated parietal cells. However, in contrast to other work they reported that the interaction of omeprazole with the $(H^+ + K^+)$-ATPase was of a freely reversible nature. This conclusion was derived from an experiment in which omeprazole, pre-incubated with $(H^+ + K^+)$-ATPase preparation at high protein concentration, and diluted 100-fold for the determination of $(H^+ + K^+)$-ATPase activity, showed little inhibition compared with the same concentration of omeprazole pre-incubated and assayed at low protein concentration.

The inhibition of $(H^+ + K^+)$-ATPase activity by omeprazole has been shown to be highly dependent upon pH [2]. Effective inhibition was only achieved if omeprazole was pre-incubated with enzyme at pH values below neutrality. Such behaviour might occur if either omeprazole or the enzyme were better able to react with each other in a protonated form. Alternatively, omeprazole may be converted, in a reaction which is favoured by acidic conditions, to an intermediate which itself inhibits the $(H^+ + K^+)$-ATPase. Indeed, omeprazole has been reported to be unstable in acidic solutions [3]. Early speculations on such acid-degradation suggested mechanisms which

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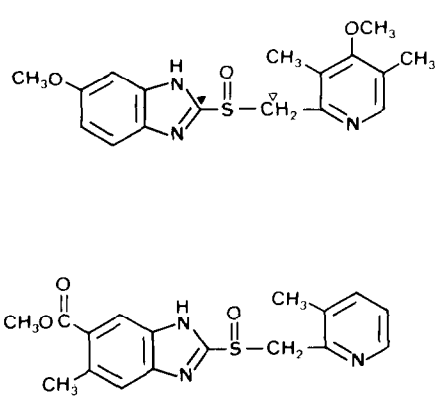

Fig. 1. Structure of omeprazole (A) and picoprazole (B). The position of radiolabel in [^{14}C]omeprazole is also indicated; benzimidazole-2 (▼), α-methylene (▽).

involved cleavage of the omeprazole molecule, particularly around the reactive sulphoxide moiety. Such a mechanism might not lead to the incorporation of the whole omeprazole molecule into the protein. To investigate this possibility, we have followed the incorporation of omeprazole radiolabelled in one of three positions (the sulphur atom and each adjacent carbon atom, Fig. 1).

In this paper we also describe work on the reversibility, pH dependence and selectivity of the interaction of omeprazole with the $(H^+ + K^+)$-ATPase.

MATERIALS AND METHODS

Chemicals. Omeprazole was dissolved in polyethylene glycol, type 400 (PEG 400) and was stored at −20°. For some experiments omeprazole was dissolved in dimethylsulphoxide (DMSO) and was used within 2 hr. Omeprazole was synthesised by SK & F. [benzimidazole-2-^{14}C]Omeprazole (50 mCi/mmole), [α-methylene-^{14}C]omeprazole (50 mCi/mmole) and [^{35}S]omeprazole (50 mCi/mmole) were synthesised by SK & F (D. Saunders, M. B. Mitchell and A. M. Crowe, methods to be published). Radiolabelled omeprazole was film dried and stored at −20°. For each series of experiments an aliquot of film dried material was taken up in PEG 400 and stored for up to 1 month at −20°. Radiochemical purity of all compounds was greater than 96%. $(Na^+ + K^+)$-ATPase (grade IV), from dog kidney, and all other chemicals were supplied by Sigma Chemical Co. (Poole, U.K.).

Acid degradation of omeprazole. For some experiments omeprazole was degraded under acidic conditions by incubating in the presence of 0.1 M HCl for 15 min at 37°. The resulting orange solution was then adjusted to pH 7.4 with NaOH and 100 mM Pipes/Tris* buffer pH 7.4 and was held on ice for no longer than 20 min until used in the experiment.

Preparation of $(H^+ + K^+)$-ATPase from pig gastric mucosa. $(H^+ + K^+)$-ATPase was prepared after the method of Saccomani *et al.* [1, 8]. The fundic regions of 3–4 pig stomachs, obtained within 15 min of slaughter, were packed in ice and transported to the laboratory within 45 min. All subsequent operations were performed at 4°. The mucosa was stripped from the stomach wall and was soaked in saturated NaCl. After vigorous wiping with paper towels to remove mucus and some surface cells the mucosa was scraped from the underlying connective tissue and was finely minced in approx. 5 vol. of 0.25 M sucrose containing 5 mM Pipes/Tris buffer pH 7.4 (isolation medium). The suspension was homogenised using a tight-fitting Teflon–glass homogeniser with 10 strokes at approximately 2000 rpm.

The homogenate was centrifuged for 45 min at 11,700 rpm (Sorvall RC5B, SA 600 rotor) and the pellets discarded. The supernatant was further centrifuged for 60 min at 29,000 rpm (Beckman L8-55M, Type 30 rotor). The pellets were resuspended in a total of 24 ml isolation medium and were layered on to discontinuous density gradients consisting of 30% (w/v) sucrose, 5 mM Pipes/Tris pH 7.4 and isolation medium containing 9% Ficoll (w/w). After centrifugation for 90 min at 24,000 rpm (Beckman L8-55M, Type Sw28 rotor) the material at the 0.25 M sucrose/9% Ficoll interface was taken and diluted 4-fold with 5 mM Pipes/Tris pH 7.4. This was centrifuged for 60 min at 29,000 rpm (Beckman L8-55M, Type 30 rotor) and the pellets resuspended in a minimum volume of 5 mM Pipes/Tris pH 7.4. This material was lyophilised and stored at −40°. The basal ATPase activity (no KCl) and the $(H^+ + K^+)$-ATPase activity (10 mM KCl) in five such $(H^+ + K^+)$-ATPase preparations were 0.10 ± 0.02 and 2.1 ± 0.1 I.U./mg protein, respectively (mean ± S.E.). This $(H^+ + K^+)$-ATPase activity represented a 30–40-fold enrichment over the crude gastric homogenate.

ATPase activity. ATPase activity was determined as follows. Enzyme sample (3–9 μg protein) was incubated at 37° in 1 ml of a medium consisting of 80 mM Pipes/Tris buffer, pH 7.4, 2 mM $MgCl_2$ and 2 mM Na_2ATP. $(H^+ + K^+)$-ATPase activity was determined in the presence of 10 mM KCl and $(Na^+ + K^+)$-ATPase activity in the presence of 100 mM NaCl and 10 mM KCl. After incubation for 5–15 min the inorganic phosphate released was determined by the method of Yoda and Hokin [9].

Assays of the $(H^+ + K^+)$-ATPase preparation contained 5 μg/ml nigericin. Nigericin was dissolved in methanol, which, at the concentration present in the incubation (0.5%), did not affect the enzyme activity. All enzyme activities were determined in the linear ranges of the assays and were corrected for the amount of ATP hydrolysis occurring in the absence of enzyme sample.

Pre-incubation of enzyme preparations with omeprazole. Enzyme preparations were pre-incubated with omeprazole (or acid-degraded omeprazole) at 37° in 10 mM Pipes/Tris buffer pH 6.1 or 7.4. After a defined time (usually 30 min) a sample containing 3–9 μg protein was taken for the determination of ATPase activity.

Reversibility of the inhibition by omeprazole. In order to determine the reversibility of the inhibition of $(H^+ + K^+)$-ATPase activity by omeprazole the following method was used. $(H^+ + K^+)$-ATPase

* Abbreviations used: Pipes, piperazine, *N,N'*-bis[2-ethanesulphonic acid].

preparation (30 μg/ml) was pre-incubated with two concentrations of omeprazole (10 μM, 100 μM) in 10 mM Pipes/Tris buffer, pH 6.1 at 37°. At intervals of 10 min, aliquots containing 3 μg protein were taken for ATPase assay. After 30 min each tube was diluted 10-fold in buffer such that the final conditions were 100 mM Pipes/Tris buffer, pH 7.4, 2 mM $MgCl_2$, 10 mM KCl and 5 μg/ml nigericin. Where necessary further omeprazole was added such that the final concentration in each tube was 10 μM. At intervals of 10 min aliquots containing 3 μg protein were taken for ATPase assay. A parallel tube containing enzyme and the equivalent concentration of PEG 400 (used to dissolve the omeprazole) was used as a control.

Incorporation of radiolabelled omeprazole. Radiolabelled omeprazole (10 μM unless otherwise stated) was incubated with enzyme preparation (30 μg protein/ml) in 10 mM Pipes/Tris buffer, pH 6.1 or 7.4 at 37°. Parallel incubations were performed in which radiolabelled omeprazole was also incubated in the absence of protein. At timed intervals, aliquots (100–200 μl) were transferred to tubes containing an excess of non-radioactively-labelled omeprazole (100 nmoles in 20 μl PEG 400) and 750 μl dimethylsulphoxide. After rapid vortexing the tube contents were rapidly filtered through pre-soaked Whatman GFB filters. Each filter was washed with 3 × 15 ml of ice-cold perchloric acid (5% w/v) before being transferred to a scintillation vial. Radioactivity was determined using 10 ml Picofluor-15 scintillant in a Beckman LS 1800 counter.

Incorporation was defined as the difference between the filter-associated radioactivity obtained from incubations in the presence and absence of the enzyme preparation.

Protein. Protein was determined by the method of Lowry [10] except the solutions contained 1% (w/v) sodium dodecylsulphate. Bovine serum albumin (fraction V) was used as a standard.

Analysis of data. Inhibition curves were fitted to the logistic equation by computer using the ALLFIT program [11]. Errors quoted for IC_{50} values are approximate standard errors derived from this fitting procedure.

RESULTS

Inhibition of $(H^+ + K^+)$*-ATPase activity by omeprazole*

Pre-incubation of $(H^+ + K^+)$-ATPase preparation (0.1 mg protein/ml) with omeprazole (10 μM) at pH 6.1 resulted in a time-dependent inhibition of $(H^+ + K^+)$-ATPase activity (Fig. 2). Approximately 50% of the total activity was lost after 30 min.

The degree of inhibition produced by 10 μM omeprazole when pre-incubated for 30 min at pH 6.1 was found to depend on the protein concentration of $(H^+ + K^+)$-ATPase preparation present in the pre-incubation (Fig. 3). At protein concentrations greater than 0.5 mg/ml, 10 μM omeprazole produced negligible inhibition of the $(H^+ + K^+)$-ATPase activity. For all subsequent experiments, the protein concentration of $(H^+ + K^+)$-ATPase preparation in pre-incubations with omeprazole was 30 μg/ml.

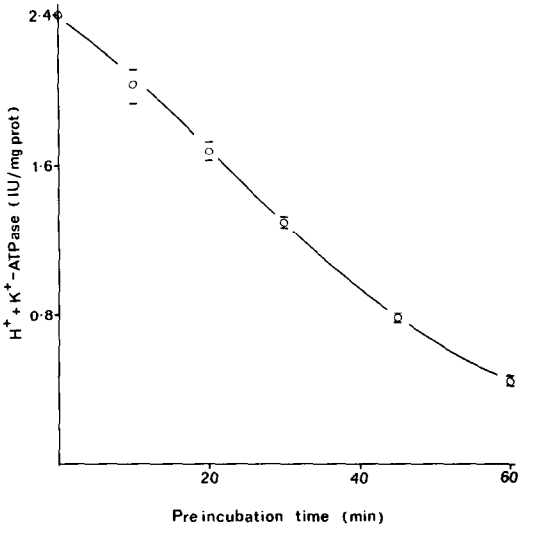

Fig. 2. Time dependence of inhibition by omeprazole. $(H^+ + K^+)$-ATPase preparation (100 μg protein/ml) was preincubated at 37° for the time indicated with 10 μM omeprazole in 10 mM Pipes/Tris buffer, pH 6.1. The $(H^+ + K^+)$-ATPase activity was then determined at pH 7.4. Values are means ± range (N = 2 assays).

The inhibition of $(H^+ + K^+)$-ATPase by omeprazole was both pH- and concentration-dependent. Following a 30-min pre-incubation at either pH 6.1 or pH 7.4 the IC_{50} values were 3.9 ± 0.4 and 36 ± 2 μM respectively (Fig. 4).

The reversibility of the inhibition by omeprazole was studied. Pre-incubation at pH 6.1 produced a time-dependent inhibition (Fig. 5). The inhibition was more pronounced at higher omeprazole concentrations. Adjusting the pH to 7.4 prevented any further increase in the degree of inhibition. Although

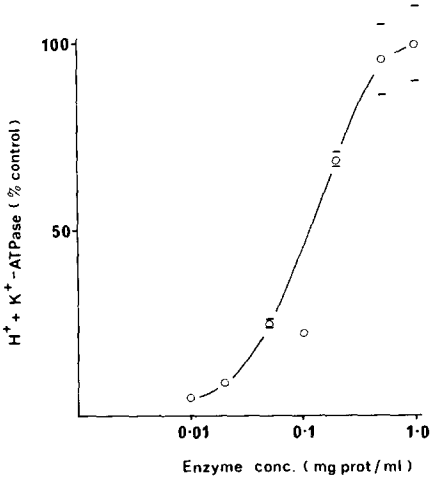

Fig. 3. Effect of enzyme concentration on inhibition by omeprazole. $(H^+ + K^+)$-ATPase preparation was pre-incubated at the concentration indicated for 30 min with 10 μM omeprazole in 10 mM Pipes/Tris buffer, pH 6.1 at 37°. The $(H^+ + K^+)$-ATPase activity was then determined at pH 7.4. Control activity (100%) was 2.52 ± 0.25 I.U./mg protein. Values are means ± S.E. (N = 3 assays).

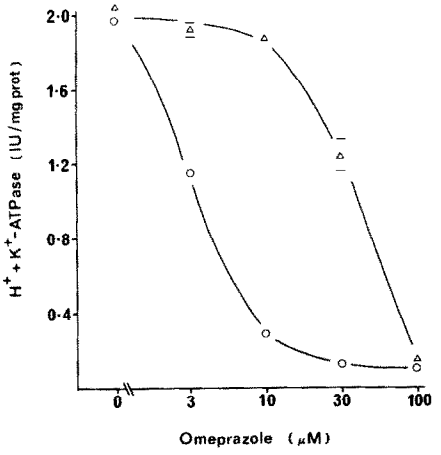

Fig. 4. Potency of omeprazole at pH 6.1 and pH 7.4. $(H^+ + K^+)$-ATPase preparation (30 μg protein/ml) was pre-incubated with omeprazole at 37° for 30 min in 10 mM Pipes/Tris buffer at either pH 6.1 (○) or pH 7.4 (△). $(H^+ + K^+)$-ATPase activity was then determined at pH 7.4. Values are means ± S.E. (N = 3 assays).

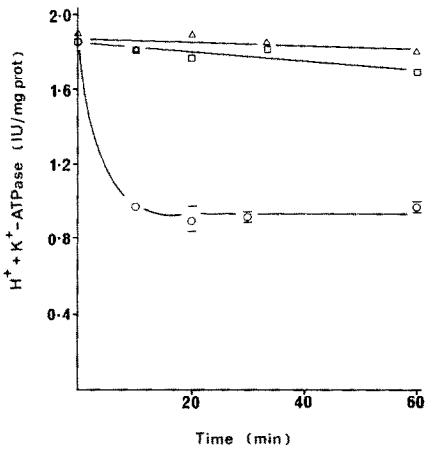

Fig. 6. Effect of transient acidification of omeprazole on its inhibitory actions at pH 7.4. Omeprazole (0.2 mM) was held at 37° for the times indicated in the following buffers; 10 mM sodium acetate pH 4.0 (○), 10 mM Pipes/Tris pH 6.1 (□), 10 mM Pipes/Tris pH 7.4 (△). Each solution was then diluted 20-fold and preincubated with $(H^+ + K^+)$-ATPase preparation (5 μg protein/ml) for 30 min at 37° in 80 mM Pipes/Tris pH 7.4 containing 2 mM $MgCl_2$, 10 mM KCl and 5 μg/ml nigericin. After preincubation, the $(H^+ + K^+)$-ATPase activity and determined by the addition of Na_2ATP (2 mM). Values are means ± S.E. (N = 3 assays).

the omeprazole concentration in all tubes was constant after the pH adjustment, differences in $(H^+ + K^+)$-ATPase activity remained over a further pre-incubation period of 20 min. The $(H^+ + K^+)$-ATPase activity following inhibition with omeprazole was linearly related to the amount of pre-incubation material assayed ruling out the possibility that the inhibition observed was due to the time-dependent formation of a reversible inhibitor. Thus the interaction of omeprazole with the $(H^+ + K^+)$-ATPase preparation was not freely reversible.

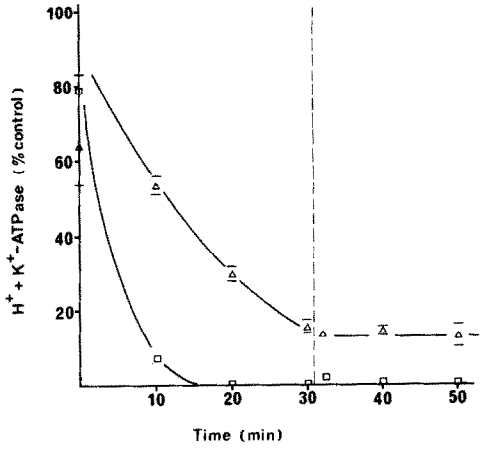

Fig. 5. Irreversibility of $(H^+ + K^+)$-ATPase inhibition by omeprazole. $(H^+ + K^+)$-ATPase preparation (30 μg protein/ml) was preincubated at 37° with omeprazole, 10 μM (△) or 100 μM (○), in 10 mM Pipes/Tris buffer, pH 6.1. At 10 min intervals aliquots containing 3 μg protein were taken and assayed for $(H^+ + K^+)$-ATPase activity at pH 7.4. After 30 min each incubation was diluted 10-fold into 100 mM Pipes/Tris buffer, pH 7.4 such that the final omeprazole concentration was 10 μM. Further aliquots were then taken at the times indicated for the determination of $(H^+ + K^+)$-ATPase activity at pH 7.4. Enzyme activities are expressed as a percentage of control activity determined by incubating $(H^+ + K^+)$-ATPase preparation for the same length of time in the absence of omeprazole. Values are means ± range (N = 2).

Since omeprazole appeared to be a more potent inhibitor of the $(H^+ + K^+)$-ATPase activity at lower pH values, the effect of transiently acidifying omeprazole for various times before a 30-min pre-incubation with $(H^+ + K^+)$-ATPase preparation at pH 7.4 was studied. Exposure of omeprazole (10 μM) at 37° for up to 60 min at pH 6.1 or pH 7.4 resulted in no significant inhibition of the $(H^+ + K^+)$-ATPase activity (Fig. 6). However, acidification to pH 4.0 for greater than 10 min resulted in a 50% inhibition of the enzyme activity.

Acid-degraded omeprazole (prepared as described in the Methods) was also found to be an effective inhibitor of the $(H^+ + K^+)$-ATPase activity at pH 7.4. When increasing concentrations of acid-degraded omeprazole were pre-incubated at pH 7.4 with $(H^+ + K^+)$-ATPase preparation (30 μg/ml), more potent inhibition was observed (IC_{50}, 5.2 ± 0.3 μM) than with non-degraded omeprazole (IC_{50}, 36 ± 2 μM; Fig. 7).

Incorporation of radiolabelled omeprazole into $(H^+ + K^+)$-ATPase preparation

Incubation of $(H^+ + K^+)$-ATPase preparation (30 μg/ml) with radiolabelled omeprazole resulted in a time-dependent incorporation of radiolabel into the material collected by filtration onto Whatman GFB filter discs. Parallel incubations in the absence of $(H^+ + K^+)$-ATPase preparation showed no time-dependent incorporation. Filter-associated radioactivity in the absence of $(H^+ + K^+)$-ATPase preparation was in the range 400–700 dpm compared with

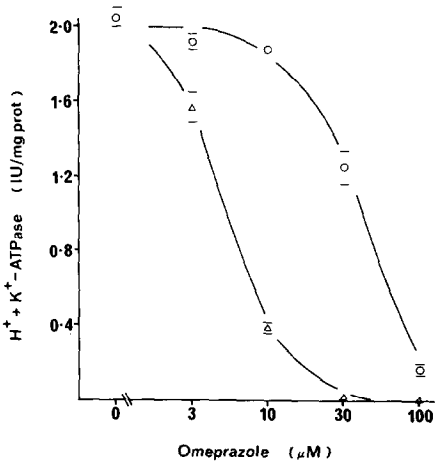

Fig. 7. Effect of acid-degraded omeprazole on $(H^+ + K^+)$-ATPase activity. Omeprazole, either non-degraded (○) or acid-degraded as described in the text (△), was pre-incubated with $(H^+ + K^+)$-ATPase preparation (30 μg protein/ml) for 30 min at 37° in 10 mM Pipes/Tris buffer, pH 7.4. The $(H^+ + K^+)$-ATPase activity was then determined at pH 7.4. Values are means ± S.E. (N = 3 assays).

up to 25,000 dpm in the presence of $(H^+ + K^+)$-ATPase preparation.

[benzimidazole-2-^{14}C]Omeprazole (10 μM) showed incorporation at pH 6.1 over a 300-min time course (Fig. 8). The rate of incorporation decreased steadily over the time course until a maximal level of 30 nmoles omeprazole/mg protein was obtained after 300 min. [α-methylene-^{14}C]Omeprazole and [^{35}S]omeprazole (both 10 μM) also incorporated into $(H^+ + K^+)$-ATPase preparation with similar time courses to the [benzimidazole-2-^{14}C]-omeprazole reaching maximal incorporations of 30 nmoles/mg protein after 300 min (Fig. 8).

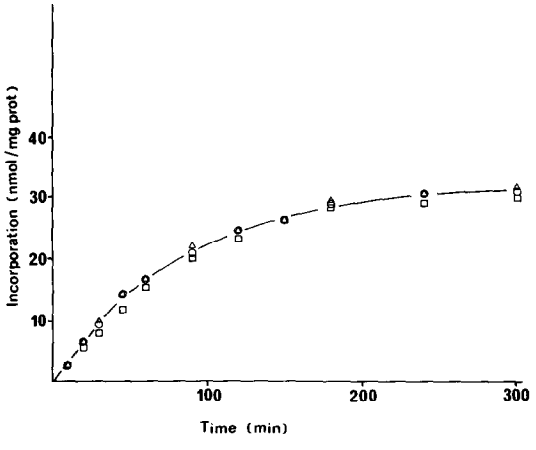

Fig. 8. Incorporation of omeprazole into the $(H^+ + K^+)$-ATPase (30 μg protein/ml) at 37° in 10 mM Pipes/Tris buffer pH 6.1. At timed intervals 200 μl aliquots were taken and the amount of radiolabel incorporated was determined in duplicate. Omeprazole was radiolabelled in the following positions; [benzimidazole-2-^{14}C] (○), [α-methylene ^{14}C] (△), [^{35}S] (□), see Fig. 1. Values are means of duplicate determinations.

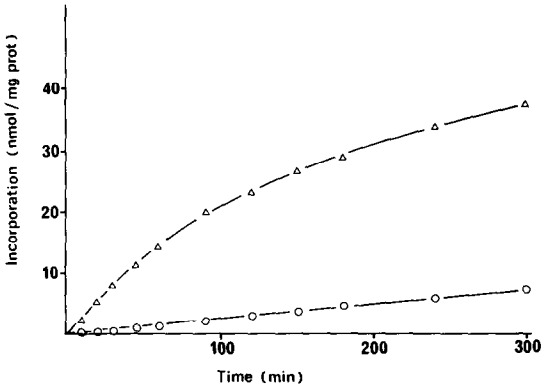

Fig. 9. Effect of pH on incorporation of [^{14}C]omeprazole into the $(H^+ + K^+)$-ATPase preparation. [benzimidazole-2-^{14}C]Omeprazole (10 μM) was incubated with $(H^+ + K^+)$-ATPase preparation at 37° in 10 mM Pipes/Tris buffer pH 6.1 (△) or pH 7.4 (○). At timed intervals 200 μl aliquots were taken and the amount of radiolabel incorporated was determined. Values are means of duplicate determinations.

The rate of incorporation of [benzimidazole-2-^{14}C]-omeprazole was lower at pH 7.4, and appeared to remain constant over the 300-min time course (Fig. 9). Acid-degraded [benzimidazole-2-^{14}C]-omeprazole incorporated into the $(H^+ + K^+)$-ATPase preparation over a much shorter time course compared with non-degraded omeprazole. Maximum incorporation was obtained at 30 min after which the level remained constant for the remaining 270 min of the time course (Fig. 10). The level of incorporation was higher at pH 6.1 (25 nmoles/mg protein) than at pH 7.4 (15 nmoles/mg protein).

Effects of omeprazole on the $(Na^+ + K^+)$*-ATPase preparation*

Omeprazole inhibited the $(Na^+ + K^+)$-ATPase activity of the $(Na^+ + K^+)$-ATPase preparation. Fol-

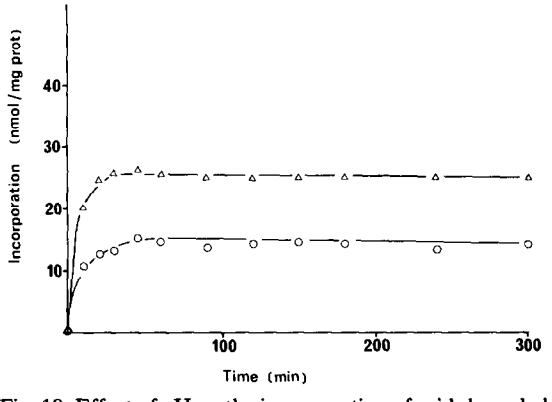

Fig. 10. Effect of pH on the incorporation of acid-degraded [^{14}C]omeprazole into $(H^+ + K^+)$-ATPase preparation. [benzimidazole-2-^{14}C]Omeprazole (1 mM) was acid degraded as described in the text. The resulting solution was diluted 100-fold and was then incubated with $(H^+ + K^+)$-ATPase preparation (30 μg protein/ml) at 37° in 10 mM Pipes/Tris buffer pH 6.1 (△) or pH 7.4 (○). At timed intervals, 200 μl aliquots were taken and the radiolabel incorporated determined in duplicate. Values are means.

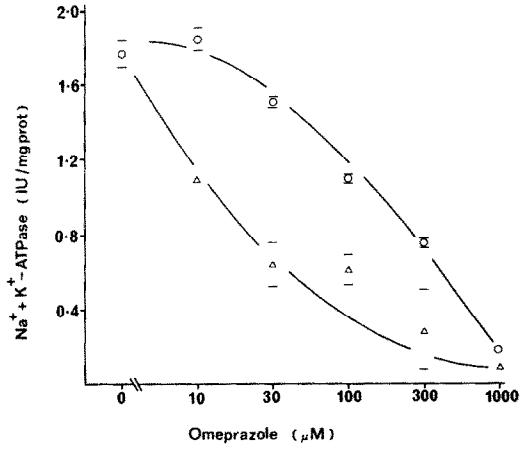

Fig. 11. Effects of omeprazole on the $(Na^+ + K^+)$-ATPase. Omeprazole, either non-degraded (○) or acid-degraded (△), was incubated with $(Na^+ + K^+)$-ATPase preparation (30 μg protein/ml) for 30 min at 37° in 10 mM Pipes/Tris buffer, pH 7.4. The $(Na + K^+)$-ATPase activity was then determined at pH 7.4. Values are means ± S.E. (N = 3 assays).

lowing pre-incubation for 30 min at pH 7.4, omeprazole was found to have an IC_{50} value of 186 ± 27 μM (Fig. 11).

Acid-degraded omeprazole was a more effective inhibitor of the $(Na^+ + K^+)$-ATPase activity following a 30-min incubation at pH 7.4 (Fig. 11), with an IC_{50} value of 19 ± 5 μM.

[benzimidazole-2-^{14}C]Omeprazole incorporated into the $(Na^+ + K^+)$-ATPase preparation with a time course similar to that obtained for the $(H^+ + K^+)$-ATPase preparation (Fig. 12). Maximum incorporation of 6 nmoles/mg protein was observed after

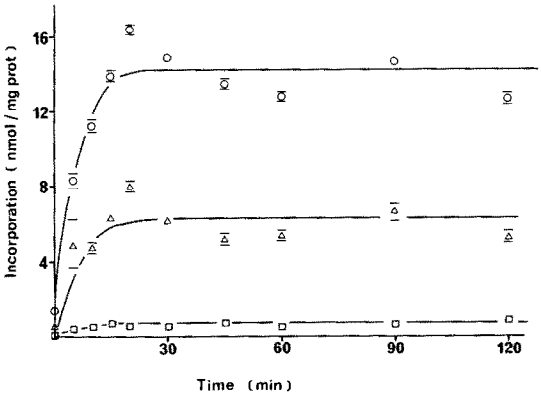

Fig. 12. Incorporation of acid-degraded omeprazole into the $(Na^+ + K^+)$-ATPase preparation. [benzimidazole-2-^{14}C]Omeprazole (1 mM) was acid-degraded as described in the text. This solution was then diluted 100-fold and was preincubated at 37° in 10 mM Pipes/Tris buffer pH 7.4 with the following proteins; $(H^+ + K^+)$-ATPase (30 μg protein/ml; ○), $(Na^+ + K^+)$-ATPase (16 μg protein/ml; △) and bovine serum albumin (30 μg protein/ml; □). At timed intervals 200 μl aliquots were taken and the radiolabel incorporated determined in duplicate. Values are means ± range.

a 20-min incubation at pH 7.4. This was lower than the incorporation into the $(H^+ + K^+)$-ATPase preparation in the same experiment (14 nmoles/mg protein). For comparative purposes, the incorporation into another protein was followed. A low level of incorporation into bovine serum albumin was observed of 0.5 nmoles/mg protein (Fig. 12).

DISCUSSION

The relatively slow onset of inhibition by omeprazole and picoprazole, recorded in this paper and by others [1], is suggestive either of an irreversible inhibitor, or the slow generation of an active inhibitory species from a less active precursor under the conditions employed. These two possibilities are not mutually exclusive, and, in fact, the evidence presented herein supports both ideas.

By taking advantage of the fact that omeprazole is a considerably more active inhibitor at pH 6.1 than at pH 7.4 we have been able to demonstrate that the inhibition is irreversible under our experimental conditions. In the experiment illustrated in Fig. 5, the $(H^+ + K^+)$-ATPase preparation was preincubated at pH 6.1 with two different concentrations of omeprazole. This resulted in different degrees of inhibition. This difference was maintained when the enzyme preparations were diluted to a constant omeprazole concentration and further incubated for 20 min at pH 7.4. Inhibition was not due to the slow formation of a freely reversible inhibitor since the enzyme activity after the preincubation with omeprazole was not altered by further dilution.

This conclusion (that omeprazole is an irreversible inhibitor) is consistent with the observation that radiolabelled omeprazole can be incorporated into the enzyme preparation, but differs from that of Beil and Sewing [7]. These authors presented evidence that the $(H^+ + K^+)$-ATPase in guinea-pig gastric vesicles was reversibly inhibited by omeprazole. The reversibility was inferred from experiments in which $(H^+ + K^+)$-ATPase, pre-incubated with 5 μM omeprazole and subsequently diluted and assayed at 0.05 μM omeprazole, showed less inhibition than if pre-incubated and assayed at 5 μM omeprazole. However, the former pre-incubation was performed at a high concentration of $(H^+ + K^+)$-ATPase preparation (500 μg protein/ml) and the latter at a low concentration (5 μg protein/ml). An alternative interpretation of their data might be that the inhibition by 5 μM omeprazole was less pronounced at the higher protein concentrations. Our data (Fig. 3) would support this view.

The increased potency of omeprazole when preincubated with $(H^+ + K^+)$-ATPase preparation at pH values below 7 ([2], and this work) suggested some form of acid activation in the inhibition process. Since omeprazole has been reported to be unstable in acidic conditions [3], it was possible that the actual inhibitor was not omeprazole itself but an acid-generated product of omeprazole. If this hypothesis is true then the interactions of omeprazole with the $(H^+ + K^+)$-ATPase at pH values around neutrality may have been limited by the rate at which such a product was formed as well as the speed of reaction with the enzyme. Consistent with this, acidification

of omeprazole before pre-incubation with the $(H^+ + K^+)$-ATPase preparation rendered omeprazole more potent at pH 7.4, (Fig. 7) and increased the rate at which it incorporated into the enzyme preparation (compare Figs. 9 and 10). Furthermore, such a mechanism could explain the decreased potency of omeprazole at high concentrations of the $(H^+ + K^+)$-ATPase preparation, since insufficient "active omeprazole" may be formed to achieve significant inhibition of large amounts of $(H^+ + K^+)$-ATPase. These observations, therefore, support the idea that omeprazole is the precursor of an irreversible inhibitor whose rate of formation is enhanced by acidic conditions.

The acid-generated product does not appear to be highly selective towards the $(H^+ + K^+)$-ATPase since it also inhibited the $(Na^+ + K^+)$-ATPase of dog kidney with a potency about 20% of that towards the $(H^+ + K^+)$-ATPase. This lack of selectivity is also consistent with the observation that the maximum number of moles of omeprazole which can incorporate into the enzyme preparation was about 30 times greater than the number of ATP sites, defined by phosphorylation with [γ-^{32}]ATP ([12] and unpublished observations in this laboratory). Furthermore, complete inhibition of the enzyme occurred when only about one third of the maximum incorporation of omeprazole had taken place suggesting that not all the incorporated omeprazole was essential for inhibition.

The nature of the inhibitory species is not clear. However, our finding that omeprazoles labelled in the sulphur position or in either of the two adjacent carbon atoms, all incorporated to the same extent and with very similar time-courses at pH 6.1 (Fig. 8) suggests that the omeprazole molecule was wholly incorporated and that the generation of an active inhibitory species did not involve any fragmentation of the molecule particularly around the reactive sulphoxide moiety.

In conclusion these results suggest that omeprazole is the precursor of a relatively non-specific, irreversible inhibitor whose formation is catalysed by hydrogen ions. Despite the lack of selectivity shown by omeprazole *in vitro*, one might still expect a high degree of selectivity towards the $(H^+ + K^+)$-ATPase *in vivo* since the active acid-generated product of omeprazole will be preferentially formed in the acidic compartments of the parietal cell in close proximity to this enzyme.

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