J. Enzyme Inhibition, 1992, Vol. 5, pp. 293–298
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INHIBITION OF GASTRIC H^+, K^+-ATPase BY QUERCETIN

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(Received 13 February 1991)

The effects of the naturally occurring flavonoid, quercetin, on gastric H^+, K^+-ATPase were investigated. Quercetin inhibited hog gastric H^+, K^+-ATPase and K^+-stimulated *p*-nitrophenyl phosphatase (K^+-pNPPase) activity in a dose dependent manner with IC_{50} values of 2.3 μM, and 6.0 μM respectively. The inhibition of H^+, K^+-ATPase by quercetin is competitive with ATP and is noncompetitive with K^+. The steady-state phosphorylation level of the enzyme was also dose-dependently reduced by quercetin with an IC_{50} value of 4.5 μM. These results suggest that quercetin reduces the phosphorylated enzyme level by competition with ATP, and thereby inhibits the H^+, K^+-ATPase activity.

KEY WORDS: Flavonoid, Quercetin, H^+, K^+-ATPase.

INTRODUCTION

Quercetin is a typical bioflavonoid which is widely distributed in plants, and has a variety of pharmacological and biochemical properties.[1,2] Quercetin has also been shown to inhibit a broad spectrum of enzymes including aldose reductase,[3,4] Na^+, K^+-ATPase,[5,6] Ca^{2+}, Mg^{2+}-ATPase,[7] cyclic AMP phosphodiesterase,[8] aromatase,[9] kinase[10] and cytochrome P-450 activity.[11] Gastric H^+, K^+-ATPase is a membrane bound enzyme which catalyzes H^+ transport from parietal cells into the gastric cavity at the response of ATP hydrolysis.[12,13] This is an important final step in gastric acid secretion. This enzyme shares many features with other cation transporting ATPases such as Na^+, K^+-ATPase in the primary amino acid sequences of the catalytic site[14] and enzymatic mechanisms.[15] In this communication, the effect of quercetin on gastric H^+, K^+-ATPase activity is investigated.

MATERIALS AND METHODS

Materials

Quercetin, ATP disodium salt and pNPP were purchased from Sigma Chemical Co., St. Louis, MO. [γ-^{32}P]ATP was purchased from NEN. All other chemicals were of the highest purity grade available. ATP Tris salt was prepared from ATP disodium salt in our laboratory. Fresh hog stomachs were purchased from the local slaughterhouse.

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Abbreviations: K^+-pNPPase, K^+-stimulated *p*-nitrophenyl phosphatase; PIPES, piperazine-N,N′-bis(2-ethanesulfonic acid).

Preparation of Gastric H^+, K^+-ATPase

Stomachs from freshly slaughtered hogs were flushed with tap water and were cleaned with paper towel, then the mucosal layer was removed from the underlying tissue. The membrane vesicle fraction containing H^+, K^+-ATPase was prepared by Ficoll-sucrose density gradient centrifugation as previously described.[16] The membrane fraction, which was fractionated above the Ficoll interface, was employed in the experiment. The vesicles obtained were collected and lyophilized to render them freely permeable to cations, and were stored at $-80°C$.

Assay of H^+, K^+-ATPase

Membrane protein (5 μg) was incubated in 1 ml of 40 mM Tris-HCl buffer pH 7.4 containing 2 mM $MgCl_2$ and 2 mM ATP Tris salt with or without 20 mM KCl for 20 min at 37°C. The reaction was stopped by the addition of 1 ml 10% ice-cold trichloroacetic acid and assayed for inorganic phosphate according to the method of Fiske and Subbarow.[17] Quercetin was dissolved in dimethylsulfoxide at a final concentration of 1%. This concentration of dimethylsulfoxide did not affect the enzyme activity. H^+, K^+-ATPase activity was obtained by subtracting the basal Mg^{2+}-stimulated activity from the enzyme activity in the presence of K^+ and Mg^{2+}. Protein was determined according to the method of Lowry *et al.*,[18] using bovine serum alubumin as a standard.

Assay of K^+-pNPPase

The assay medium contained, in a total volume of 1 ml, 40 mM Tris-HCl buffer, 5 mM $MgCl_2$, 5 mM *p*-nitrophenyl phosphate, pH 7.4, and 5 μg membrane protein with or without 20 mM KCl. After 20 min of incubation at 37°C, the reaction was stopped by the addition of 1 ml 1 M NaOH. The absorbance of the reaction medium was measured at 410 nm.

Determination of Intermediate Phosphoenzyme

The incubation medium consisted of 10 mM Tris-PIPES buffer pH 7.0, 2 mM $MgCl_2$, 2.5 μM [γ-^{32}P]ATP and 50 μg membrane vesicles, with or without quercetin in a final volume of 0.5 ml. After 15 s of incubation at room temperature, the reaction was quenched with 1 ml ice-cold 10% (w/v) perchloric acid containing 5 mM non-labeled ATP and 40 mM NaH_2PO_4. The precipitated membrane was then collected by filtration on a Whatman GF/B filter. The filter was washed 10 times with ice-cold 5 ml 5% (w/v) perchloric acid containing 10 mM NaH_2PO_4 and was transfered to a liquid scintillation vial. Its radioactivity was counted in 10 ml of Aquasol.

RESULTS AND DISCUSSION

The results show that the naturally occurring flavonoid, quercetin, is a potent inhibitor of gastric H^+, K^+-ATPase *in vitro*. Quercetin inhibited gastric H^+, K^+-ATPase from hog stomach in a dose-dependent manner with IC_{50} value of 2.3 μM (Figure 1). The basal Mg^{2+}-ATPase was also inhibited by quercetin, with an IC_{50} value of 10 μM (Figure 2). The gastric H^+, K^+-ATPase preparation contains K^+-pNPPase as well as

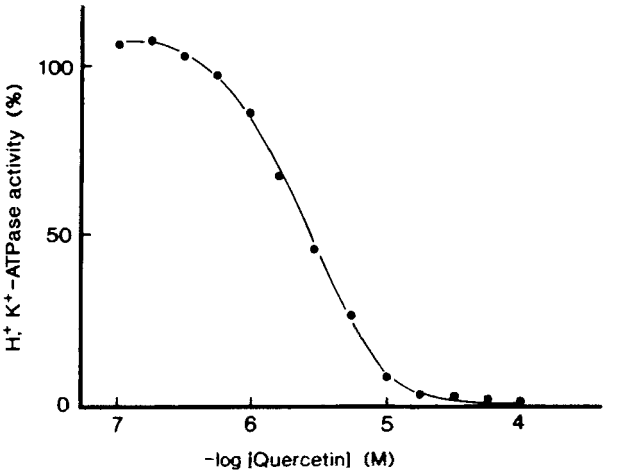

FIGURE 1 Effect of quercetin on H^+, K^+-ATPase from hog gastric mucosa. Microsome membranes (5 μg protein) were incubated in 40 mM Tris-HCl buffer (pH 7.4) containing 2 mM $MgCl_2$ and 2 mM ATP Tris salt with or without 20 mM KCl in a total volume of 1 ml for 20 min at 37°C. The reaction was terminated by the addition of trichloroacetic acid (final concentration of 5%) and liberated inorganic phosphate was determined. Each value represents the mean of duplicate assays of two different experiments (n = 4). The 100% value was 143 μmol P_i/mg protein/h.

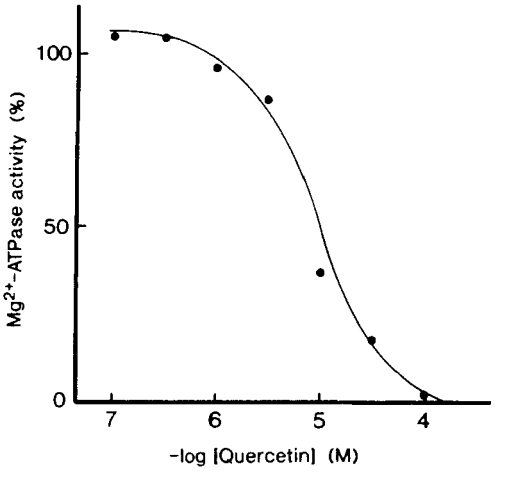

FIGURE 2 Effect of quercetin on basal Mg^{2+}-ATPase from hog gastric mucosa. Microsome membranes (5 μg protein) were incubated in 40 mM Tris-HCl buffer (pH 7.4) containing 2 mM ATP Tris salt with or without 2 mM $MgCl_2$ in a total volume of 1 ml for 20 min at 37°C. The reaction was terminated by the addition of trichloroacetic acid (final concentration of 5%) and liberated inorganic phosphate was determined. Each value represents the mean of duplicate assays of two different experiments (n = 4). The 100% value was 55 μmol P_i/mg protein/h.

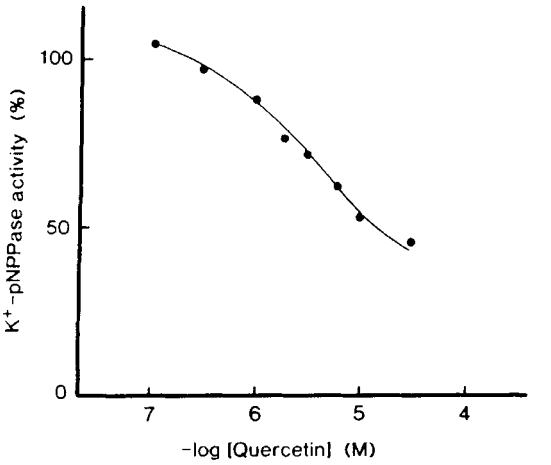

FIGURE 3 Effect of quercetin on K^+-pNPPase from hog gastric mucosa. Microsome membranes (5 μg protein) were incubated in 40 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ and 5 mM *p*-nitrophenyl phosphate with or without 20 mM KCl in a total volume of 1 ml for 20 min at 37°C. The reaction was terminated by the addition of 1 ml 1 M NaOH and the absorbance was measured at 410 nm. Each value represents the mean of duplicate assays of two different experiments ($n = 4$). The 100% value was 52 μmol pNPP/mg protein/h.

H^+, K^+-ATPase activity. Quercetin inhibited K^+-pNPPase activity in a dose-dependent manner with an IC_{50} value of 6.0 μM (Figure 3). The inhibition of H^+, K^+-ATPase activity by quercetin was measured as a function of both ATP as well as K^+ concentration to elucidate the mechanism of action by which quercetin inhibits the enzyme. Double reciprocal plots showed that quercetin inhibited H^+, K^+-ATPase competitively with ATP. Quercetin, at 1.5 μM and 3.0 μM concentration increased the apparent K_m value from 1.4 to 2.4 and 3.7 mM respectively while the V_{max} value did not change (184 μmol P_i/mg protein/h) (Figure 4). The calculated K_i value was 2.0 μM. Variation of the K^+ concentration at a fixed ATP concentration showed that 1.4 μM and 2.8 μM quercetin decreased the V_{max} values from 188 to 130 and 83 μmol P_i/mg protein/h respectively. The K_m value of 0.59 mM did not change (Figure 5). The Calculated K_i value was 1.9 μM. The effect of quercetin on the H^+, K^+-ATPase was therefore noncompetitive with respect to K^+

The ATP hydrolytic site of H^+, K^+-ATPase is located on the cytosolic side whereas the high affinity K^+ site is on the luminal face across the membrane.[19] The H^+, K^+-ATPase is phosphorylated on the cytosolic site by ATP in the presence of Mg^{2+}. The phosphorylated enzyme is then dephophorylated by luminal K^+. In the absence of K^+, the steady-state phosphorylation level was dose-dependently reduced by quercetin (Figure 6) with an IC_{50} value of 4.5 μM. These findings suggest that quercetin reduces the phosphoenzyme level by competing with ATP.

Quercetin has been reported to inhibit other cation transporting ATPases such as Na^+, K^+-ATPase[6] and Ca^{2+}, Mg^{2+}-ATPase.[7] Kuriki and Racker[6] reported that quercetin does not inhibit the phosphorylation of Na^+, K^+-ATPase and inhibits the formation of ADP insensitive E_2-P form as well as the hydrolysis of phosphoenzyme. In the case of Ca^{2+}, Mg^{2+}-ATPase,[7] quercetin does not inhibit the phosphorylation enzyme level but rather enhances it. At low concentrations of ATP ($< 10\,\mu$M),

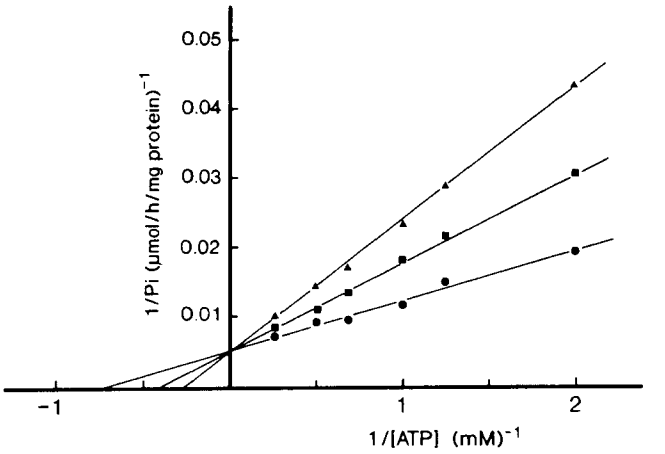

FIGURE 4 Double reciprocal plots of the hydrolysis rates of ATP by H^+, K^+-ATPase *vs.* concentrations of ATP in the presence of 0 (●), 1.5 (■) and 3.0 μM (▲) quercetin. Each value represents the mean of duplicate assays of two different experiments (n = 4).

quercetin seems to inhibit Ca^{2+}, Mg^{2+}-ATPase by competition with ATP. However, at higher concentrations of ATP, another effect of quercetin caused the inhibition. The present kinetic studies revealed that quercetin inhibits gastric H^+, K^+-ATPase competitively with ATP, and reduced the phosphorylated enzyme level. This indicates that quercetin inhibits phosphorylation of the enzyme protein by competing with ATP at the ATP site which is located on the cytosolic face, thereby inhibiting the H^+, K^+-ATPase activity. This is the same inhibitory action of quercetin as observed against Ca^{2+}, Mg^{2+}-ATPase at a low ATP concentration. Whether the reduction in

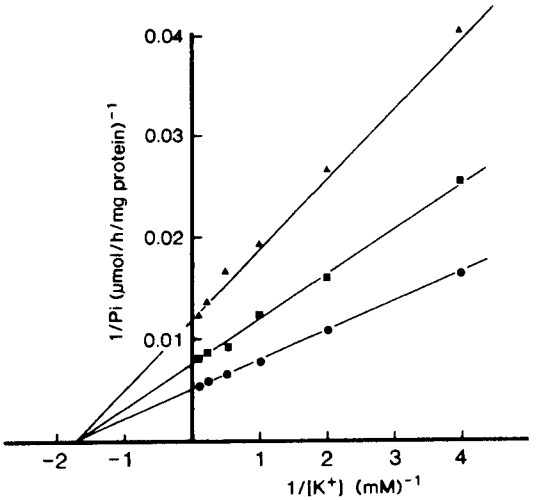

FIGURE 5 Double reciprocal plots of the hydrolysis rates of ATP by H^+, K^+-ATPase *vs.* concentrations of K^+ in the presence of 0 (●), 1.4 (■) anmd 2.8 μM (▲) quercetin. Each value represents the mean of duplicate assays of two different experiments (n = 4).

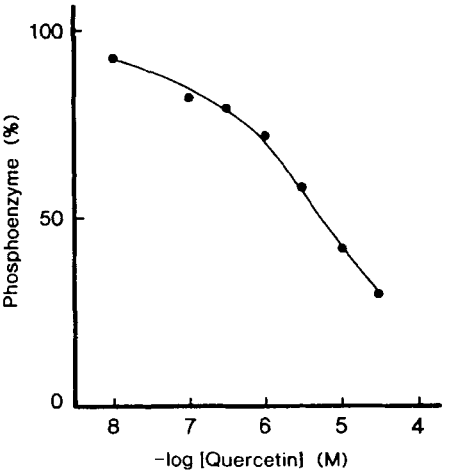

FIGURE 6 Effect of quercetin on steady-state phosphoenzyme level of H^+, K^+-ATPase. Membrane vesicles, 50 μg, were incubated in 10 mM Tris-PIPES buffer (pH 7.0) containing 2 mM $MgCl_2$ and 2.5 μM [γ-^{32}P]ATP with various concentrations of quercetin for 15 s at room temperature. Determination of phosphoenzyme level was carried out as described in Materials and method. Each value represents the mean of duplicate assays of two different experiments (n = 4).

the phosphorylated enzyme level by quercetin is due to inhibition of formation or stimulation of breakdown, remains unknown.

Acknowledgement

We thank Miss Y. Ago for typing the manuscript.

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