Acta Chemica Scandinavica B 36 (1982) 649–653

Short Communications

Effects of Phospholipase C on Gastric Vesicle Membranes Containing H^+,K^+-ATPase *

HANS OLAISSON, SVEN MÅRDH and GÖSTA ARVIDSON

Institute of Medical and Physiological Chemistry, Biomedical Center, University of Uppsala, Box 575, S-75123 Uppsala, Sweden

Vesicular membranes of the gastric mucosa contain the H^+,K^+-ATPase which is part of the HCl secreting machinery of the stomach.[1] When ATP is added to vesicles containing KCl the ATP is hydrolyzed by the enzyme and hydrogen ions are transported into the vesicles in exchange for intravesicular K^+ which results in the accumulation of hydrochloric acid in the vesicles. The vesicular membranes are able to maintain a concentration gradient of hydrogen ions of more than 10^6 *in vivo*.[2] On the external (cytoplasmic) side of the membrane the H^+,K^+-ATPase is able to hydrolyze *p*-nitrophenylphosphate in the presence of extravesicular KCl.[3] The present experiments were conducted in order to gain some information on the organization and function of the lipids in the membranes of intact gastric vesicles. Such vesicles were treated with phospholipase C and the effects on the phospholipid content and composition, hydrogen ion transport and the *p*-nitrophenylphosphatase activity of the H^+,K^+-ATPase were recorded.

Experimental. Phospholipase C (4000 U/mg) from *Bacillus cereus* was obtained from Boehringer, Mannheim. Silica Gel H was a product of Merck, Darmstadt. ATP and *p*-nitrophenylphosphate (pNPP) were from Sigma. Ficoll 70 was from Pharmacia Fine Chemicals, Uppsala. Vesicular membranes were prepared from a homogenate of pig gastric mucosa by differential centrifugations in order to obtain a microsomal fraction.[2,3] This fraction was then layered on top of a step gradient, from the bottom consisting of 40 ml of 37% sucrose and 25 ml of 7.5% Ficoll in 0.25 M sucrose. After centrifugation at 75 000 × g for 2 h in a 6 × 100 ml MSE angle rotor, a vesicular fraction was collected from the top of the sucrose-Ficoll layer. Gastric vesicles at a protein concentration of 1 mg/ml were incubated with phospholipase C in 10 mM Hepes-Tris buffer, pH 7.5, containing 0.25 mM $CaCl_2$, 2 mM $MgCl_2$, 150 mM KCl at 30 °C. The phospholipase was added at a concentration of 3 units per mg of vesicular protein. Phospholipids were separated on thin layers of silica gel impregnated with magnesium acetate.[4] The plates were developed in two dimensions, first in chloroform–methanol–25% ammonia–water, 65:35:5:2 (v/v) and then in chloroform–methanol–acetic acid–water, 60:30:8:5 (v/v). The phospholipids were visualized and quantitated by phosphorus determination as previously described.[5]

Hydrogen ion transport in vesicles which had been equilibrated with 150 mM KCl was assayed at 21 °C in 2 mM $MgCl_2$, 150 mM choline-Cl, 10 μM acridine orange in 10 mM Hepes-Tris buffer, pH 7.0. The change of the absorbance at 493 nm upon the addition of 100 μM ATP as hydrogen ions were accumulated inside the vesicles was registered by means of a standard spectrophotometer equipped with a recorder.[6] The *p*-nitrophenylphosphatase activity of the H^+,K^+-ATPase was assayed by incubation of vesicles at 21 °C in 3 mM pNPP, 3 mM $MgCl_2$ and 10 mM KCl in 10 mM Hepes-Tris buffer, pH 7.5. The absorbance at 410 nm was measured.[7] Protein was measured according to the method of Lowry *et al.*[8]

Results and discussion. Approximately 50% of the membrane phospholipids were rapidly degraded by phospholipase C (Fig. 1). Concomitantly, marked changes occurred in the composition of the phospholipids (Fig. 2). These results indicate that sphingomyelin and phosphatidylinositol were left intact while the major part (80% of the original amounts) of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine seemed to be immediately accessible to hydrolysis by phospholipase C. This might reflect a preferential localization of these glycerophospholipids to the outer half, *i.e.* the cytoplasmic half of the lipid bilayer in the vesicles. Such a conclusion would be in general

*Communication at the Meeting of the Swedish Biochemical Society in Gothenburg, 7–8th June, 1982.

0302-4369/82/090649-02$02.50

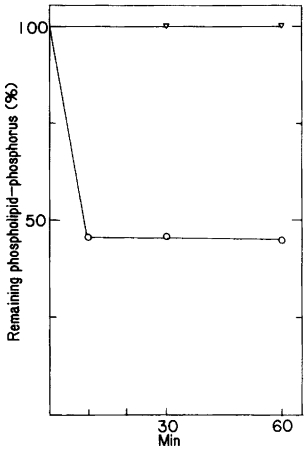

Fig. 1. Time dependent hydrolysis of phospholipids in gastric vesicles upon incubation with phospholipase C; ○, 3 units of phospholipase C per mg of vesicular protein were added at zero time; ▽, control, no phospholipase was added.

agreement with the findings of Saccomani *et al.*[9] who studied the effects of phospholipase A_2 on gastric vesicles. The *p*-nitrophenylphosphatase activity of the H^+,K^+-ATPase was inactivated in parallel with the hydrolysis of total phospholipids while the accumulation of hydrogen ions was more sensitive and decreased much more rapidly (Fig. 3).

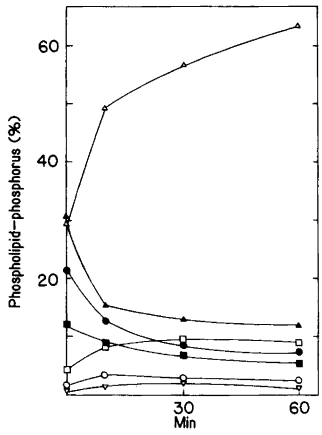

Fig. 2. Time dependent change of the phospholipid composition in gastric vesicles upon incubation with phospholipase C. ▲, phosphatidylethanolamine; ●, phosphatidylcholine; ■, phosphatidylserine; △, sphingomyelin; □, phosphatidylinositol; ○, lysophosphatidylethanolamine; ▽, lysophosphatidylcholine.

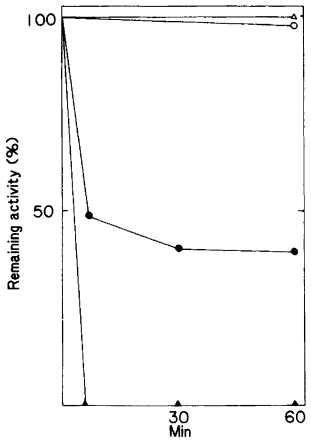

Fig. 3. Activities of p-nitrophenylphosphatase and hydrogen ion transport after incubation of gastric vesicles with phospholipase C. ○, ●, p-nitrophenylphosphatase; △, ▲, hydrogen ion transport. Open symbols represent control experiments in the absence of phospholipase C.

Acknowledgement. This investigation was supported by the Swedish Medical Research Council, Project 13X-4965 and by the Swedish Natural Science Research Council.

1. DiBona, D. R., Ito, S., Berglindh, T. and Sachs, G. *Proc. Natl. Acad. Sci. U.S.A. 76* (1979) 6689.
2. Reenstra, W. W. and Forte, J. G. *J. Membr. Biol. 61* (1981) 55.
3. Saccomani, G., Dailey, D. W. and Sachs, G. *J. Biol. Chem. 254* (1979) 2821.
4. Tolbert, M. E. M., White, A. C., Aspry, K., Cutts, J. and Fain, J. N. *J. Biol. Chem. 255* (1980) 1938.
5. Arvidson, G. A. E. *Eur. J. Biochem. 4* (1968) 478.
6. Lee, H. C. and Forte, J. *Biochim. Biophys. Acta 508* (1978) 339.
7. Mårdh, S. *Acta Chem. Scand. B 36* (1982) 269.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem. 193* (1951) 265.
9. Saccomani, G., Chang, H. H., Spisni, A., Helander, H. F., Spitzer, H. L. and Sachs, G. *J. Supramol. Struct. 11* (1979) 429.

Received June 23, 1982.