Regulation of Intermediary Phosphorylation of K+ -ATPase from Pig Gastric Mucosa by Sodium Ions * , **

MAGNUS LJUNGSTROM, a
BJÖRN WALLMARK b and SVEN MÅRDH a

a Inst. of Medical and Physiological Chemistry, Biomedical Centre, Uppsala University, Box 575, S-751 23 Uppsala, Sweden
b AB Hässle, Fack, S-431 20 Mölndal 1, Sweden

Vesicles from the microsomal fraction of gastric mucosa hydrolyze ATP with a concomitant K+-dependent uptake of H+.1 Broken membranes derived from these vesicles contain a K+-stimulated ATPase which is believed to constitute an integral part of the proton pump. In the presence of Mg2+ and ATP a phosphorylated form of the ATPase appears.2 The extent of phosphorylation is reduced by K+. In a recent report evidence was presented that the phosphoenzyme is an intermediate in the hydrolysis of ATP.3 It was found also that Na+ inhibited the K+-stimulated hydrolysis of ATP. This study shows that already low concentrations of Na+ effectively reduce the rate of formation of the phosphoenzyme intermediate.

Experimental. The Tris-salt of ATP was prepared as described previously.4 [γ-32P]ATP was a product of New England Nuclear. K+-ATPase was prepared from the gastric mucosa of pig stomachs (fraction GII, Ref. 5). The ATPase activity was about 6 μmol (mg protein)−1 h−1 at 21°C in the presence of 5 μM ATP, 2 mM MgCl2 and 10 mM KCl in 40 mM Tris-HCl buffer, pH 7.4. Phosphorylation experiments were carried out at 20−22°C at 5 μM ATP by means of a rapid-mixing apparatus.4 Maximal amount of phosphoenzyme was obtained by phosphorylation of the enzyme in this apparatus, or by calculation of the upper limit to which the experimental values extrapolated in a time-dependent study. Both methods gave maximally about 1.5 mmol per mg protein. Curve fitting of experimental data points was performed by the method of least squares on a Wang 600 calculator assuming first-order or pseudo first-order kinetics. The correlation coefficient was 0.997 or better in all experiments. Results and discussion. In order to investigate further interactions of Na+ with the K+ -ATPase, the rate of formation of the phosphoenzyme intermediate was studied at various

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** The abbreviations used are: K+-ATPase, potassium-stimulated ATP phosphohydrodase; (Na+,K+)-ATPase, sodium plus potassium ion transport ATP phosphohydrodase.
These results indicate that Na\(^+\) has a regulatory role in the K\(^+\)-stimulated ATPase reaction by an inhibitory effect on the intermediary phosphorylation. Since the function of the K\(^+\)-ATPase is essential for the acid secretion in the stomach,\(^1\) Na\(^+\) may also have a regulatory role in the production of acid. In unstimulated normal cells intracellular concentrations of 35 ans 23 meq Na\(^+\)/kg have been reported.\(^5\)\(^,\)\(^7\)

Depletion of the ATP in the gastric mucosa by treatment with 2,4-dinitrophenol increases the Na\(^+\) content of the tissue.\(^7\) From the present investigation it is not possible to determine from which side of the membrane Na\(^+\) inhibits the K\(^+\)-stimulated ATPase. The results, however, infer an important regulatory function of the Na\(^+\)-pump (Na\(^+\),K\(^+\)-ATPase) in the regulation of gastric ion secretion. In addition, the results show the importance of having a strict control of the Na\(^+\) concentration in kinetic studies on the K\(^+\)-ATPase.

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