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Kinetic characteristics of purified H^+,K^+-ATPase from parietal cells plasma membrane under stress-induced stomach ulcer

Charakterystyka kinetyczna oczyszczonej H^+,K^+-ATPazy z błon plazmatycznych komórek ściennych w warunkach wrzodów żołądka indukowanych stresem

INTRODUCTION

The upper gastrointestinal tract comprising the lower esophagus, the stomach, and the proximal duodenum are frequently exposed to extremely acidic pH. A large majority of the population tolerates the acidic environment, without major pathological consequences. However, in about 10% of the population, lesions of the epithelium occur that range from superficial destruction of the mucosa to ulcers penetrating the full thickness of the tissue [7]. In Ukraine in recent years ulcer disease has taken the second place which is 18–20% of digestion diseases [2].

Gastric acid secretion belongs to the main factors of gastric mucosa ulceration. Gastric acid is secreted by parietal cells in the stomach. Gastric H,K-ATPase in the parietal cell of the gastric mucosa is responsible for the transport of HCl through membrane by H^+ for K^+ exchange catalyzed by ATP driven phosphorylation/dephosphorylation [4]. It catalyzes an electroneutral exchange of cytoplasmic protons for extracytoplasmic potassium. In the resting parietal cell, the pump enzyme is present in smooth surfaced cytoplasmic membrane tubules. Upon stimulation of acid secretion, the pump is on the microvilli of the secretory canaliculus of the parietal cell. This morphological change results in a several fold expansion of this structure. In addition to this transition, there is activation of K^+ and perhaps Cl^+ conductance in the pump membrane, which allows K to access the extracytoplasmic face of the pump, enabling dephosphorylation and recycling of the pump [8].

It is important to notice that H,K-ATPase catalyzes transport by means of conformational changes driven by cyclic phosphorylation and dephosphorylation of the catalytic subunit of the ATPase.

It is well known that gastric ulcer development positively correlates with increasing H,K-ATPase activity; however, we must admit that the biochemical mechanism which leads to this increase remains unclear. That is why the aim of our work was to study kinetic characteristics of the H^+,K^+-ATPase purified from rat gastric parietal cells apical membranes in control as well as under stress-induced experimental ulcer development.

MATERIAL AND METHODS

Experiments were performed on male rats, weighing 220–250 g. The animals were not fasted for 24 h before the experiment but with free access to water. Experimental gastric ulcer development was induced by immobilization stress [3]. H,K-ATPase activity measurement as well as H,K-ATPase purification were performed on the second day after stomach ulcer appearance. The ulcer index (UI) of gastric mucosal lesions was evaluated by the score system.

H,K-ATPase was purified by the previously described method [5]. A membrane-bound fraction, accounting for about one-third of the total H,K-ATPase preparation, was obtained by zonal centrifugation from a 30% sucrose. The enzyme was washed and subsequently resuspended in 5 mM Pipes/Tris, pH 6.8, and stored at -80ºC. The ATPase activity of these preparations was measured by the previously described method [5] at 37°C using the quantity of oxidized NADH.

Parietal cells were isolated from gastric mucosa cell fraction and calculated [9]. H,K-ATPase was chromatographically purified using Reactive Red-agarose. H,K-ATPase was eluted with buffer solution which contained 550 mM KCl. SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli [6] with slight modifications using 10–20% polyacrylamide gel plates in the presence of 0.1% SDS. ATP at 0.01 mmol/l up to 0.2 mmol/l was used for enzyme kinetic measurement. Protein concentration was determined by the method of Bradford [1] using bovine serum albumin as a standard.

All data were expressed as mean ± SD. The statistical differences between different groups were analyzed by Student's *t*-test using Excel – 2000 and Origin Pro 8.0. $P<0.05$ was considered significant.

RESULTS

In order to study some of the kinetic features upon ulceration, H,K-ATPase was obtained and purified from rat parietal cells apical membranes. For H,K-ATPase purification the following steps were performed: 1) obtaining rat parietal cells apical membranes fraction; 2) solubilisation obtained rat parietal cells apical membranes fraction with n-dodecyl-β-D-maltoside (DOM); 3) affinity chromatographic purification using Reactive Red-agarose. H,K-ATPase was eluted with buffer solution which contained 550 mM KCl. We must admit that there was only one H,K-ATPase elution peak in control samples; however, there were two elution peaks in samples obtained under ulceration (Fig. 1). After purification, all of the elution peaks were tested for ATPase activity as well as in electrophoresis mobility.

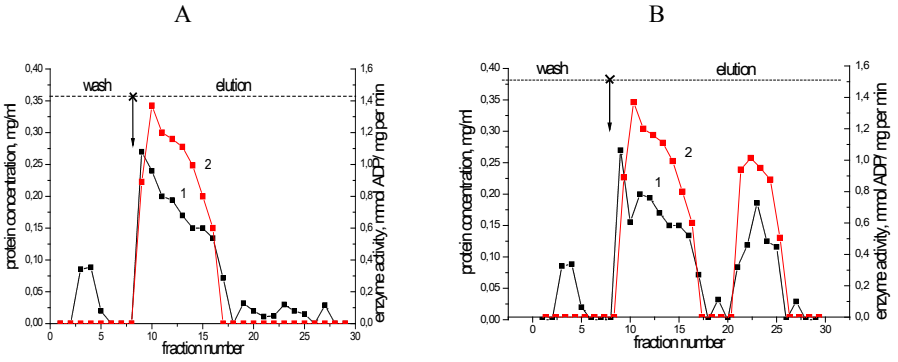

Fig. 1. Chromatography elution profiles of the H^+,K^+-ATPase purified from rat parietal cells apical membranes: A – control animal group; B – purification upon gastric ulcer development; 1 – protein profile; 2 – H^+,K^+-ATPase activity profile

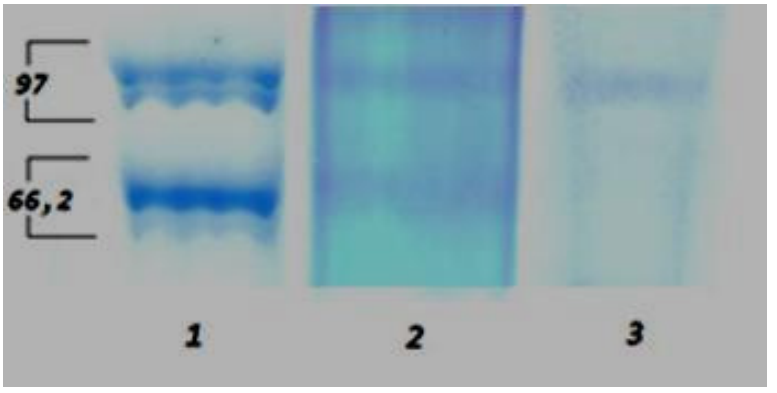

Fig. 2. Electrophoretic pattern of fraction obtained during the purification of H^+,K^+-ATPase-enriched membranes from gastric mucosa: lane 1 – markers of the molecular weight (97; 66.2; kDa), lane 2 – fraction of the plasma apical membranes, lane 3 – purified H^+,K^+-ATPase

Figure 2 shows typical patterns of SDS polyacrylamide gel electrophoresis of the fraction of purified H^+,K^+-ATPase. The electrophoretic pattern of purified enzyme corresponded to molecular weight near 97 kDa which corresponded to the molecular weight of α-subunit of H^+,K^+-ATPase consisting of 95–114 kDa depending on the mammalian species [1].

In order to elucidate catalytic properties of the purified H^+,K^+-ATPase, the dependence of ATP hydrolysis reaction velocity on ATP-Mg^{2+} concentration as well as kinetic characteristics (V_{max}, K_m, K_a, pH_{opt}) were measured (Table 1). As shown in Table 1, upon ulceration V_{max} increases 5 times compared to control samples. On the other hand, K_m decreased 4 times (see Table 1). These results suggest that upon ulceration H^+,K^+-ATPase affinity to potassium increases.

Table 1. Kinetic characteristics of the H^+,K^+-ATPase purified from rat gastric parietal cells apical

membranes (in control as well as under stress-induced experimental ulcer development) (M±m, n=7–10)

Kinetic features	Control	Stress-induced experimental ulcer
Dependence of the velocity of hydrolysis reaction on ATP concentration; (M±m, n=7)		
V_{max}, mmol ADP/ mg per min	0.46±0.04	2.3±0.18*
K_m, mmol/l	0.02±0.004	0.005±0.001*
Dependence of the velocity of hydrolysis reaction on K^+ concentration; (M±m, n=7)		
K_a, mmol/l	3.5±0.175	1.53±0.076*
Dependence of the velocity of hydrolysis reaction on pH; (M±m, n=7)		
pH_{optim}	7.0	5.0

* $p \leq 0.05$ compared to control group

It was also noticed that upon water immersion-restraint stress pH_{opt} shifts from 7.0 to 5.0, which proves essential changes in active site functional groups ionization resulting in pH-dendent changes of H^+,K^+-ATPase conformation.

CONLUSIONS

We can conclude that changes of some kinetic characteristics as well as chromatography elution shifting may result from conformation changes and activity regulation changes in the cell upon stress-induced gastric ulceration.

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SUMMARY

In the present study of kinetic parameters of purified H^+,K^+-ATPase of rats plasma membrane under stress-induced stomach ulcer were investigated. It was established that elution profile of the protein had one peak under control conditions and two peaks – under the stomach ulcer. Results of SDS-PAGE of purified enzyme visualized a single band, with an apparent molecular weight of about 97 kDa. Analysis of kinetic parameters of purified H^+,K^+-ATPase had the following features: decrease of K_m and increase V_{max} in ATP hydrolysis reaction, increase of K^+-affinity and shift of enzyme pH_{opt} to 5.0.

Key words: H^+,K^+-ATPase, parietal cells, stomach ulcer

STRESZCZENIE

W pracy zbadano parametry kinetyczne oczyszczonej H^+,K^+-ATPazy błon plazmatycznych szczurów w warunkach wrzodu żołądka indukowanego stresem. Stwierdzono, że profil elucyjny białka ma jeden pik w warunkach kontrolnych i dwa piki w warunkach wrzodu. Wyniki SDS-PAGE oczyszczonego enzymu wykazały pojedyncze pasmo o masie cząsteczkowej około 97 kDa. Analiza parametrów kinetycznych oczyszczonej H^+,K^+-ATPazy wykazała obniżone K_m i zwiększone V_{max} w reakcji hydrolizy ATP, zwiększone powinowactwo do K^+ i przesunięcie pH_{opt} do 5.0.

Słowa kluczowe: H^+,K^+-ATPaza, komórki ścienne, wrzód żołądka