Kinetic Properties of Potassium Stimulated ATPase Purified from Gastric Mucosa

Seyhan Tükel and Turgay İsbir

University of Çukurova, Medical Faculty, Department of Biochemistry, Balcali, Adana, Turkey

In this study, partially purified K$^+$-H$^+$ ATPase from frog gastric mucosa were obtained by using differential and density gradient centrifugation.

Optimum activity of K$^+$-H$^+$ ATPase (V_{max}), Michealis-Menten constant (K_m) and Hill coefficient (h) were found as 83.3 µmol Pi·mg prot^{-1}·h^{-1}, 95.2 µM and 0.91, respectively. Enzyme preparations were more stable in glycerol solutions stored at -40 °C. Minimum activity lost was determined for samples stored in 40% (v/v) glycerol solution at -40 °C for two months.

Introduction

Ganser and Forte [1] have described a potassium-activated proton-transport adenosine triphosphatase (K$^+$-H$^+$ ATPase; EC 3.6.1.36) in microsomal fraction of bullfrog cells. The K$^+$-H$^+$ ATPase system existing in tubulovesicular system of gastric parietal cell is able to exchange K$^+$ for H$^+$ in electroneutral fashion and is thought to play a major role in gastric acid secretion [2–4].

Although considerable progress was made on K$^+$-H$^+$ ATPase enzyme system there is several issues still remain unresolved.

In the present study, attempts were made to estimate the specific activity, kinetic parameters and storage conditions of K$^+$-H$^+$ ATPase in frog gastric mucosa.

Materials and Methods

Materials

All reagents were of highest purity available and were obtained from BDH (England), Merck (F.R.G.) or Sigma Chemical Company (U.S.A.).

Preparation of K$^+$-H$^+$ ATPase containing membrane

A membrane fraction G$_1$ (containing low density microsomal membrane vesicles) derived from the apical plasma membrane of parietal cell was obtained from the frog gastric mucosa by a combination of differential and zonal density gradient (ficoll-sucrose) centrifugation by the methods detailed previously [5, 6]. Briefly the antral and cardiac regions were cut away and the glandular mucosa was removed from the muscle by scraping. The scrapings from the fundic mucosa of stomach were homogenized in an icecold medium containing 250 mM sucrose, 0.2 mM EDTA, 5 mM Tris-HCl (pH 7.4) to give a suspension containing 10 g wet weight of tissue per 100 ml. Crude microsomal pellet prepared by centrifuging the post 12,000 × g supernatant at 100,000 × g for 90 min. The pellet was resuspended at a concentration of 1 mg/ml protein again in homogenizing buffer. This suspension was layered on top of a discontinuous gradient of 7.5% (w/v) ficoll and 37% sucrose, and centrifuged in IEC-B Damon, SP-110 type rotor for 1 h at 78,000 × g. Fraction G$_1$ (on the top of the gradient) was suspended in homogenizing solution and was used throughout in the experiments. All operations were carried out at 0–4 °C.

Assays of marker enzymes

Both succinic dehydrogenase and Na$^+$-K$^+$ ATPase used as mitochondrial enzyme markers. Methods for measuring succinic dehydrogenase activity described by King [7] and Na$^+$-K$^+$ ATPase activity as reported by Reading and İsbir [8] were employed.

K$^+$-stimulated phosphatase (PNPPase) was assayed as the release of p-nitrophenol from p-nitrophenyl phosphate according to the method described by Forte et al. [9].

K$^+$-H$^+$ ATPase activity

K$^+$-H$^+$ ATPase activity was determined by measuring the release of Pi from ATP. Assays were

carried out in a final volume of 1 ml containing 25−50 µg membrane protein as the enzyme source, 2 mM $MgCl_2$, 40 mM Tris-HCl buffer (pH 7.5), 2 mM Na_2ATP and the presence or absence of 20 mM KCl. Membranes were preincubated in the mixture for 10 min at 37 °C before starting the reaction by adding the substrate, ATP. After 30 min incubation with substrate reaction was stopped by adding 1 ml of 15% trichloroacetic acid. The released Pi was assayed by the method of Atkinson *et al.* [10], and proteins were assayed by the method of Lowry *et al.* [11]. K^+-H^+ ATPase activity was calculated by subtracting the activity with Mg^{2+} as the only cation (Mg^{2+} ATPase) from the activity in the presence of both Mg^{2+} and K^+.

Kinetic studies and storage conditions

Hydrolysis rate of ATP at pH 6.5 and pH 7.5 was determined. Lineweaver-Burk plot, Hill plot and Arrhenius plot showing the effects of temperature changes (10−55 °C) were constructed.

Freshly prepared fraction G_1 stored at room temperature, +4 °C, −40 °C and in 30%, 40%, 50% (v/v) glycerol solutions at −40 °C in small fractions. Activities were measured at different times during two months.

Results

Fraction G_1 is devoid of mitochondrial enzyme markers, succinic dehydrogenase and Na^+-K^+ ATPase but is enriched by a factor of 10.47 in the activity of K^+-H^+ ATPase and 6.59 in the activity of PNPPase which is copurified with K^+-H^+ ATPase. The specific K^+-H^+ ATPase activity was 83.34 ± 2.00 µmol Pi·mg prot^{-1}·h^{-1}.

The rate of hydrolysis of ATP was increased by increasing its concentration up to 2 mM. ATP concentrations higher than 2 mM were inhibitory. This inhibition was found as 20% at pH 6.5 and 13% at pH 7.5 for 3 mM ATP concentration.

Fig. 1 A shows the Lineweaver-Burk plot as a function of the ATP concentrations. K_m for the ATP has been determined as 95.2 µM. As shown from Fig. 1B Hill plot was linear and Hill coefficient was 0.91.

The response to the temperature changes in the range of 10−55 °C on the K^+-H^+ ATPase activity was examined. Arrhenius plot Fig. 2 clearly revealed two breaks at 15 °C and 25 °C. The activation energies calculated as 12.5 kcal·mol^{-1} below 15 °C and
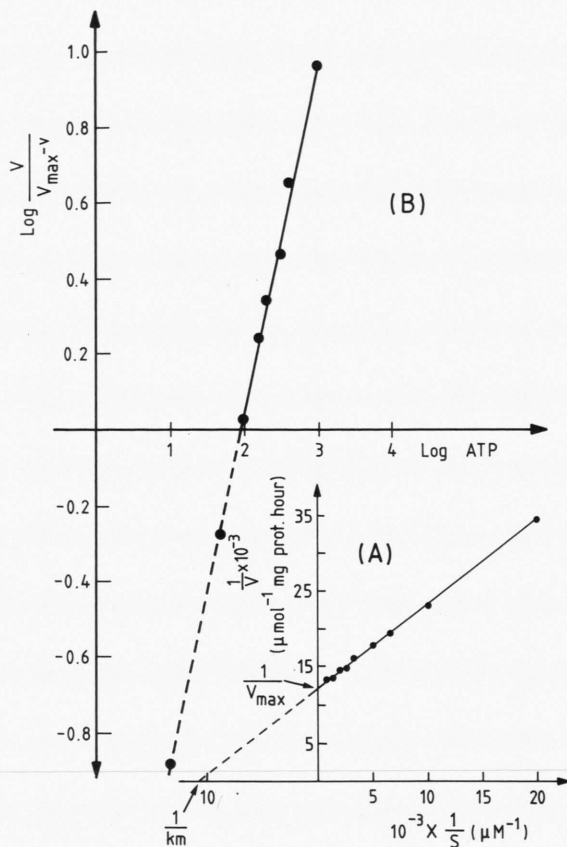

Fig. 1. A. K^+-H^+ ATPase activity as a function of ATP at 37 °C and pH 7.5. The data are plotted as a Lineweaver-Burk plot of $1/V$ against $1/S$. B. Hill plot for K^+-H^+ ATPase.
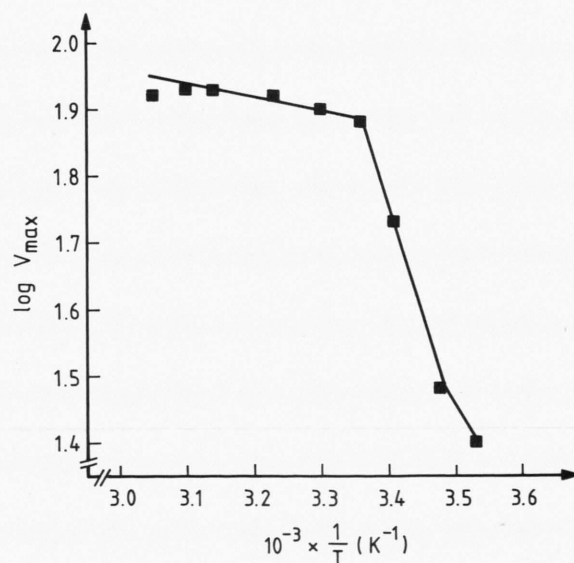

Fig. 2. Arrhenius plot of the response to the temperature of K^+-H^+ ATPase activity.
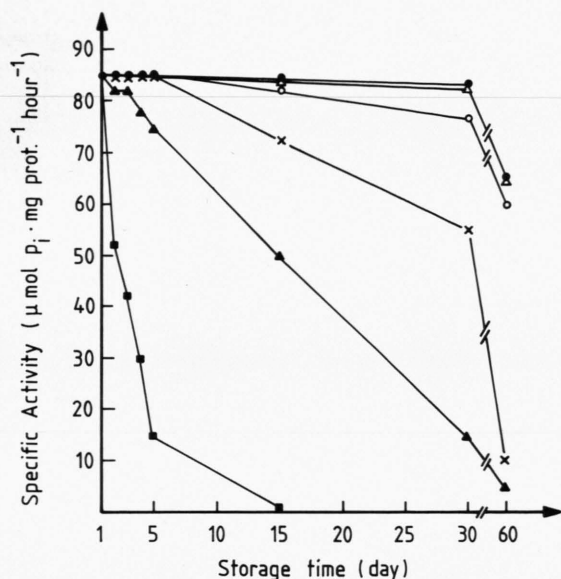
Fig. 3. Effect of storage conditions on K$^+$-H$^+$ ATPase activity ■———■ at room temperature; ▷———▷ at +4 °C; ×———× at −40 °C; ●———● at −40 °C in 30% (v/v) glycerol; ○———○ at −40 °C in 40% (v/v) glycerol; ▷———▷ at −40 °C in 50% (v/v) glycerol.

39.1 kcal·mol^{-1} between 15 °C and 25 °C. After 25 °C Arrhenius plot was almost linear and activation energy was 0.9 kcal·mol^{-1}.

Activity changes upon storage was shown in Fig. 3. A sharp decrease (about 40%) occurred for the samples stored at room temperature by the end of a day. On the other hand, samples stored at −40 °C in glycerol solutions were stable. After two months, minimum activity lost was observed for the samples stored at −40 °C in 40% glycerol solution. In this condition, activity lost was 24%.

Discussion

The overall ATPase reaction measured as the release of Pi is a complex reaction as substrate ATP have both stimulating and inhibiting effects.

Significant substrate inhibition was found at pH 6.5 and 7.5 for ATP concentrations greater than 2 mM. Inhibition was more pronounced at pH 6.5. It was reported [12] that Mg^{2+} ATP complex had not any inhibitory effect but free ATP causes inhibition

at both pH for ATP concentrations greater than 2 mM in the presence of 2 mM Mg^{2+}. Ljungström and Mardh [13] have reported that 2 mM ATP was inhibitory at pH 7.4. They used similar incubation medium with us but the only difference was in the K$^+$ concentration. They were used 5 mM K$^+$. Most probably differences between our and their results were due to this indicating that the increased K$^+$ concentration prevents the ATP inhibition at pH 7.5.

In our study a linear Lineweaver-Burk plot Fig. 1 A was obtained and a single K_m was found. These results indicate that enzyme shows normal Michealis-Menten kinetics. On the other hand, Hill coefficient was 0.91 which may indicate Michealis-Menten kinetics or a negative cooperativity.

Arrhenius plot Fig. 3 showed two transition points at 15 °C and 25 °C indicating altered lipid domain at these temperatures. A transition temperature of 28 °C has been obtained by Sachs et al. [14]. Lee et al. [15] reported that only the valinomycin stimulated part of enzyme shows a transition temperature of 14 °C, while ionophore insensitive K$^+$-stimulated activity shows no transition point at this temperature. In contrast to this finding, we found two transition temperatures although we did not use any K$^+$ ionophore.

In the literature there was no detailed study examining the effect of storages on the enzyme. Lee and Forte [16] have reported no significant change after two months storage of frozen samples. Wolosin and Forte [17] stored the enzyme at −20 °C for one month without any activity lost. Schrijen et al. [18] have reported that enzyme was stable for six months at −20 °C but they reported enzyme activity as 90−140 μmol Pi·mg prot^{-1}·h^{-1}. In our opinion, their unchanged activity was most probably due to the broad range of activity they based on. Im et al. [6] stored their samples in 20% (v/v) glycerol in liquid nitrogen and Soumarmon et al. [19] stored in 20% (v/v) glycerol at −40 °C. In these two reports there were no information about storage time.

Our study showed that samples stored in 40% (v/v) glycerol solutions at −40 °C were very stable.

[1] A. L. Ganser and J. G. Forte, Biochim. Biophys. Acta **307**, 169–180 (1973).
[2] G. Sachs, J. G. Spenney, and M. L. Lewin, Physiol. Rev. **58**, 106–173 (1978).
[3] J. G. Forte, T. T. E. Machen, and K. J. Öbrink, Annu. Rev. Physiol. **42**, 111–126 (1980).
[4] F. M. A. H. Schuurmens Stekhoven and S. L. Bonting, Physiol. Rev. **61**, 1–75 (1981).
[5] G. Saccamoni, H. B. Stewart, S. Show, M. Lewin, and G. Sachs, Biochim. Biophys. Acta **465**, 311–330 (1977).
[6] W. B. Im, D. P. Blakeman, J. Mendlein, and G. Sachs, Biochim. Biophys. Acta **770**, 65–72 (1984).
[7] T. E. King, Methods Enzymol. **10**, 322–331 (1967).
[8] H. W. Reading and T. İsbir, Q. J. Exp. Physiol. **65**, 105–115 (1980).
[9] J. G. Forte, J. L. Poulter, R. Dykstra, J. Rivas, and H. C. Lee, Biochim. Biophys. Acta **644** (2), 257–265 (1981).
[10] A. Atkinson, A. D. Getenby, and A. G. Lowe, Biochim. Biophys. Acta **320**, 195–204 (1973).
[11] O. H. Lowry, N. J. Rosenbrough, A. L. Ferr, and R. J. Randel, J. Biol. Chem. **193**, 261–275 (1951).
[12] S. E. Helmich, S. E. Van Ernst de Vries, F. M. A. H. Swerts, F. M. A. H. Schuurmens Stekhoven, and J. J. H. H. M. DePont, Biochim. Biophys. Acta **860**, 641–649 (1986).
[13] M. Ljungström and S. Mardh, J. Biol. Chem. **260** (9), 5440–5444 (1985).
[14] G. Sachs, T. Berglindh, F. Rabon, H. B. Stewart, M. L. Barvellona, B. Wallmark, and G. Saccomani, Ann. N. Y. Acad. Sci. **341**, 312–334 (1980).
[15] H. C. Lee, H. Breitbort, M. Berman, and J. G. Forte, Biochim. Biophys. Acta **553**, 107–131 (1979).
[16] H. C. Lee and J. G. Forte, Biochim. Biophys. Acta **508**, 337–356 (1978).
[17] J. M. Wolosin and J. G. Forte, J. Biol. Chem. **256** (7), 3149–3152 (1981).
[18] J. J. Schrijen, A. Omechi, L. W. A. Van Groningen, J. J. DePont, and S. L. Bonting, Biochim. Biophys. Acta **649** (1), 1–12 (1981).
[19] A. Sourmarmon, F. Grelac, and M. J. Levin, Biochim. Biophys. Acta **732** (3), 579–585 (1983).