Effects of valinomycin on ATP-ADP exchange in K^+-loaded erythrocyte ghosts

Takeo YAMAGUCHI*

（Received November 22, 2021）

Abstract

Na^+,K^+-ATPase shows the different response to the absence of extracellular K ions. In Na^+-containing but K^+-free medium, this enzyme exhibits the ATP-ADP exchange reaction. So, properties of this exchange were examined using K^+-loaded human erythrocyte ghosts. Upon addition of valinomycin, this exchange reaction was facilitated depending on the concentration of loaded K ions. However, such facilitation by valinomycin was inhibited by oligomycin. These results suggest that the ATP-ADP exchange reaction is affected by valinomycin, i.e., membrane potential.

Keywords: Na^+,K^+-ATPase, ATP-ADP exchange, erythrocyte membrane, oligomycin, valinomycin

1. Introduction

Na^+,K^+-ATPase or Na^+,K^+-pump exists as a transmembrane protein on the plasma membrane in the cells of all higher eukaryotes [1]. This pump in medium containing $Na^+$and K^+ transports two K ions inward across the plasma membrane and three Na ions outward per ATP molecule hydrolyzed, as demonstrated in forward reaction in the Post-Albers scheme (Fig. 1) [1-3]. Such unequal translocation of ions produces a net separation of charges across the membrane [4]. The resulting membrane potential plays an important role in the activation of nerve cells and the transport processes of numerous nutrients [5,6]. On the other hand, in K^+-free medium this pump (sodium pump) catalyzes Na^+-Na^+ exchange [7] and ATP-ADP exchange [8], which are shown as the backward reaction in Fig. 1. In Na^+-Na^+ exchange, the influx of extracellular Na^+ is coupled to the efflux of intracellular Na^+ [7]. Thus, Na^+-Na^+ exchange is electroneutral, requires the presence of intracellular ADP and ATP, and occurs without net ATP hydrolysis [9, 10]. The ATP-ADP exchange reaction is carried out at the cytoplasmic surface [11]. Under conditions of the Na^+-Na^+ exchange, ATP-ADP exchange is also induced [12]. However, ATP-ADP exchange occurs in the absence of extracellular Na^+ [13]. Moreover, in oligomycin-treated erythrocytes ATP-ADP exchange is observed but the Na^+-Na^+ exchange reaction is not [1,14]. These data suggest that ATP-ADP exchange is loosely coupled to Na^+-Na^+ exchange [12].

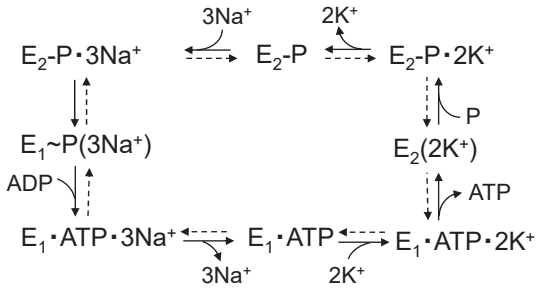

Fig. 1 Post-Albers scheme. Solid and broken lines indicate the backward and forward reactions in Na^+,K^+-ATPase, respectively. For simplicity, detail of forward reaction is omitted. Parenthesis indicates occlusion of ions.

Interestingly, the Na^+-Na^+ exchange reaction is affected by membrane potential; the uptake of extracellular Na^+ into the cell is accelerated by negative voltage or hyperpolarization [15,16]. However, the effect of membrane potential on ATP-ADP exchange remains to be determined. The membrane potential is induced using ionophores such as valinomycin [17]. The present work describes the effect of valinomycin, i.e., membrane potential, on ATP-ADP exchange in K^+-loaded erythrocyte ghosts.

2. Methods

2. 1 Ghost preparation

Freshly heparinized blood from author was centrifuged at 3,000 g for 10 min at 4 ˚C, and the plasma and buffy coat were carefully removed by aspiration. The cells were washed three times in 140 mM choline-chloride (Cl),

Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma 8-19-1 Jonan-ku, Fukuoka 814-0180, Japan
*Corresponding author: T. Yamaguchi (e-mail: takeo@fukuoka-u.ac.jp)

10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), pH 7.6 (with tris [hydroxymethyl] aminomethane (Tris)) (henceforth, buffer A) and suspended at a 25% hematocrit (2 mL in 6 mL buffer A). Such cell suspension was cooled in ice and applied to the top of the gel filtration column, which was cooled at 4 ˚C in the cold room. The column was filled with Bio-Gel A-50m beads (Bio-Rad Laboratories, Richmond, CA) and equilibrated in 10 mM piperazine-N,N'-bis (2-ethanesulfonic acid) (PIPES) buffer (pH 6.0 with Tris) containing 0.1 mM EDTA [13]. Open ghosts were eluted from the column at a rate of ~ 2 mL min^{-1} and collected in tubes. The ghosts were packed by centrifugation at 10,000 g for 5 min at 4 ˚C and suspended in the isotonic mixture of salts and nucleotides. These mixtures contained 140 mM salts consisted of KCl, NaCl, and choline-Cl, 10 mM HEPES (pH 7.4), 10 μM $MgCl_2$, 75 μM Caged ATP, 25 μM [^{3}H] ADP, and 0.1 mM P1,P5-di (adenosine-5') pentaphosphate (Ap5A) [13]. The ghost suspensions were incubated for 10 min at 0 ˚C, 20 min at 4 ˚C, and finally 45 min at 37 ˚C. The resealed ghosts were washed three times in chilled buffer A, Such ghosts were packed in plastic tubes by centrifugation at 10,000 g for 10 min at 4 ˚C and held at room temperature (23 ˚C) until use.

2. 2 ATP-ADP exchange

ATP-ADP exchange was taken as the ouabain-sensitive [^{3}H]ATP production from [^{3}H]ADP loaded within resealed ghosts [13]. Packed ghosts, which were loaded with salts and nucleotides, were suspended at a 11% hematocrit in 10 mM HEPES buffer (pH 7.4) containing140 mM NaCl and 0.1 mM KCl. Ouabain (0.2 mM) was added to the cell suspension and incubated for 1 min at 23 ˚C. Then, valinomycin in C_2H_5OH was added to the suspension (final concentration 1 μM) [17]. Oligomycin treatment of ghosts was performed prior to addition of ouabain and valinomycin. Namely, oligomycin in C_2H_5OH was added to the ghost suspension (final concentration 0.2 ~ 1.0 μg/mL) and incubated for 5 min at 23 ˚C [18]. Such ghost suspensions (225 μL) were irradiated for 15 sec at 340 nm [8, 13]. Aliquots (25 μL) were taken at time intervals of 30, 60, 90, and 120 sec and added to ice-cold 10% trichloroacetic acid (25 μL). The samples were rapidly mixed, held for 10 min at 0 ˚C, and centrifuged at 13,000 g for 1 min at 4 ˚C. The supernatants were used for the detection of nucleotides.

The separation of nucleotides was carried out using the thin-layer chromatography (plate, polyethyleneimine; developer, 0.4 M potassium phosphate, pH 3.5). Each nucleotide on the plate was detected by an ultraviolet lamp. The measurement of radioactivity in nucleotides was performed using a liquid scintillation counter (7500, Beckman Instruments, Inc.), as previously described [13].

3. Results

3. 1 Effects of intracellular K ion concentration on ATP-ADP exchange in the erythrocyte membrane

It is reported that ATP-ADP exchange by sodium pump is inhibited by extracellular K ions but not by intracellular K ions (17 mM) under high concentration of intracellular Na^+ [13]. Here, the influence of intracellular K ions on such exchange reaction was examined in the wide concentration range up to130 mM. As shown in Fig. 2, the ouabain-sensitive ATP-ADP exchange rate was decreased at high intracellular K ion concentration (130 mM).

3. 2 Effects of valinomycin on ATP-ADP exchange in K^+-loaded erythrocyte ghosts

As seen in Fig. 2, the ouabain-sensitive ATP-ADP exchange rate was decreased with increasing intracellular K ion concentration. Upon addition of valinomycin to the system, such exchange rate was restored as shown in Fig. 3A. To clarify the effect of valiomycin, the ratio of such exchange rate in the presence (Rval (+)) and absence (Rval (-)) of valinomycin, Rval (+) / Rval (-), was plotted against intracellular KCl concentration (Fig. 3B). The results indicate that the ouabain-sensitive ATP-ADP exchange rate is increased upon addition of valinomycin to the K^+-loaded ghosts.

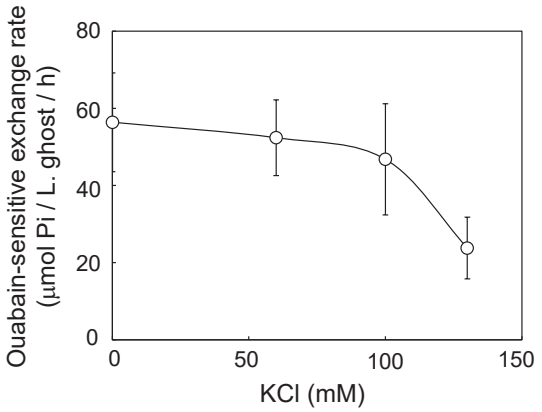

Fig. 2 Effects of intracellular KCl concentration on ouabain-sensitive ATP-ADP exchange in the erythrocyte membrane. The ghosts loaded with 0.1 mM KCl/10 mM NaCl/130 mM choline-Cl, 60 mM KCl/10 mM NaCl/70 mM choline-Cl, 100 mM KCl/10 mM NaCl/30 mM choline-Cl, or 130 mM KCl/10 mM NaCl as major salts were suspended in 10 mM HEPES buffer (pH 7.4) containing140 mM NaCl and 0.1 mM KCl. Values are means ± standard deviations for at least three independent experiments.

3. 3 Effects of oligomycin on ATP-ADP exchange in valinomycin-treated erythrocyte membrane

The ouabain-sensitive ATP-ADP exchange rate increased upon oligomycin treatment (Fig. 4A). On the other hand, such exchange rate decreased upon valiomycin treatment of oligomycin-treated erythrocyte membrane (Fig. 4A). To clarify the effect of valiomycin and oligomycin on ATP-ADP exchange, values of Rval (+) / Rval (-) were plotted against oligomycin concentration. Values of Rval (+) / Rval (-) were decreased by oligomycin (Fig. 4B). These results suggest that ouabain-sensitive ATP-ADP exchange facilitated by valinomycin is inhibited by oligomycin.

4. Discussion

The ouabain-sensitive ATP-ADP exchange reaction in the human erythrocyte membrane is generally inhibited by extracellular K^+, but not by K^+ (0.07% vs. Na ions) diluted with high concentration of extracellular Na ions, as demonstrated here. In the case of intracellular K^+, it is reported that this exchange reaction is affected by K^+ (14 mM) under low concentration of intracellular Na^+ [13]. The present data show the inhibition of ATP-ADP exchange by the intracellular K ions at their high concentrations. However, such inhibition seems to be relaxed by addition of valinomycin, K^+ ionophore. Namely, the ouabain-sensitive ATP-ADP exchange is restored to the level in K^+-unloaded ghosts. However, a large decrease of intracellular K^+ concentration due to valinomycin under present conditions is not considered, judging from data of Hoffmann and Laris used a fluorescence probe [17]. Importantly, valinomycin is widely used to generate membrane potential ($\Delta\Psi$) in K^+-loaded membranes [17, 18]. Although membrane potential is not measured in this work, it can be estimated from the following relation: $\Delta\Psi = (RT/F)\ \ln\ \{[\alpha[K^+]_o + [Cl^-]_i] / [\alpha[K^+]_i + [Cl^-]_o]\}$, where R, gas constant; T, absolute temperature; F, Faraday's constant; α = permeability constant for K^+ (P_{K+}) / permeability constant for Cl^- (P_{Cl-}); $[\ ]_i$ and $[\ ]_o$, intracellular and extracellular concentrations, respectively. Hoffman and Laris determined a value of 3 as α in human erythrocytes with intracellular K^+ of 152 mM [17]. Applying α= 3 in the present case, i.e., 130 mM KCl-loaded

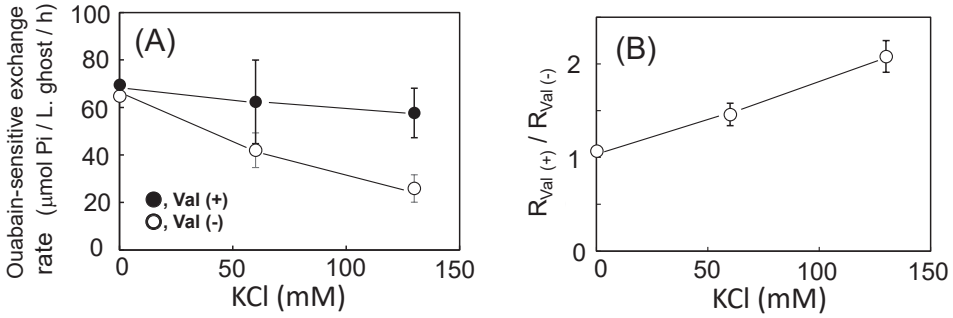

Fig. 3 Effects of valinomycin on ouabain-sensitive ATP-ADP exchange in the erythrocyte membrane. (A) The ghosts loaded with 0.1 mM KCl/10 mM NaCl/130 mM choline-Cl, 60 mM KCl/10 mM NaCl/70 mM choline-Cl, or 130 mM KCl/10 mM NaCl as major salts were suspended in 10 mM HEPES buffer (pH 7.4) containing 140 mM NaCl and 0.1 mM KCl. Valinomycin (Val, 1 μM) was added to the ghost suspension. (B) Ouabain-sensitive ATP-ADP exchange rate (R) was determined in the presence (Rval (+)) or absence (Rval (-)) of 1 μM valinomycin. Values of Rval (+) / Rval (-) were plotted against intracellular KCl concentrations. Error bars show standard deviations for at least two independent experiments.

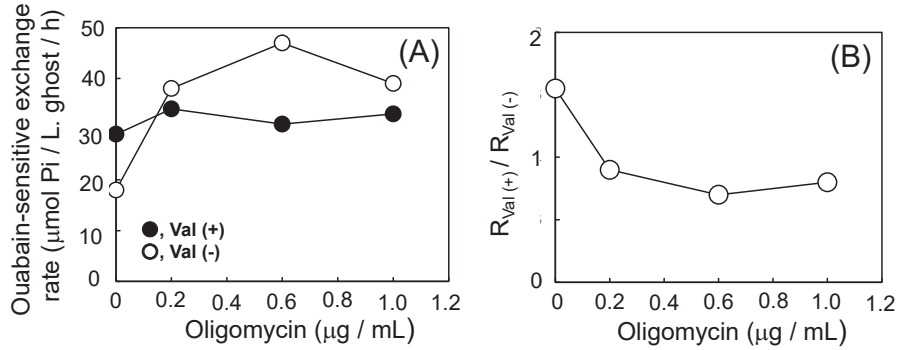

Fig. 4 Effects of oligomycin on ouabain-sensitive ATP-ADP exchange in the valinomycin-treated ghost membrane. (A) Ghosts loaded with 130 mM KCl and 10 mM NaCl were suspended in 10 mM HEPES buffer (pH 7.4) containing 140 mM NaCl and 0.1 mM KCl. Oligomycin (0.2 ~ 1.0 μg/mL) was added into the ghost suspension. Ouabain (0.2 mM) and valinomycin (1 μM) were added as described in text. (B) Ouabain sensitive ATP-ADP exchange rate (R) was determined in the presence (Rval (+)) or absence (Rval (-)) of 1 μM valinomycin. Values of Rval (+) / Rval (-) were plotted against oligomycin concentration.

ghosts, a value of – 34 mV as a membrane potential is obtained. This value is near to that obtained from the fluorescence probe [17]. Thus, the ATP-ADP exchange reaction in K^+-loaded erythrocyte ghosts is facilitated by valinomycin, i.e., membrane potential, as shown in Fig. 3B.

Interestingly, Na^+-Na^+ exchange is also affected by membrane potential [15]. Extracellular Na ions travel up to binding site of sodium pump through its ion pathway under electrical potential and their translocation rate increases under hyperpolarizing potential [15,16]. The resulting increment of intracellular Na ions facilitates the backward reaction in the Post-Albers scheme. Thus, the formation of E_2-P · $3Na^+$ from E_2-P in sodium pump is accelerated so that the Na^+ release to the cell interior readily occurs. Therefore, the Na^+-Na^+ exchange reaction is facilitated by hyperpolarization [16]. On the other hand, each step involved in ATP-ADP exchange takes place at the cytoplasmic surface so that it is not directly subjected to membrane potential. The enhancement of E_2-P · $3Na^+$formation, which is accelerated by hyperpolarization of the membrane, can facilitate the backward reaction toward ATP production from ADP. Therefore, the facilitation of Na^+-Na^+ exchange under hyperpolarization results in the enhancement of ATP-ADP exchange. Thus, ATP-ADP exchange as well as Na^+-Na^+ exchange is also affected by membrane potential via the formation of E_2-P · $3Na^+$.

Oligomycin binds to sodium pump and blocks the conversion between Na^+-occluded form E_1~P($3Na^+$) and E_2-P · $3Na^+$ in the Post-Albers scheme [1,19]. Thus, oligomycin inhibits the Na^+-Na^+ exchange reaction [14, 19]. On the other hand, oligomycin does not inhibit ATP-ADP exchange [14], whereas reduces the enhancement of ATP-ADP exchange by valinomycin. The results from oligomycin also suggest that the facilitation of ATP-ADP exchange by valinomycin is associated with the increase of Na^+-Na^+ exchange. Although it is well known that membrane potential generates upon addition of valinomycin to K^+-loaded erythrocyte membranes, the measurement of membrane potential in the present system is necessary to understand sufficiently the data given here.

Acknowledgment

I thank Prof. Jack H Kaplan (University of Pennsylvania) for his encouragement, insightful comments, and support during this work. This work was carried out in the laboratory of Jack H Kaplan.

References

[1] I. M. Glynn, J. Physiol., **462**, 1 (1993).
[2] R. L. Post, S. Kume, T. Tobin, B. Orcutt, A. K. Sen, J. Gen. Physiol., **54**, 306 (1969).
[3] R. W. Albers, G. J. Koval, G. J. Siegel, Mol. Pharmacol., **4**, 324 (1968).
[4] J. A. Reynolds, E. A. Johnson, C. Tanford, Proc. Natl. Acad. Sci. U. S. A., **82**, 6869 (1985).
[5] A. S. Pivovarov, F. Calahorro, R. J. Walker, Invert. Neurosci., **19**, 1 (2018).
[6] K. B. Gagnon and E. Delpire, Front. Physiol., **11**, 588664 (2021).
[7] P. J. Garrahan and I. M. Glynn, J. Physiol., **192**, 159 (1967).
[8] J. H. Kaplan and R. J. Hollis, Nature, **288**, 587 (1980).
[9] P. J. Garrahan and I. M. Glynn, J. Physiol., **192**, 217 (1967).
[10] J. D. Cavieres and I. M. Glynn, J. Physiol., **297**, 637 (1979).
[11] I. M. Glynn and S. J. Karlish, J. Physiol., **256**, 465 (1976).
[12] J. H. Kaplan, Am. J. Physiol., **245**, G327 (1983).
[13] J. H. Kaplan, J. Gen. Physiol., **80**, 915 (1982).
[14] R. Blostein, J. Biol. Chem., **245**, 270 (1970).
[15] R. D. Peluffo, Biophys. J., **87**, 883 (2004).
[16] M. Nakao and D. C. Gadsby, Nature, **323**, 628 (1986).
[17] J. F. Hoffman and P. C. Laris, J. Physiol., **239**, 519 (1974).
[18] X. S. Yang and K. Kamino, Jpn. J. Physiol., **47**, 217 (1997).
[19] H. Homareda and M. Ushimaru, FEBS. J., **272**, 673 (2005).