ELSEVIER

Biochimica et Biophysica Acta 1330 (1997) 110–112

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Rapid report

Transport energetics of the Cl^- pump in *Aplysia* gut

George A. Gerencser *

Department of Physiology, College of Medicine, University of Florida, Gainesville, FL 32610, USA

Received 23 July 1997; accepted 31 July 1997

Abstract

Basolateral membranes of *Aplysia* foregut epithelia contain an ATP-dependent Cl^- transporter (Cl^- pump). Increased activity of the Cl^- pump, coupled to apical and basolateral membrane depolarization, changed the Cl^- transport energetics across the apical membrane but did not change the vectorially-opposite Cl^- transport energetics across the basolateral membrane. © 1997 Elsevier Science B.V.

Keywords: Gut; Active transport; Cl^- energetics; *(Aplysia)*

Active Cl^- absorption by the *Aplysia californica* (seahare) foregut is mediated by a basolaterally-located Na^+-independent, electrogenic, Cl^--stimulated ATPase [1,2]. This Cl^- pump accounts for the intracellular Cl^- electrochemical potential ($\bar{\mu}$) being less than the extracellular Cl^- $\bar{\mu}$ [3]. Cl^- transport across the apical membrane into the cytosol of these cells is mediated via voltage-regulated channels [4]. In addition, aminoisobutyric acid (AIB), a nonmetabolizable amino acid, is actively accumulated by the *Aplysia* foregut in the presence of Na^+ [5]. This Na^+-dependent AIB transport enhanced the net mucosal-to-serosal Cl^- flux across the *Aplysia* foregut [5]. In view of this apical membrane coupling of Na^+, AIB and Cl^-, the present study was undertaken to determine whether this event altered the energy gradient across the basolateral membrane where the Cl^- pump transports Cl^- uphill.

Adult seahares (*Aplysia californica*) were obtained from Marinus (Westchester, CA) and were maintained at 25°C in circulating filtered sea water. The animals were sacrificed and their posterior foreguts were removed, slit longitudinally, rinsed and then positioned between two halves of a Lucite chamber described previously [3] which allowed measurement of transepithelial electrical potential (Ψ_{ms}) and, simultaneously, the introduction of microelectrodes into the surface epithelial cells. The chamber exposed the tissue to an oxygenated seawater medium. The formula for the seawater medium was (in mM): NaCl, 462.0; $MgSO_4 \cdot 7H_2O$, 2.4; KCl, 10.0; $KHCO_3$, 2.4; $MgCl_2$, 9.8; $CaCl_2$, 11.4. The total osmolality of the bathing medium was 1000 mosmol/l and the final pH was 7.8. Microelectrodes for measurements of mucosal membrane potential (Ψ_m) and $\bar{\mu}_{Cl}$ were constructed and utilized as previously described [3]. Briefly, the experimental protocol was as follows: after the excised tissue was placed into cells lining the gut villi to obtain an independent estimate of Ψ_m, after which, Cl^--selective microelectrodes were passed into the villus ep-

* Fax: (+1)352 846 0270.

PII S0005-2736(97)00188-0

ithelial cells to measure the intracellular $\bar{\mu}_{Cl}$. The intracellular Cl^- activity (a^i_{Cl}) was calculated using

$$a^i_{Cl} = a''_{Cl}/e^{2.303[(\Psi_i - \Psi_m) - \Psi'']/S} \quad (1)$$

where Ψ_i is the potential of the Cl^- electrode in the cell, a^i_{Cl} the activity of Cl, Ψ'' the potential of the Cl^- electrode in 500 mM NaCl, Ψ_m the mean mucosal membrane potential, and S is the slope of the electrode response defined as described previously [3,6]. In Table 1, $\bar{\mu}_j$ is expressed as reversible work, in joules, required to transfer one equivalent of Cl^- across the mucosal or basolateral (serosal) membrane of a foregut villus epithelial cell and is calculated from

$$\bar{\mu}_j = RT \ln a^i_j/a^0_j + z\Psi F \quad (2)$$

where R, T, z and F have their usual physicochemical meanings and Ψ can either be Ψ_m or Ψ_s (basolateral or serosal membrane potential). The data obtained were analyzed statistically by Student's t-test.

As demonstrated in the present study (Table 1), mucosally-applied 80 mM AIB significantly depolarized both Ψ_m and Ψ_s and significantly hyperpolarized Ψ_{ms}. Therefore the calculated change in Ψ_s exceeded the empirically determined change in Ψ_m. Also seen in Table 1 are the findings that mucosally-applied AIB significantly increased a^i_{Cl} above control and significantly lowered the difference in $\bar{\mu}_{Cl}$ across the basolateral membrane.

Mucosally-applied AIB caused a depolarization of Ψ_m, a hyperpolarization of Ψ_{ms} and an increase in a^i_{Cl} in the foregut cells of *Aplysia californica* (Table 1). These observations can be explained as follows: If an actively transported amino acid such as AIB is Na^+-coupled at the apical membrane [5], this would depolarize the Ψ_m because of the electrogenic or rheogenic nature of the mechanism [2,7]. This depolarization would then lower the electrochemical or thermodynamic energy gradient ($\bar{\mu}^m_{Cl}$) opposing intracellular Cl^- entry from the mucosal bathing solution as shown in Table 1. This would then allow Cl^- to move across the mucosal membrane into the intracellular space with greater ease via the voltage-regulated Cl^- channels which were further opened by the Ψ_m depolarization [4]. The finding that a^i_{Cl} is significantly higher in the presence of mucosal AIB than in its absence (Table 1) lends strong support for this notion. The basolaterally-located electrogenic Cl^- pump or Cl^--ATPase [1] would then accommodate the increased thermodynamic activity of Cl^- (Cl^- transport pool) by increasing its rate of work much as a variable output device which was described by Michaelis–Menten kinetics in an isolated Cl^- pump protein system [8]. In addition, this was previously demonstrated both by an increase in short-circuit current (SCC) and unidirectional mucosal-to-serosal Cl^- flux after mucosal AIB addition to the voltage-clamped, isolated foregut [5]. Since it has been shown that the major portion of the SCC before and after AIB addition to the mucosal solution is a Cl^- current [5], there would be a decrease in the negativity of Ψ_s by both the increased electrogenic Cl^- pump activity and the linkage of Ψ_m to Ψ_s through a low resistance extracellular shunt [9] which would lead to a greater serosally-negative Ψ_{ms} [10] as shown in Table 1. Whether AIB is present or absent in the mucosal bathing solution the thermodynamic energy gradient against which the Cl^- pump is moving Cl^- ($\bar{\mu}^s_{Cl}$)

Table 1
Potential profiles, intracellular Cl^- activities and transmembrane Cl^- electrochemical potential differences in *Aplysia californica* foregut bathed in NaCl seawater medium in the presence and absence of mucosal aminoisobutyric acid

	Ψ_m (mV)	Ψ_s (mV)	Ψ_{ms} (mV)	a^i_{Cl} (mM)	$\bar{\mu}^m_{Cl}$ (J/equiv.)	$\bar{\mu}^s_{Cl}$ (J/equiv.)	n
Before AIB addition	-68.2 ± 1.8 (35)	$+67.9 \pm 1.4$ (35)	-0.3 ± 0.1 (35)	8.6 ± 0.5 (22)	$+7066 \pm 66$ (22)	-7095 ± 70 (22)	12
After AIB addition	-61.1 ± 1.8 (35) $p < 0.01$	$+57.8 \pm 1.5$ (35) $p < 0.01$	-3.3 ± 0.4 (35) $p < 0.01$	13.8 ± 0.7 (22) $p < 0.01$	$+6663 \pm 55$ (22) $p < 0.01$	-6981 ± 48 (22) N.S.	12

Values are means ± S.E. Numbers in parentheses are number of observations; n is the number of animals. Polarity of Ψ_m and Ψ_{ms} are relative to the mucosal solution. Polarity of calculated Ψ_s is relative to cytoplasm. a^i_{Cl} was calculated by means of Eq. (1). $\bar{\mu}^m_{Cl}$ and $\bar{\mu}^s_{Cl}$ were calculated by means of Eq. (2). (+) for $\bar{\mu}^m_{Cl}$ represents downhill energy gradient from mucosal solution to cytosol. (−) for $\bar{\mu}^s_{Cl}$ represents uphill energy gradient from cytosol to serosal solution.

does not change (Table 1). This is because in the presence of a maximally stimulating 80 mM AIB concentration [5], the increase in a^{i}_{Cl} (which favors a decrease in the energy gradient) almost equals the thermodynamic equivalent for the decrease in Ψ_s (which favors an increase in the energy gradient). These two forces (chemical and electrical potentials), which define the overall energy gradient against which the basolaterally-localized Cl^- pump must move Cl^-, quantitatively cancel one another because they are vectorially oriented in opposite directions and their respective quantitative changes are equivalent in the presence of AIB.

My thanks to Dr. William McD. Armstrong for helpful discussions during the course of the study. I would like to acknowledge the excellent technical assistance of F. Robbins. This investigation was supported by the Eppley Foundation for Research, Inc.

References

[1] G.A. Gerencser, B. Zelezna, Reaction sequence and molecular mass of a Cl^--translocating P-type ATPase, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 7970–7974.

[2] G.A. Gerencser, The chloride pump: A Cl^--translocating P-type ATPase, Crit. Rev. Biochem. Mol. Biol. 31 (4) (1996) 303–337.

[3] G.A. Gerencser, Stoichiometry of a Cl^--translocating ATPase, FEBS Lett. 33 (1,2) (1993) 137–140.

[4] G.A. Gerencser, in: (G.A. Gerencser (Ed.), Chloride Channels in Molluscs Electrogenic Cl^- Transporters in Biological Membranes, as part of Advances in Comparative and Environmental Physiology, Springer, Berlin, 1994, pp. 133–153

[5] G.A. Gerencser, Effects of amino acids on chloride transport in *Aplysia* intestine, Am. J. Physiol. 240 (1981) R61–R69.

[6] J.F. White, Bicarbonate-dependent chloride absorption in small intestine: Ion fluxes and intracellular chloride activities, J. Membr. Biol. 53 (1980) 95–107.

[7] R.C. Rose, S.G. Schultz, Sugar and amino acid effects on the electrical potential profile across rabbit ileum, Biochim. Biophys. Acta. 211 (1970) 376–378.

[8] G.A. Gerencser, K.R. Purushotham, Reconstituted Cl^- pump protein: a novel ion (Cl^-)-motive ATPase, J. Bioenerg. Biomembr. 28 (6) (1996) 461–471.

[9] G.A. Gerencser, G.M. Loughlin, Extracellular transport properties of *Aplysia californica* intestine, Comp. Biochem. Physiol. 74A (1983) 201–204.

[10] S.G. Schultz, Sodium-coupled solute transport by small intestine: A status report, Am. J. Physiol. 233 (1977) E249–E254.