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HCO_3^--ACTIVATED ADENOSINE TRIPHOSPHATASE IN INTESTINAL MUCOSA OF THE EEL

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Summary

An HCO_3^--activated and SCN^--inhibited ATPase (ATP phosphohydrolase, EC 3.6.1.3) found in homogenates of intestinal mucosa of the eel was solubilized by Triton X-100. Optimal HCO_3^- concentration and pH for the enzyme were 25 mM and 8.7, respectively. HCO_3^--ATPase activity in both homogenate and solubilized preparations increased after seawater adaptation. This adaptive increase in enzyme activity was also observed in the gills and the kidney. The HCO_3^--ATPase seems to be related to transport mechanisms, especially for Cl^-, in osmoregulatory surfaces of the eel.

Introduction

It has been well established that the intestine is an important osmoregulatory organ for teleosts in sea water; they ingest sea water and absorb it from the intestine to replace water lost osmotically through the body surface [1]. Recent investigations have revealed that the increased water absorption observed in seawater eel intestine is closely related to the development of a chloride pump [2,3]. Furthermore, presence of Cl^-/HCO_3^- exchange has been shown in flounder intestine as well as in the gills of some teleosts [1].

Since Kasbeker and Durbin described an HCO_3^--activated and SCN^--inhibited ATPase (ATP phosphohydrolase, EC 3.6.1.3) from frog gastric mucosa [4], evidence has accumulated suggesting that this enzyme is responsible for HCl secretion accompanied by transport of HCO_3^- [5—7] in the vertebrate stomach generally. A similar enzyme occurs in mammalian pancreas [8,9] and in the gills of *Necturus* [5], rainbow trout [10] and eel [11], suggesting a role for the enzyme in Cl^-/HCO_3^- exchange.

In the present investigation, the presence, properties and role of HCO_3^--ATPase from the intestinal mucosa and other tissues of the eel were studied.

Materials and Methods

Homogenate

Japanese cultured eels (*Anguilla japonica*) were maintained in freshwater or seawater aquaria as described in the previous paper [12]. After decapitation, gills, stomach, intestine, kidney and liver were excised & washed 3 times with 0.25 M sucrose. The stomach and intestine were opened and washed again with the same solution. After blotting with filter paper, the mucosa was removed by scraping with a surgical knife. The gills, kidney and liver were minced with scissors. Tissues weighing about 0.1—0.5 g were homogenized in 3 ml of 0.25 M sucrose buffered with 20 mM Tris · HCl at pH 7.4, by 15 strokes of a Potter-Elvehjem glass homogenizer equipped with a Teflon pestle at 1200 rev./min. In order to show that oligomycin blocks authentic mitochondrial ATPase of the eel intestine, the homogenate was centrifuged to produce nuclear (800 × *g*), mitochondrial (10 000 × *g*), microsomal (100 000 × *g*) and supernatant fractions.

Solubilization of the enzyme

Mucosal scrapings of the intestine were homogenized by 10 strokes of the glass homogenizer in 10 vols. of 20 mM *N*-2-hydroxyethylpiperazin-2-ethansulfonic acid/NaOH (HEPES/NaOH) (pH 7.4) at 1200 rev./min. The homogenate was mixed with Triton X-100 at final concentration 1 or 6% and incubated for 2 h at 2° C with constant stirring. The supernatant was centrifuged at 100 000 × *g* for 60 min and the final supernatant was used as enzyme solution.

Enzyme assay

The activity of HCO_3^--ATPase and Mg^{2+}-ATPase was measured in a standard system consisting of 3 mM ATP, 5 mM $MgCl_2$, 100 mM Tris · HCl (pH 8.7) and 0.1 ml of the homogenate or the solubilized enzyme solution with or without appropriate concentrations of $NaHCO_3$. Final volume was adjusted to 2.0 ml. The reaction mixtures were incubated at 25° C for 20 min. The reaction was stopped by adding 0.5 ml of 30% trichloroacetic acid. After centrifugation, 2.0 ml of the deproteinized supernatant was analyzed for inorganic phosphate by the method of Fiske and SubbaRow [13]. HCO_3^--ATPase activity was expressed as the difference between the activity in the presence and the absence of $NaHCO_3$. Parallel blanks were always run. Cytochrome *c* oxidase activity was measured by the decrease in absorbance at 550 nm. The cytochrome *c* was reduced with ascorbic acid prior to use. Oligomycin, ouabain and NaSCN were added to the incubation medium containing 25 mM $NaHCO_3$. The protein content was measured by the method of Lowry et al. [14].

The reagents used were disodium salt of ATP, Tris and Oligomycin from Sigma Chemical Co., and ouabain from Merck AG. Other reagents were of analytical grade.

Results

Properties of HCO_3^--ATPase in intestinal mucosa

The HCO_3^- concentration dependence of the enzyme activity in mucosal ho-

mogenate and Triton X-extract of the seawater eel intestine is shown in Fig. 1. Maximum activity was attained at 25 mM and the activity remained virtually constant. This concentration was used in all subsequent experiments. NaCl at concentrations from 25 to 100 mM had no detectable effect on the ATPase activity in the absence of $NaHCO_3$, indicating that the activation of the enzyme is due to HCO_3^- rather than to Na^+.

The effect of pH on HCO_3^--stimulated ATP hydrolysis was studied in mucosal homogenates of both seawater- and freshwater-adapted eel intestines over a pH range from 7.0 to 9.0 (Fig. 2). Tris · HCl buffer at a final concentration of 100 mM was used to obtain the pH range. The optimal pH was 8.7 in both seawater and freshwater eel intestines, and subsequent experiments were performed at pH 8.7. Next the effect of various concentrations of Triton X-100 on solubilization of the enzyme was examined. As shown in Fig. 3, 1% Triton X-100 resulted in a maximum extraction and gave the most reproducible results. As is also shown in Fig. 1, optimal HCO_3^- concentration of the solubilized enzyme was similar to that of the homogenate, whereas the degree of activation was 5 times higher in the solubilized fraction than in the homogenate. Substrate specificity of the 6% Triton-solubilized enzyme from seawater eel intestine is in Table I. ATP was the most effective nucleotide. GTP, UTP and ITP were also effective, but CTP, ADP and AMP were without effect.

Effects of various inhibitors on the extracted enzyme were next examined. As shown in Fig. 4, NaSCN also inhibited the intestinal enzyme at all concentrations studied, and maximum inhibition was attained at a concentration of 5 mM. Ouabain did not affect bicarbonate activation of the ATPase at a concentration of 0.5 mM. Diamox, a potent inhibitor of carbonic anhydrase, was also without effect at 0.5 mM. Oligomycin at concentrations of 5 and 10 μg/ml inhibited Mg^{2+}-ATPase activity in the mitochondrial fraction by 70%, which

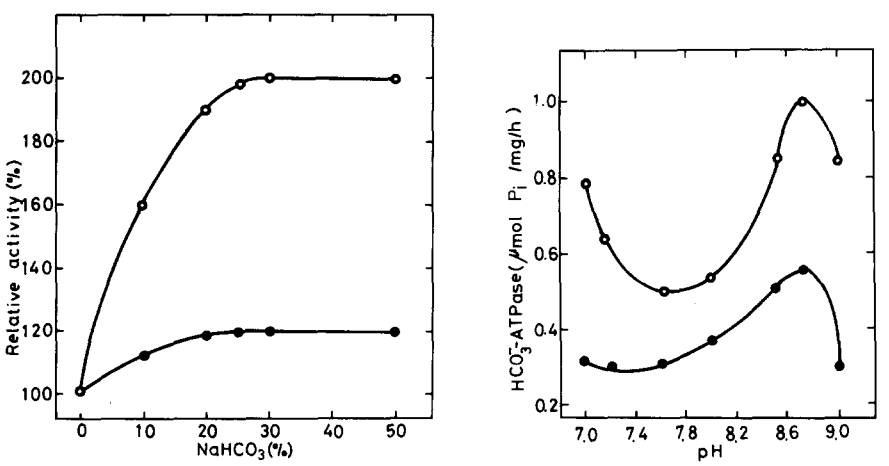

Fig. 1. Effect of HCO_3^- on ATPase activity in intestinal mucosa of the eel adapted to sea water for 5 days. ●———●, homogenate. ○———○, 6% Triton X-100 extract. Experiments used three different preparations; representative results are presented.

Fig. 2. Effect of pH on HCO_3^--ATPase activity in the homogenate of intestinal mucosa of eels. ●———●, freshwater eel, ○———○, seawater eel (6 days). Experiments as in Fig. 1.

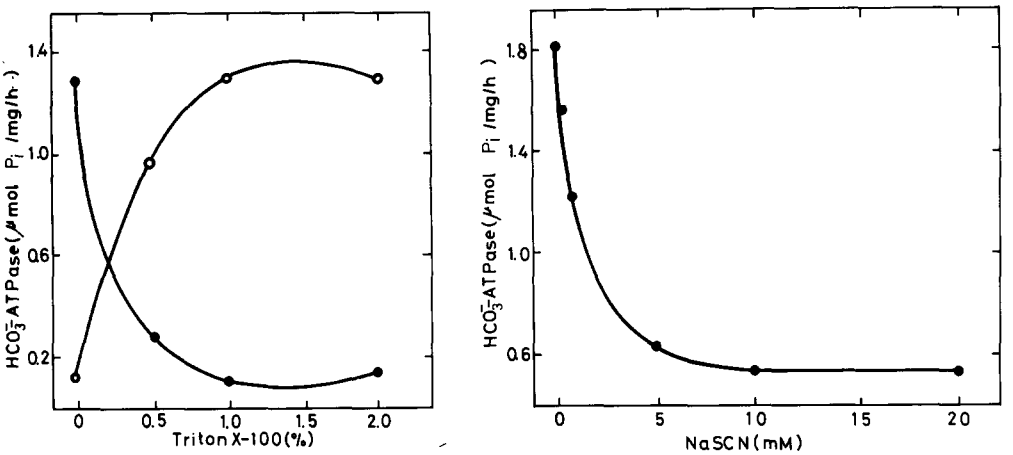

Fig. 3. Effect of various concentrations of 1% Triton X-100 on solubilization of HCO_3^--ATPase from eel intestinal mucosa. ●——●, precipitate. ○——○, supernatant.

Fig. 4. Effect of NaSCN on HCO_3^--ATPase activity in Triton X-100 extract of the intestinal mucosa of the eel adapted to sea water for 5 days.

contained 82% of cytochrome *c* oxidase activity of all fractions. In contrast, oligomycin did not affect the HCO_3^--ATPase activity in either homogenate or solubilized fraction at concentrations between 0.5 and 10 μg/ml. The Mg^{2+}-ATPase activity in the microsomal fraction containing 13% of cytochrome *c* oxidase activity was inhibited only by 13% with oligomycin.

Effect of seawater adaptation on HCO_3^--ATPase in various tissues

In order to consider the possible involvement of HCO_3^--ATPase in ion transport, the change in the enzyme activity in various tissues of the eel adapted to sea water for 7 days was examined. The enzyme activity increased in the homogenate of the osmoregulatory organs such as the intestine, gills and kidney after seawater adaptation. In contrast, adaptation to seawater did not affect the enzyme activity in the gastric mucosa and the liver (Table II). Erythrocytes isolated from eel blood by centrifugation at 10 000 × *g* 5 min, had no HCO_3^--ATPase activity, indicating that the enzyme activity in both homogenate and solubilized preparation was not derived from red cell ATPase.

TABLE I

SUBSTRATE SPECIFICITY OF SOLUBILIZED HCO_3^--ATPase

Substrate *	$(HCO_3^- + Mg^{2+})/Mg^{2+}$-ATPase activity
ATP	2.04
GTP	1.27
UTP	1.28
ITP	1.33
CTP	1.00
ADP	1.08
AMP	1.00

* 3 mM nucleoside phosphate were added to the reaction mixture.

TABLE II

HCO_3^--ATPase ACTIVITY IN VARIOUS TISSUES OF THE EEL

Values given as mean ± S.E. (n = 3)

Tissues	HCO_3^--ATPase (μmol P_i/mg/h)		Activity in seawater eel / Activity in freshwater eel
	Freshwater eel	Seawater eel	
Gill	0.42 ± 0.05	0.70 ± 0.08	1.67
Intestinal mucosa	0.42 ± 0.02	0.71 ± 0.05	1.69
Kidney	0.57 ± 0.06	0.74 ± 0.10	1.30
Gastric mucosa	1.36 ± 0.18	1.39 ± 0.18	1.02
Liver	0.63 ± 0.09	0.54 ± 0.09	0.86

Discussion

There is much evidence suggesting that HCO_3^--ATPase is related to HCl secretion by the gastric mucosa of amphibians [4,5] and mammals [6,7] and to HCO_3^- secretion in the pancreas of mammals [8,9]. Several properties of the enzyme of the intestinal mucosa of the eel are similar to those of amphibians and mammals. The optimal HCO_3^- concentration (25 mM) [4,9], substrate specificity [6,9] and solubility in Triton X-100 [5] are identical. HCO_3-ATPase activity in the gastric mucosa and the pancreas [4,5,8] and acid secretion in the gastric mucosa [18,19] were both inhibited by SCN^-. In the eel intestine, this reagent also inhibited the enzyme activity. Diamox is known to inhibit the secretion of HCl and HCO_3^- in the gastric mucosa and the pancreas, [20,21] but has no effect on HCO_3^--ATPase in these tissues [4,8]. This reagent also has no inhibitory effect on the enzyme activity in the eel intestine. Ouabain was without effect on HCO_3^--ATPase activity in the eel intestine, as in the frog gastric mucosa [4].

In contrast, optimal pH (8.7) which is identical to that of intestinal fluid [12] of the eel, was different from pH optimum of the enzyme from gastric mucosa (7.4) [6] and the pancreas (7.6) [9] of mammals.

In the eel intestine, recent electrophysiological experiments have shown that active transport of Cl^- coupled with water transport markedly increases during the course of seawater adaptation [2,3]. The observed increase in Cl^- transport raises the question of an associated increase in an enzyme contributing to the transport mechanism.

In the present study, HCO_3^--ATPase activity of the homogenate of osmoregulatory organs increased during seawater adaptation of the eel. This increase in the enzyme activity was most prominent in the intestinal mucosa. The enzyme activity of the intestinal homogenate increased three times in the 5th day after transfer to sea water, and then gradually decreased. The pattern of increasing enzyme activity is similar to the changes in ion and water transport: the net lumen-to-plasma Cl^- and Na^+ transport increased in seawater-adapted eel [15], the rate of water absorption, which is associated with NaCl transport [3], and Na^+ transport also increased to a maximum on the 5th day both in vivo and in isolated intestine [16,17]. The activity of the solubilized enzyme from the eel

adapted to sea water for 5 days was also higher than that from the freshwater eel. Maetz et al. [11] also demonstrated an increase in enzyme activity in the gills of the eel on seawater adaptation.

From these considerations, it seems probable that HCO_3^--ATPase plays an important role on Cl^- transport and in water absorption in the eel intestine. The enzyme and its mechanism of action seem to be similar in the eel intestine and in the gastric mucosa [7] and pancreas [8].

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