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# ALLOSTERIC COOPERATIVITY DURING INTESTINAL COTRANSPORT OF SODIUM AND CHLORIDE IN FRESHWATER PRAWNS

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## Summary

Coupled influxes of sodium and chloride across the mucosal border of a freshwater prawn intestine were sigmoidal functions of luminal ion concentrations, indicative of a cooperative allosteric transport process. This process had a higher affinity for Cl ($K_{Cl}$ = 94 mM) than for Na ($K_{Na}$ = 155 mM), maximally transported twice as much cation as anion ($J^{Na}_{max}$ = 1.6; $J^{Cl}_{max}$ = 0.75 $\mu$mol · $cm^{-2} \cdot h^{-1}$), and exhibited identical Hill interaction indices for both ions ($n_{Na}$ = 3.4; $n_{Cl}$ = 3.5). The suggestion is made that this cooperative carrier mechanism may be regulatory, maintaining relatively constant luminal ion concentrations which, in turn may facilitate ion-dependent absorption of non-electrolytes.

## Introduction

In many organisms the intestine is an important site for the control of ion balance. Ion transport from intestinal lumen to blood is considered to be a two-step process involving first, the entry of the respective ion across the brush border membrane into the epithelial cell layer, and second, the transfer of this intracellular solute across the serosal membrane into the circulatory system [1,2]. Studies examining intestinal preparations from amphibians to mammals have suggested that sodium and chloride may each be transferred across the epithelium by the combination of shared and unshared processes [3–5]. Detailed examination of the rabbit ileum has disclosed that sodium and chloride influxes across the mucosal membrane are each mediated by at least two carrier processes and that a fraction of these influxes is coupled to form a neutral influx system, while independent mechanisms account for the remaining transmembrane transport of both ions [5,6]. Recently we have shown that in the intestine of the freshwater prawn, *Macrobrachium rosenbergii*, these two ions are only translocated across the epithelial mucosal border by a coupled

mechanism having a 2 $Na^+$/1 $Cl^-$ stoichiometry [7]. No evidence was found for the presence of independent transport mechanisms for sodium or chloride in the apical cell membrane as has been reported for vertebrate intestine. In the present investigation, we report further information on the characteristics of this coupled entry process, specifically the finding that both sodium and chloride influxes exhibit allosteric cooperativity.

## Materials and Methods

*Incubation media*

Ionic composition and osmotic pressure of standard intestinal incubation medium were based on data obtained from flame photometry, chloride titration, and osmometry of haemolymph and intestinal content samples. This saline had an osmotic pressure of 430 mosM/kg, a pH of 7.4 and consisted of the following ion concentrations in mM: $Na^+$, 221.1; $K^+$, 8.1; $Ca^{2+}$, 12.0; $Mg^{2+}$, 4.8; $Cl^-$, 211.3; $SO_4^{2-}$, 25.1; $PO_4^{3-}$, 0.4; $HCO_3^-$, 0.7. When incubation media of altered sodium or chloride concentrations were used, choline chloride and $Na_2SO_4$ replaced or were added to NaCl, while mannitol was included where appropriate to maintain osmotic conditions.

*Experimental procedures*

Intestines were removed from prawns and the fecal contents gently flushed with saline. The isolated intestine was mounted on epoxy-coated 18-gauge stainless steel needles with surgical thread and immersed in 10 ml of aerated prawn saline in a lucite chamber. The mounted gut was then perfused with saline by means of a peristaltic pump (Buchler Instruments) at a flow rate of approx. 125 $\mu l \cdot min^{-1}$. Sodium influx (55, 110, 165, 221, 275 mM Na) and chloride influx (50, 100, 150, 211 mM Cl) across the apical cell membrane were measured by briefly exposing the mucosal surface to saline containing either $^{22}Na$ or $^{36}Cl$ (New England Nuclear Corp.) for periods of time ranging from 7.5 s to 16 min. Because the average luminal volume of the isolated intestinal preparation was approximately 8.0 $\mu l$, at the flow rate used, complete isotope mixing within this compartment and total epithelial exposure occurred only after approximately 3.5 s of incubation. Since the interval of incomplete isotope exposure represented a significant fraction of the shortest incubation time used (i.e., 7.5 s), most experiments were conducted with exposures equal to or greater than 15 s, where complete isotope mixing was ensured for 80—100% of the incubation period. The serosal bath contained 221 mM Na and 211 mM Cl at all times except during experiments using 275 mM Na where identical Na concentrations were used on both sides of the gut. Immediately after this radioactivity pulse, a 15-s rinse (350 $\mu l \cdot min^{-1}$) with the respective unlabelled saline followed to wash out the bulk of luminal isotope. The tissue was then removed from the perfusion chamber, digested in Protosol Tissue Solubilizer (New England Nuclear Corp.) and analyzed for radioactivity using a toluene-based scintillation cocktail and Beckman scintillation counter. Radioactivity in the rinsed tissue was considered to represent labelled ions present in both cellular and extracellular compartments, the former increasing in isotope concentration with time, and the latter containing a fixed isotope concentration in equilib-

rium with the bulk luminal solution throughout the incubation interval. All influx values are expressed on the basis of intestinal surface area ($cm^2$) determined by considering the intestine a cylinder.

## Results

*Time course of tissue ion uptake*

Both sodium and chloride uptake by the intestinal epithelium were linear functions of time over the selected exposure intervals for all luminal ion concentrations examined (Fig. 1). In each case a positive vertical axis intercept was obtained by extrapolating the uptake curve to zero time. Influxes of $^{22}Na$ and $^{36}Cl$ across the apical cell membrane were determined by regression analysis from the slopes of the tissue accumulation curves and are presented as slope ±1 S.E. in Table I. In each case the regression line, and therefore the influx, was highly significant ($P < 0.02$). The vertical axis intercepts, representing the magnitude of extracellular $^{22}Na$ and $^{36}Cl$ throughout the tissue accumulation experiments, increased with elevations of either sodium or chloride concentration in the perfusate, but due to the variability in the data, signficance of this observation is unclear. In several cases these intercepts were not significantly different than zero ($P > 0.05$), indicating that a minimal quantity of tissue activity was confined to this compartment following the routine rinsing procedure.

*Influx dependence on luminal ion concentrations*

Both sodium and chloride influx kinetics were found to be sigmoidal rather

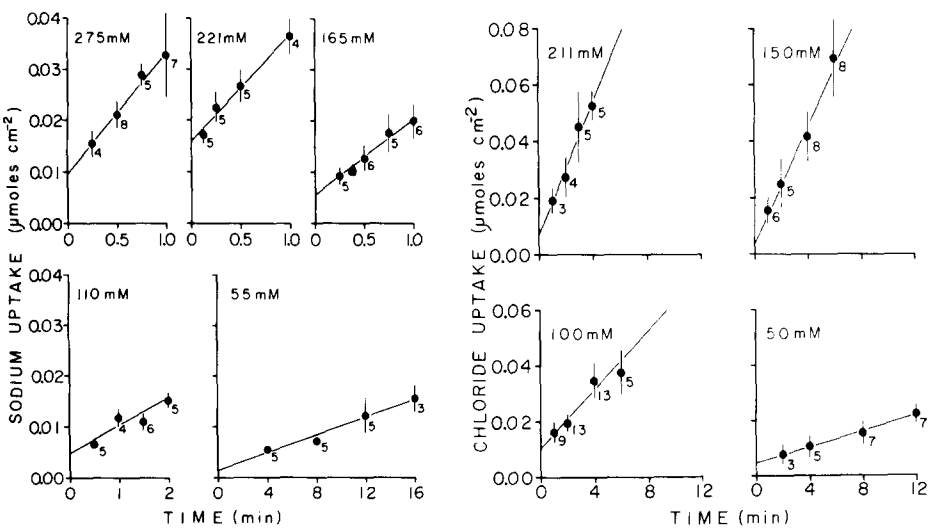


Fig. 1. Time course of $^{22}Na$ (left) and $^{36}Cl$ (right) accumulation by the perfused intestine of *M. rosenbergii* at a series of luminal ion concentrations. The serosal bath contained 221 mM Na and 211 mM Cl at all times except for the experiments using 275 mM Na where identical Na concentrations were used on both sides of the gut. Closed circles represent mean uptake values, vertical lines are ±1 S.E., and numbers signify sample size. Lines drawn through the data were calculated by regression analysis.

TABLE I

INFLUX OF $^{22}Na$ AND $^{36}Cl$ ACROSS THE MUCOSAL BORDER OF THE FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII*

| Ion | Concentration (mM) | Influx ($\mu mol \cdot cm^{-2} \cdot h^{-1}$) * | $n$ † | $P$ ‡ |
|---|---|---|---|---|
| Na | 55 | 0.052 ± 0.015 | 18 | $P < 0.01$ |
| | 110 | 0.316 ± 0.076 | 20 | $P < 0.01$ |
| | 165 | 0.888 ± 0.308 | 22 | $P < 0.01$ |
| | 221 | 1.260 ± 0.200 | 19 | $P < 0.01$ |
| | 275 | 1.423 ± 0.567 | 24 | $P < 0.02$ |
| Cl | 50 | 0.084 ± 0.031 | 22 | $P < 0.02$ |
| | 100 | 0.312 ± 0.096 | 40 | $P < 0.01$ |
| | 150 | 0.624 ± 0.148 | 24 | $P < 0.01$ |
| | 211 | 0.710 ± 0.230 | 17 | $P < 0.01$ |

* Influx values determined from the slope ± 1 S.E. of tissue accumulation curves.
† Number of intestines used at each ion concentration.
‡ Significance of slopes from tissue accumulation curves for each ion concentration.

than hyperbolic (Figs. 2 and 3), indicative of a departure from the standard Michaelis-Menten relationship between substrate entry rate and substrate concentration. Plotting the sigmoidal influx data for both ions in a log-log fashion (insets, Figs. 2 and 3), resulted in linear relationships between the variables. In both instances regression lines calculated from the log-log transformations were

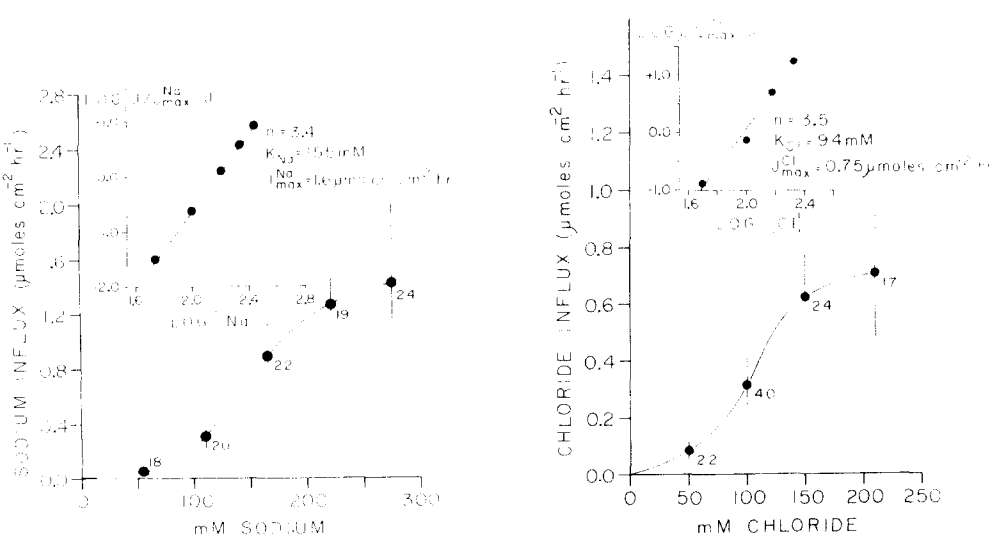


Fig. 2. Influx of $^{22}Na$ into the intestinal epithelium of *M. rosenbergii* as a function of luminal Na concentration. Symbols and conditions are as described in Fig. 1. The inset is a log-log transformation of the mean sigmoidal influx values at each Na concentration. The line drawn through the log-log data was calculated by regression analysis.

Fig. 3. Influx of $^{36}Cl$ into the intestinal epithelium of *M. rosenbergii* as a function of luminal Cl concentration. Symbols and conditions are as described in Fig. 1. The inset is a log-log transformation of the mean sigmoidal influx values at each Cl concentration. The line drawn through the log-log data was calculated by regression analysis.

highly signficant ($P < 0.01$). The linear relationships between log $[J/J^{Na}_{max} - J]$ vs. log [Na] and log $[J/J^{Cl}_{max} - J]$ vs. log [Cl] suggest that the influxes of sodium and chloride into the intestinal epithelium follow Hill equations for cooperativity:

$$\log[J/J^{Na}_{max} - J] = n_{Na} \log[Na] - \log K_{Na} \quad (1)$$

$$\log[J/J^{Cl}_{max} - J] = n_{Cl} \log[Cl] - \log K_{Cl} \quad (2)$$

where $J$ is either sodium or chloride influx, $J^{Na}_{max}$ and $J^{Cl}_{max}$ are the maximal influxes for the respective ions, $n_{Na}$ and $n_{Cl}$ are the slopes of the log-log plots and represent in each case an index of the number of interacting sodium and chloride binding sites and their strength of interaction, and $K_{Na}$ and $K_{Cl}$ signify the luminal sodium and chloride concentrations at one-half maximal influx, respectively. The maximal transport rates for the two ions ($J^{Na}_{max}$ = 1.6 $\mu$mols · $cm^{-2} \cdot h^{-1}$; $J^{Cl}_{max}$ = 0.75 $\mu$mols · $cm^{-2} \cdot hr^{-1}$) were estimated from the sigmoidal influx curves, while $K_{Na}$ (155 mM Na) and $K_{Cl}$ (94 mM Cl) were calculated from the log-log transformations as the respective concentrations at which log $[J/J^{Na}_{max} - J]$ = 0.0 and log $[J/J^{Cl}_{max} - J]$ = 0.0. The interaction indices for the two ions ($n_{Na}$ = 3.4 ± 0.2; $n_{Cl}$ = 3.5 ± 0.4; calculated slopes ±1 S.E.) were virtually identical.

## Discussion

This investigation is the first to identify sigmoidal influx kinetics for a cotransport carrier system in the intestine of any organism. The cooperative kinetics found for Na and Cl uptake in the freshwater prawn intestine deviate considerably from the hyperbolic kinetics previously defined for influxes of glucose [8—11] and lysine (refs. 12, 13 and Brick, R.W. and Ahearn, G.A. (1977), unpublished) in this same preparation and suggest the occurrence of major differences in the types of epithelial carrier proteins involved in solute transfer in this crustacean. Other coupled transport systems from gastrointestinal organs of a variety of animal species involving ion-ion cotransport [5,6], ion-amino acid cotransport [14—17], ion-sugar cotransport [18—20], and ion-vitamin cotransport [21] all exhibit hyperbolic influx kinetics without cooperativity between the binding ligands. On the other hand, sodium efflux from red cells by way of (Na + K)-activated ATPase is known to be a sigmoidal function of internal Na concentration and is thought to occur as a result of the operation of a 3 Na/2K exchange process involving cooperative interactions between the ligand binding sites on either side of the membrane [22—24]. Transmembrane transfer processes for other ion species are also known to deviate from Michaelis-Menten kinetics. Calcium efflux from squid axons [25] and barnacle muscle fibers [26] occur by sigmoidal processes, the divalent cation apparently exchanging for external Na by way of a carrier mechanism having multiple binding sites for the ions involved. Furthermore, membranes of cellular organelles such as those from rat liver mitochondria have cooperative uptake carrier mechanisms for divalent cations including calcium, magnesium, barium and manganese, which assist in maintaining low concentrations of these ions in the cytosol [27,28].

Allosteric cooperativity between enzyme (or carrier) binding sites occurs when the protein has multiple attachment sites for various ligands and the reaction of one of these sites with substrate significantly alters the affinity of the other sites for the ligand [29—31]. Often this increase in affinity comes about by a conformational change in the protein structure itself. Cooperative allosteric interactions appear to be a characteristic of many regulatory enzymes (or carriers), because as the steepness of the velocity curve about the inflection point indicates, small changes in substrate concentration will produce relatively large changes in velocity [31,32]. Hemoglobin oxygen dissociation curves are sigmoidal, and this protein is considered regulatory because small changes in plasma oxygen tension near the $P_{50}$ (as occurs at respiring tissues) result in the release of large amounts of oxygen.

Data from the present investigation and work done previously by our group concerning intestinal sodium and chloride transport in the freshwater prawn, suggest that both ions are cotransported by a regulatory carrier that possesses multiple binding sites for the two ligands which are present in the ratio 2 Na/1Cl site. This carrier mechanism apparently has a somewhat higher affinity for Cl ($K_{Cl}$ = 94 mM) than for Na ($K_{Na}$ = 155 mM), but maximally transports twice as many sodium ions ($J^{Na}_{max}$ = 1.6 $\mu$mols · cm$^{-2}$ · h$^{-1}$) as chloride ions ($J^{Cl}_{max}$ = 0.75 $\mu$mols · cm$^{-2}$ · h$^{-1}$). Both ions exhibit identical interaction indices ($n_{Na}$ = 3.4; $n_{Cl}$ = 3.5) providing suggestive evidence for their association with a common carrier. Additional data supporting the concept of coupled transmembrane fluxes of sodium and chloride are provided by experiments showing complete influx cessation of both ions from luminal solutions lacking the respective counterions [7]. The nature of the regulatory activity afforded by this particular carrier process remains obscure, but may be involved with the maintenance of stable luminal Na and/or Cl concentrations. Our previous work [7] indicates that luminal Na and Cl concentrations vary considerably less than do those of Ca or K. Such stable ion concentrations in a known site of nutrient uptake in crustaceans [10,13,17,33] would have obvious benefit for ion-dependent absorption of non-electrolytes.

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