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Sulfatide Content and $(Na^+ + K^+)$-ATPase Activity of Skin and Gill during Larval Development of the Chilean Frog, *Calyptocephalella caudiverbera*

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Summary. The sulfatide content, phospholipid concentration, and $(Na^+ + K^+)$-ATPase activity from skin and gills of different stages of larval development of *Calyptocephalella caudiverbera* (a Chilean frog) were analyzed. Additionally, the short-circuit current in skin was studied. When skin and gills, depending on the stage of larval development, present $(Na^+ + K^+)$-ATPase activity, they have a high ratio of sulfatide to amount of membrane and the phosphatidylserine concentration remains unchanged. Sulfatide content and $(Na^+ + K^+)$-ATPase activity in skin are in direct relationship with the level of sodium flux present during development. The specific enzymatic hydrolysis of sulfatide with partially purified arylsulfatase of pig kidney inhibits 100% of the ouabain-sensitive $(Na^+ + K^+)$-ATPase. The ouabain-insensitive ATPase remains virtually unchanged with the treatment, even with a high concentration of arylsulfatase or with ouabain present in the medium. These experiments strongly suggest a role of sulfatides in the $(Na^+ + K^+)$-ATPase activity and, as a consequence, in sodium ion transport.

The active transport of Na^+ through the abdominal skin is an important mechanism in the regulation of ionic equilibrium of anurans [14]. However, when in earlier stages of development the abdominal skin of larvae is isolated and bathed with Ringer's solution, it shows incapacity to transport Na^+ actively. Moreover, the skin lacks a transepithelial potential difference, the gill being the only site where this transport occurs [1, 7, 36].

In cell membranes [32, 33] and epithelium [3, 4, 18, 30] the active transport is mediated by $(Na^+ + K^+)$-ATPase, a specific membrane-bound enzyme which is of lipoprotein nature [37]. To perform its catalytic activity, the enzyme needs a lipidic microenvironment because of its membrane localization, as has been postulated for integral proteins [31, 34]. However, in spite of many attempts, it has been impossible to establish the kind of lipid which would activate ATPase, through a specific interaction with the enzyme.

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Phosphatidylserine has been proposed as the lipid requirement of $(Na^+ + K^+)$-ATPase [13]. This lipid is able to restore the hydrolytic activity of ATPase when it is added to membrane preparations depleted of lipids [20, 26, 35, 39]. However, recent work has shown that total phosphatidylserine decarboxylation *in situ* does not affect the enzymatic activity of the $(Na^+ + K^+)$-ATPase [6]. This suggests that the lipid requirement is other than phosphatidylserine.

Recently, the polar moiety of sulfatides, sphingolipids with a carbohydrate residue which are common components of some membranes, has been indicated as the requirement for a specific cellular function, the Na^+ transport [15]. Measurements of sulfatide content in organs with a high Na^+ transport and $(Na^+ + K^+)$-ATPase ouabain-sensitive activity, such as kidney outer medulla, salt gland of herring gull, rectal gland of spiny dogfish [16, 17], indicate that sulfatide may play a role in Na^+ transport [15].

This hypothesis has also received support from enzymatic studies; thus if ducks are fed with hypertonic salt solutions, it is found that the level of $(Na^+ + K^+)$-ATPase activity and the sulfatide content in their salt gland increases 200% [16].

Based on this last finding, we have examined the relationship between sodium transport, $(Na^+ + K^+)$-ATPase activity, and sulfatide content. Anuran gills and skin are very useful for this kind of study. It is known that during larval development the site of active Na^+ and K^+ transport changes its location from the gill to the abdominal skin, allowing for the opportunity to study some probably related events before and after the above-mentioned mechanism of transport begins its function.

To establish if sulfatides are required for ATPase activity, we used arylsulfatase A, a lysosomal enzyme involved in cerebroside-3-sulfate hydrolysis, giving cerebroside and sulfate as products of the catalysis.

Materials and Methods

Tadpoles of *Calyptocephalella caudiverbera* (a Chilean frog) were collected and stored in tap water at room temperature (20 °C). The tadpoles were classified, considering mouth and limb development, in three principal groups which ranged from stage 30 to 44 [22].

The animals were immobilized by spinal transection, and both skin and gill were quickly removed and collected separately and kept with 250 mM sucrose on ice. Skins and gills from at least five animals were weighed and pooled for each cell fraction preparation. The pool, cut in small pieces, was homogenized in 4 volumes per g of solution

containing 250 mM sucrose; 1 mM EDTA; 0.1% deoxycholate and 10 mM Tris adjusted to pH 7.4 with acetic acid, using a Potter-Elvehjem glass homogenizer with three passes each with a pestle of 0.012 inch clearance [4, 18]. The homogenate was filtered through a 110 mesh nylon monofilament bolting cloth. The filtered homogenate was centrifuged at $1,000 \times g$ for 10 min. The supernatant was then centrifuged at $100,000 \times g$ for 60 min. Pellets were suspended in 2 ml of 500 mM Tris acetate pH 7.4. As a result of differential centrifugation, three fractions were obtained. In each of these fractions, the protein concentration was determined using bovine albumin as a standard [23] and total phosphorus according to Chen *et al.* [5].

Enzymatic Assays

The $(Na^+ + K^+)$-ATPase activity was estimated as ATPase inhibited by ouabain at 35 °C, determined as the optimal temperature for the enzymatic assay for these tissues, using a final volume of 2 ml. One incubation medium contained 50 mM NaCl; 5 mM KCl; 6 mM $MgCl_2$; 50 mM Tris acetate, pH 7.4, and between 50 and 100 μg of cell fraction protein. The other incubation medium contained in addition, 1 mM ouabain (Sigma Chemical Co., St. Louis, Mo.).

Mg^{++}-ATPase was estimated in a medium containing 6 mM $MgCl_2$ and 50 mM Tris-acetate, pH 7.4. The tubes containing the medium were preincubated for 10 min at 35 °C. The reaction was started by adding 3 mM ATP-Tris, pH 7.4, and after 20 min it was stopped with 0.5 ml of 1.5 N perchloric acid. The assay system was centrifuged at $6,500 \times g$ for 5 min at 4 °C and the inorganic phosphate liberated determined in the supernatant [8].

Arylsulfatase

Partially purified arylsulfatase was prepared from pig kidney cortex [24], with some modifications. After precipitation of the crude enzyme with 60% acetone, it was further purified by chromatography on controlled-pore glass beads [2]. Crude enzyme, in 50 mM sodium acetate, ph 4.5, was applied to a controlled-pore glass bead column (5.5×6.5 cm) in deionized water loaded with 10 mg of enzyme protein per ml of column volume. The column was washed successively with 20 column volumes of 50 mM sodium acetate, pH 4.5, the same at pH 5.5, and again at pH 6.5; 50 mM Tris-acetate, pH 7.5 and at pH 8.5. The partially purified enzyme was eluted with 30 column volumes of 50 mM Tris-acetate, pH 8.5, containing 300 mM NaCl. The dilute enzyme was concentrated using an amicon PM 30 filter. Purification was 278-fold over the homogenate with a recovery of 20%. Before use, the enzyme was precipitated with 60% acetone and redissolved in 200 mM sodium acetate pH 5.4. To monitor the purification, the arylsulfatase was assayed using *p*-nitrocatechol sulfate as substrate at 37 °C and pH 5.6 [25].

To determine the ATPase activity in the presence of arylsulfatase, the same ionic conditions were employed and for each 100 μg of $100,000 \times g$ pellet, 0.7 to 7.0 μg of the partially purified enzyme protein was used.

Short-Circuit Current

The isolated abdominal skin of animals from three different larval stages used in this work was also utilized to determine the skin short-circuit current [38]. The skin was placed in a Lucite chamber of 2.1 cm^2, containing on each side of the skin, 17 ml of

Ringer's solution which contained 115 mM NaCl; 3 mM KCl; 1.8 mM $CaCl_2$; 2.4 mM $NaHCO_3$; at pH 7.5. A voltage-clamp system was used to maintain the potential difference across the skin equal to zero.

Lipid Analysis

The total homogenate and the $100{,}000 \times g$ pellets were extracted with 20 volumes of chloroform/methanol (2:1, vol/vol) at room temperature [27]. All solvent mixtures are expressed as volume ratios. The residues were collected on sintered glass filters and re-extracted with 10 volumes of chloroform/methanol (2:1, vol/vol) and refiltered as before. The extracts were combined, evaporated to a small volume, and freed of nonlipid contaminants by chromatography on Sephadex G-25 [28].

The lipids, eluted by chloroform/methanol (19:1, vol/vol) saturated with water, were evaporated by means of a flash evaporator under reduced pressure to a moist residue and suspended in a small volume of chloroform. Phosphorus determined on this fraction is referred to as lipid phosphorus.

Neutral lipids, glycolipids and phospholipids, were separated from the total lipid extract by means of a silicic acid chromatographic column (Unisil, Clarkson Chemical Co., Williamsport, Pa.), using as eluent chloroform, acetone, and methanol, respectively [28]. The excess of solvent was evaporated under nitrogen, and the moist residue was resuspended in a small volume of chloroform/methanol (2:1, vol/vol) and stored at -20 °C.

An aliquot (10 μg of lipid phosphorus) of phospholipids was then used to determine the phosphatidylserine content using two-dimensional thin layer chromatography (TLC) [29]. The solvent systems used were chloroform/methanol/ammonia (65:25:5, vol/vol) in the first dimension and chloroform/acetone/methanol/acetic acid/water (3:4:1:1:0.5, vol/vol) in the second dimension. The plates were prepared using plain silica gel, containing 10% magnesium silicate. The spots were visualized by spraying with 3% formaldehyde in concentrated sulfuric acid followed by charring, and analyzed for phosphorus [27]. Phosphatidylserine was identified by its relative migration and, in addition, by its ninhydrin-positive reaction. Sulfatide determinations were carried out on the glycolipid fraction [19]. Bovine sulfatide, obtained from Applied Science Laboratories, was used as a standard.

Results

The sulfatide content of the glycolipid fraction and the $(Na^+ + K^+)$-ATPase activity (ouabain-sensitive ATPase), measured in total homogenates of abdominal skin from tadpoles of *Calyptocephalella caudiverbera* are shown in Fig. 1. During larval development, between stages 36 and 48, the slopes of ATPase activity curve was 0.178 ± 0.0001, while that for the sulfatide content was 0.332 ± 0.0001. The ratio between slopes was 1.87, which indicates that sulfatide content is increasing almost twice as fast as $(Na^+ + K^+)$-ATPase activity. On the other hand, ATPase activity greatly increases in the larval skin during this period; the activity at stage 44 is sevenfold greater than at stage 36.

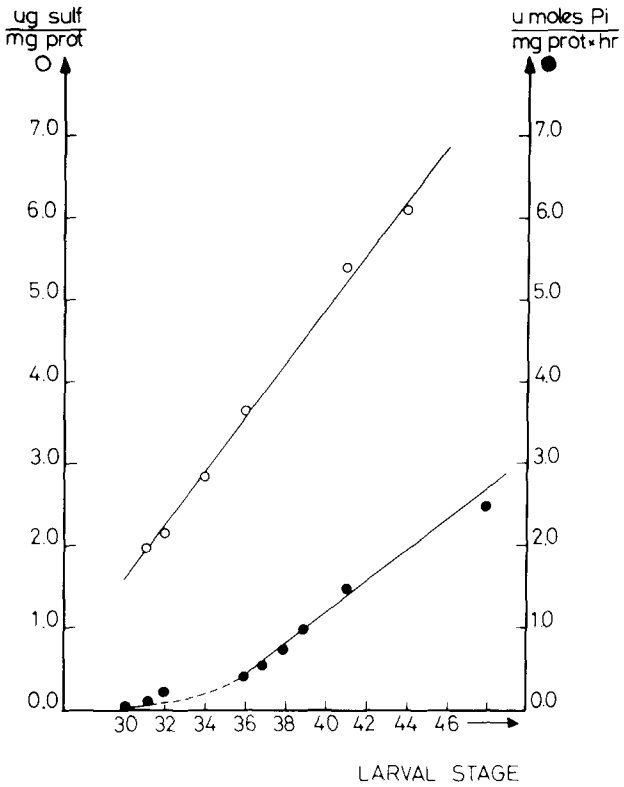

Fig. 1. Sulfatide content and $(Na^+ + K^+)$-ATPase (ouabain-sensitive) activity from total homogenate of larval *Calyptocephalella caudiverbera* skin during different stages of development was measured

Table 1 summarizes the $(Na^+ + K^+)$-ATPase distribution of cell fractions obtained by differential centrifugation of total homogenates from skin and gills of different stages of larval development. ATPase activity was not detected in the mitochondrial cell fraction (not shown).

The "microsomal" fraction is the enriched cell fraction with respect to the $(Na^+ + K^+)$-ATPase activity, while in the low spin pellet and supernatant of microsomes, activity of this enzyme was not detected. It can also be observed that about 70 to 90% of the total units of ATPase sedimented at $100{,}000 \times g$. The skin of stages 30 to 35 does not present activity of this enzyme.

It can be observed (Table 1) that as development advances there is a concomitant increase in skin $(Na^+ + K^+)$-ATPase. The gills present a similar relation until the middle of development (stage 36–39), but this activity is not detected in more advanced stages.

Table 1. The yield of $(Na^+ + K^+)$-ATPase (ouabain-sensitive) from larval *Calyptocephalella caudiverbera* skin and gill homogenate upon differential centrifugation

	Stage 30–35			Stage 36–39				Stage 40–44		
	Skin activity	Gill		Skin		Gill		Skin		Gill activity
		S.A.	% Total units	S.A.	% Total units	S.A.	% Total units	S.A.	% Total units	
Total homogenate	N.D.	0.44	100	0.35	100	1.06	100	1.28	100	N.D.
1,000 × *g* pellet	N.D.	0.00		0.00		0.00		0.00	76.3	N.D.
100,000 × *g* pellet	N.D.	0.87	71.3	1.40	93.8	3.29	80.6	2.15		N.D.
supernatant	N.D.	0.00		0.00		0.06	2.7	0.00		N.D.

S.A. = specific activity, expressed as μmol P_i/mg protein · hr^{-1}.
N.D. = not detected.
The data are the average of 4 experiments.

Table 2. Sulfatide and phospholipid content from skin and gill of different larval stages of *Calyptocephalella caudiverbera* 100,000 × *g* pellet

	Skin			Gill		
Larval stage	30–35	36–39	40–44	30–35	36–39	40–44
Ouabain-sensitive $(Na^+ + K^+)$-ATPase activity	N.D.	1.40	2.15	0.87	3.29	N.D.
μg P/mg prot	11.09	11.07	18.72	14.80	13.83	13.86
μg P of phospholipids/mg prot	2.16	2.08	3.44	3.94	3.91	3.77
μg sulfatide/mg prot	13.94	42.60	55.44	25.56	10.94	6.17
μg of phospholipids/mg prot	51.84	49.62	82.56	70.56	93.84	90.48
μg sulfatide/μg phospholipids	0.27	0.85	0.67	0.36	0.12	0.07
% phosphatidylserine/total phospholipids	8.15	8.42	11.11	10.83	7.02	9.97

N.D. = not detected.
(Na+K)-ATPase activity, expressed as μmol P_i/mg protein · hr^{-1}.
A factor of 24 was used to calculate the amount of phospholipids based on a mean mol wt of 744. The results were obtained from pools of skin and gills from 5 animals for each larval stage.

Typical values obtained for sulfatide and phospholipid of skin and gills of different stages are shown in Table 2. The phospholipid/protein ratio, that reflects the amount of membrane present, is not significantly different during the larval development in the gills; and in the skin, this ratio seems to increase along with development. During the larval development the amount of phosphatidylserine in both tissues is almost constant.

Table 3. Effect of Arylsulfatase on ATPase activity of larval *Calyptocephalella caudiverbera* skin and gill $100{,}000 \times g$ pellet

Arylsulfatase	Skin (stage 40–44)			Gill (stage 36–39)		
	Total ATPase	Ouabain insensitive ATPase	Mg^{++}-ATPase	Total ATPase	Quabain-insensitive ATPase	Mg^{++}-ATPase
None	2.30	1.15	1.15	5.84	3.76	4.17
1.43 μg prot	0.92	0.92	1.15	3.67	3.73	4.22
None	3.55	1.46	1.67	5.84	3.76	4.17
14.30 μg prot	1.54	1.54	1.54	3.56	3.46	3.91

Activity, expressed as μmol P_i/mg protein·hr^{-1}. 200 μg of $100{,}000 \times g$ pellet were incubated with Arylsulfatase for 10 min at 35 °C in the ionic medium. ATP-Tris was added to the mixture and incubation continued for 20 min. The reaction was stopped with perchloric acid and the amount of P_i released measured. The results were obtained from pools of skin and gills from 15 and 8 animals, respectively.

The table points out that the sulfatide/phospholipid ratio increases about threefold with development in the skin and decreases in the gills to about 20% of the early value in the oldest or more advanced stage tested.

Table 3 summarizes the effect of arylsulfatase on ATPase activity of active larval *C. caudiverbera* skin and gills $100{,}000 \times g$ pellet. The results obtained from two different pools of a cell fraction treated with two different amounts of arylsulfatase are shown. The use of 1.43 μg protein of arylsulfatase with or without ouabain in the medium, inhibits 60 and 37% of the total ATPase present in the cell fractions of skin and gills, respectively. On the other hand, Mg^{++}-ATPase plus unspecific hydrolysis of ATP is not affected by arylsulfatase even when its concentration is increased tenfold in the medium. This unspecific hydrolysis of ATP in the gill fraction is 3.6-fold greater than the value obtained in the skin fraction.

In the skin, the high level of 14.3 μg of arylsulfatase inhibits the activity of total ATPase about 43.4% with or without ouabain in the medium. The inhibition in gills is 39 and 41%, respectively.

The table shows that the activation by Na^+ and K^+ ions was abolished 100% in skin and gill cell fractions. The inhibition by ouabain represents about 55 and 36% of total ATPase activity of skin and gill cell fraction, respectively.

The inhibition of $(Na^+ + K^+)$-ATPase activity (ouabain sensitive) and breakdown of sulfatide by different amounts of arylsulfatase are shown

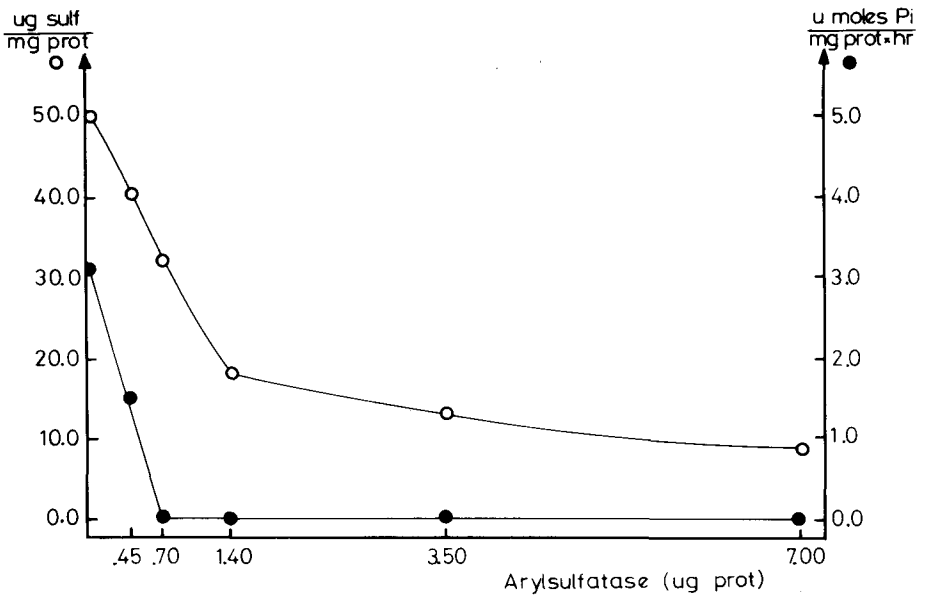

Fig. 2. 100,000 × *g* pellet from stage 40–44 skin homogenates (200 μg protein) was incubated with different amounts of Arylsulfatase (0.45–7.00 μg protein) in the appropriate ionic medium for 10 min without ATP, and incubation was continued for 10 min with ATP. Half the final volume was used to measure the P_i release, the other was extracted with chloroform/methanol (2:1, vol/vol). In the isolated glycolipid fraction, sulfatide amount was measured

in Fig. 2. Results were obtained working with the 100,000 × *g* pellet from a pool of abdominal skin of stage 40–44 of larval development. The $(Na^+ + K^+)$-ATPase activity drops to 50% using 0.45 μg protein of the lysosomal enzyme; treatment with 0.7 or more arylsulfatase produces 100% inhibition of activity.

The sulfatide shows a typical enzymatic reduction curve, 0.45 μg of protein producing a 20% decrease, and 7.0 μg of protein producing hydrolysis of 83% of the substrate structurally present in the cell fraction.

It must be pointed out that the analysis by TLC of the phospholipid part of this cell fraction treated with different amount of arylsulfatase did not show any kind of lysocompound.

The values of sodium flux of the abdominal skin from three different larval stages of development are shown in Table 4. This table indicates that in younger stages of development, 30 to 35, the skin of these larvae presents a sodium flux which remains almost unchanged by amiloride addition. In stages 36 to 39 the sodium flux presented a 75.7% inhibition by amiloride, while in more advanced stages the inhibition by amiloride

Table 4. Relationship between net sodium flux and larval development

Stage	Ringer solution		Ringer solution + amiloride	
	$\bar{X}$	Range of values	$\bar{X}$	Range of values
30–35 (6)	0.90	0.55– 1.37	0.79	0.55– 1.37
36–39 (4)	7.54	5.48– 8.91	1.83	1.37– 2.74
40–44 (4)	220.00	80.00–300.00	9.25	4.00–14.00

Sodium flux in abdominal skin of larval *Calyptocephalella caudiverbera* of three different stages of development are presented. The measurements were performed at 20 °C. Amiloride in a final concentration of 0.058 mM was used. Net sodium flux, expressed as nmol/$cm^2 \cdot hr^{-1}$. Numbers in parenthesis are number of observations.

is about 95.8%. Comparing the last two cases, the sodium flux through the abdominal epithelium is quite different, becoming 37-fold higher in the advanced stages than in the younger ones.

Discussion

The synthesis of sulfatide and its subsequent integration as plasma membrane component has been established [9, 10, 11]. This, and the knowledge that 77% of the glycolipid fraction of rat kidney plasma membrane is sulfatide [40], added to the finding of a correlation between high $(Na^+ + K^+)$-ATPase activity and sulfatide content, allow us to postulate a sulfatide role in active sodium transport [14, 19, 20, 21]. The present work compares the sulfatide content, ATPase activity, and sodium flux in skin of *Calyptocephalella caudiverbera* during its larval development.

The skin, in the advanced stages of development, becomes the site where the Na^+ translocation takes place; meanwhile the gill, in the same stages, reduces its physiological function to a very low level.

Our results show a relation between $(Na^+ + K^+)$-ATPase activity and sodium translocation obtained in both gills and skin during development. Therefore, they are in agreement with the studies that have correlated the $(Na^+ + K^+)$-ATPase activity and short-circuit current in skin of *Rana pipiens* and *Rana catesbeiana* [18, 36] as well as with the relationship shown in gill of larval *R. catesbeiana* by measuring the sodium flux [7]. The results of Karlsson *et al.* [16] have shown that the $(Na^+ + K^+)$-ATPase activity and the sulfatide content in the salt gland increases twofold when ducks are fed with hypertonic saline solution. Our results (Fig. 1) closely confirm the same relationship. It seems that

some mechanism during the larval development causes in skin of the intermediate stage an increase of the ATPase activity together with a similar increase in the sulfatide content and a high level of sodium flux. On the other hand, skin of earlier stages of development shows low content of sulfatide with almost zero ATPase activity and there is no short-circuit current.

This kind of relationship, reported for the first time here, strongly suggests a specific and important role fulfilled by cerebroside-3-sulfate in the $(Na^+ + K^+)$-ATPase activity and, as a consequence, in the sodium ion transport.

The increment of phospholipid concentration in both epithelia does not correspond specifically to phosphatidylserine, because the percentage of phosphatidylserine with respect to total phospholipids does not vary significantly in the epithelia, the values remaining between 7 and 11%. A negatively charged phospholipid, specifically phosphatidylserine, which has been postulated as the phospholipid required for $(Na^+ + K^+)$-ATPase activity [13], would be expected to show a significant concentration in those tissues that have high enzyme activity and sodium flux. Besides, it has been established that the total conversion of phosphatidylserine to phosphatidylethanolamine, by specific enzymatic decarboxylation of a purified $(Na^+ + K^+)$-ATPase from rabbit kidney outer medulla, results in a loss of only 13% of the activity [6]. De Pont *et al.* have also [6] suggested that there is not a specific phospholipid requirement for this enzyme.

Our present study seems to indicate that sulfatide may play a role in the ATPase activity.

Further experiments are needed to establish with precision the step where the ATPase action is interrupted by arylsulfatase action. However, the present work is the first direct evidence of the importance of sulfatide in the $(Na^+ + K^+)$-ATPase activity.

Preliminary experiments show that the $(Na^+ + K^+)$-ATPase activity in preparation treated with arylsulfatase can be recovered by addition of sulfatide microdispersion. The $(Na^+ + K^+)$-ATPase activity thus recovered is ouabain sensitive.

Conclusions

From these experiments and our present knowledge, the three following conclusions can be suggested:

a) Tissues rich in $(Na^+ + K^+)$-ATPase ouabain sensitive activity, associated with sodium ion transport, present a high concentration of sulfatide.

b) The specific enzymatic breakdown of sulfatide inhibit 100% of the ouabain-sensitive ATPase.

c) The total inhibition occurs without change in the structure of any phospholipid, and in particular phosphatidylserine content remains unchanged.

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References

1. Alvarado, R.H., Moody, A. 1970. Sodium and chloride transport in tadpoles of the bullfrog *Rana catesbeiana*. *Am. J. Physiol.* **218**:1510
2. Bock, H.G., Skene, P., Fleischer, S., Cassidy, P., Harshman, S. 1976. Protein purification. Adsorption chromatography and controlled pore glass with the use of chaotropic buffers. *Science* **191**:380
3. Bonting, S.L., Caravaggio, L.L. 1963. Studies on sodium-potassium-activated adenosine triphosphatase. V. Correlation of enzyme activity with cation flux in six tissues. *Arch. Biochem. Biophys.* **101**:37
4. Boonkoom, V., Alvarado, R.H. 1971. Adenosinetriphosphatase activity in gills of larval *Rana catesbeiana*. *Am. J. Physiol.* **220**:1820
5. Chen, P.S., Toribara, T.Y., Warner, H. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**:1756
6. De Pont, J.J.H.H.M., Van Prooijen-Van Eeden, A., Bonting, S.L. 1978. Role of negatively charged phospholipids in highly purified $(Na^+ + K^+)$-ATPase from rabbit kidney outer medulla. *Biochim. Biophys. Acta* **508**:464
7. Dietz, T.H., Alvarado, R.H. 1974. Na^+ and Cl^- transport across gill chamber epithelium of *Rana catesbeiana* tadpoles. *Am. J. Physiol.* **226**:764
8. Fiske, C.H., Subbarow, Y. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**:375
9. Fleischer, B., Zambrano, F. 1973. Localization of cerebroside-sulfotransferase activity in the Golgi apparatus of rat kidney. *Biochem. Biophys. Res. Commun.* **52**:951
10. Fleischer, B., Zambrano, F. 1974. Golgi apparatus of rat kidney. Preparation and role in sulfatide formation. *J. Biol. Chem.* **249**:5995
11. Fleischer, B., Zambrano, F., Fleischer, S. 1974. Biochemical characterization of the Golgi complex of mammalian cells. *J. Supramol. Struct.* **2**:737
12. Hansson, C.G., Karlsson, K.A., Samuelsson, B.E. 1978. The identification of sulfatides in human erythrocyte membrane and their relation to sodium-potassium dependent adenosine trisphosphatase. *J. Biochem.* **83**:813

13. Israel, Y. 1969. Phospholipid activation of $(Na^+ + K^+)$-ATPase. *In:* The Molecular Basis of Membrane Function. D.C. Tosteson, editor, p. 529. Prentice Hall, Englewood Cliffs
14. Jørgensen, C.B., Levi, H., Zerahn, K. 1954. On active uptake of sodium and chloride in anurans. *Acta Physiol. Scand.* **30**:178
15. Karlsson, K.A. 1976. Aspects on structure and function of sphingolipids in cell surface membranes. *In:* Structure of Biological Membranes. S. Abrahamson and I. Pascher, editors. p. 245. University of Goteborg, Goteborg (Sweden)
16. Karlsson, K.A., Samuelsson, B.E., Steen, G.O. 1971. Lipid pattern and $Na^+ - K^+$ dependent adenosine trisphosphatase activity in the salt gland of duck before and after adaptation to hypertonic saline. *J. Membrane Biol.* **5**:169
17. Karlsson, K.A., Samuelsson, B.E., Steen, G.O. 1974. The lipid composition and $Na^+ - K^+$ dependent adenosine triphosphatase activity of the salt (nasal) gland of eider duck and herring gull. A role for sulphatides in sodium ion transport. *Eur. J. Biochem.* **46**:243
18. Kawada, J., Taylor, R.E., Jr., Barker, S.B. 1975. Some biochemical properties of Na^+, K^+-ATPase in frog epidermis. *Comp. Biochem. Physiol.* **50**:297
19. Kean, E.L. 1968. Rapid, sensitive spectrophotometric method for quantitative determination of sulfatides. *J. Lipid Res.* **9**:319
20. Kimelberg, H.K., Papahadjopoulos, D. 1972. Phospholipids requirement of $(Na^+ + K^+)$-ATPase activity: head group specificity and fatty acid fluidity. *Biochim. Biophys. Acta* **282**:277
21. Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of frog skin potential. *Acta Physiol. Scand.* **42**:298
22. Limbaugh, B.A., Volpe, E.P. 1957. Early development of the Gulf coast toad, *Bufo valliceps* Wiegmann. *Am. Mus. Novit.* **1842**:1
23. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, J.R. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265
24. Mehl, E., Jatzkewitz, H. 1964. Eine cerebrosidsulfatase aus Schweineniere. *Z. Physiol. Chem.* **339**:260
25. Mehl, E., Jatzkewitz, H. 1968. Cerebroside 3-sulfate as a physiological substrate of Arylsulfatase A. *Biochim. Biophys. Acta* **151**:619
26. Roelofsen, B., Deenen, L.L.M. van 1973. Lipid requirement of membrane bound ATPase. *Eur. J. Biochem.* **40**:245
27. Rouser, G., Fleischer, S. 1967. Isolation, characterization and determination of polar lipids of mitochondria. *In:* Methods in Enzymology. R.W. Estabrook and M.E. Pullman, editors. p. 385. Academic Press, New York
28. Rouser, G., Kritchevsky, G., Yamamoto, A. 1967. Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. *In:* Lipid Chromatography Analysis. G.V. Marinetti, editor. p. 99. Marcel Dekker, New York
29. Rouser, G., Siakotos, A.N., Fleischer, S. 1966. Quantitative analysis of phospholipids by thin layer chromatography and phosphorus analysis of spots. *Lipids* **1**:85
30. Shamoo, Y.E., Brodsky, W.A. 1970. The $(Na^+ + K^+)$-dependent ATPase in the isolated mucosal cells of turtle bladder. *Biochim. Biophys. Acta* **203**:111
31. Singer, S.J., Nicholson, C.L. 1972. The fluid mosaic model of the structure of cell membranes. *Science* **175**:720
32. Skou, J.C. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta* **23**:394
33. Skou, J.C. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.* **45**:596
34. Tanford, C., Reynolds, J.A. 1976. Characterization of membrane proteins in detergent solutions. *Biochim. Biophys. Acta* **457**:133

35. Taniguchi, K., Tonomura, Y. 1971. Inactivation of $Na^+ - K^+$-dependent ATPase by phospholipase-treatment and its reactivation by phospholipids. *J. Biochem.* **69**:543
36. Taylor, R.E., Barker, S.B. 1965. Transepidermal potential difference development in anuran larvae. *Science* **148**:1612
37. Uesugi, S., Kahlenberg, A., Medzihradsky, F., Hokin, L.E. 1969. Studies on the characterization of the sodium-potassium transport adenosinetriphosphatase. IV. Properties of a Lubrol-solubilized beef brain microsomal enzyme. *Arch. Biochem. Biophys.* **130**:156
38. Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110
39. Wheeler, K.P., Whittam, R. 1970. The involvement of phosphatidylserine in adenosine triphosphatase activity of the sodium pump. *J. Physiol. (London)* **207**:303
40. Zambrano, F., Fleischer, S., Fleischer, B. 1975. Lipid composition of the Golgi apparatus of rat kidney and liver in comparison with other subcellular organelles. *Biochim. Biophys. Acta* **380**:357