Molecular Properties of Purified (Sodium + Potassium)-
activated Adenosine Triphosphatases and Their
Subunits from the Rectal Gland of Squalus
acanthias and the Electric Organ of
Electrophorus electricus*

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SUMMARY

The chemical properties of two highly purified preparations
of (sodium + potassium)-activated adenosine triphosphatase
(NaK ATPase) and their subunits have been compared. One
preparation is derived from the rectal gland of the spiny
dogfish shark, Squalus acanthias and the other preparation is
derived from the electric organ of the electric eel, Electrophorus
electricus. Ouabain binding and phosphorylation from
\[\text{[T-\text{\textsuperscript{32}P}]ATP}\] for both enzymes ranged from 4000 to 4300
pmol per mg of protein. This gives a stoichiometry for oua-
bain binding and phosphorylation of 1:1 for both enzymes.
The molar ratios of catalytic subunit to glycoprotein was 2:1
for both enzymes, suggesting a minimum molecular weight
of 250,000, which agrees with the molecular weight obtained
by radiation inactivation. Assuming that only one of the two
catalytic subunits is phosphorylated and binds ouabain per
(sodium + potassium)-activated adenosine triphosphatase
molecule the data on phosphorylation and ouabain binding
also give a molecular weight of 250,000. The data on phos-
phorylation, ouabain binding, subunit composition, and
molecular weight based on radiation inactivation are thus all
internally consistent. A technique has been developed for
isolation of pure catalytic subunit and glycoprotein in good
yields by preparative sodium dodecyl sulfate-polyacrylamide
gel electrophoresis. A variety of chemical studies have been
carried out with the purified subunits. The amino acid com-
position of the catalytic subunit was different from that of the
glycoprotein, but the amino acid composition of each of the
two subunits was essentially the same for both species. HOW-
ever, the NH\textsubscript{2}-terminal amino acid for the catalytic subunit
was alanine for the rectal gland enzyme and serine for the
glycoprotein was alanine for the two species. The glycoproteins from both species contained the same
carbohydrates but in quite differing amounts. The carbo-
hydrates were glucosamine, sialic acid, fucose, galactose,
mannose, and glucose. The release of all of the sialic acid
from the electric organ enzyme and the release of 40% of
the sialic acid from the rectal gland enzyme did not affect
(sodium + potassium)-activated adenosine triphosphatase
activity. Both enzymes contained the following phospholipids,
which accounted for 98 to 100% of the total phospholipid
phosphorus: sphingomyelin, lecithin, phosphatidylserine,
phosphatidylethanolamine, and phosphatidylglycerol. With
the exception of phosphatidylserine, the amount of any
phospholipid per mg of enzyme as well as the total phos-
pholipid content were quite different for the two enzymes.

The (sodium + potassium)-activated adenosine triphosphatase
has been purified in this laboratory to 90 to 95% homogeneity in
high yield from the rectal gland of Squalus acanthias (1) and the
electric organ of Electrophorus electricus (2). Both enzymes show
two predominant proteins on sodium dodecyl sulfate-polyacryl-
amide gel electrophoresis—a catalytic subunit with a molecular
weight of about 95,000 and a glycoprotein with a molecular
weight of about 59,000. The catalytic subunit has been directly
identified as a component of the enzyme by showing that it carries
the aspartyl\textsuperscript{\beta}-phosphate (3-5) residue in the enzyme (1, 2, 6-
13). Direct proof that the glycoprotein is an integral component
of the enzyme is still lacking, although it is present as the only
other predominant protein of NaK ATPases\textsuperscript{1} which have been
highly purified (13-12) with the exception of our report (12).
The present study has had two primary aims. First, with two
highly purified enzymes in hand, one from an elasmobranch and
the other from a teleost, it was felt that a detailed comparison

\textsuperscript{1} The abbreviations used are: NaK ATPase, (sodium + potas-
sium)-activated adenosine triphosphatase; Na dodecyl-SO\textsubscript{4}, so-
dium dodecyl sulfate.
of the chemical anatomy of these two enzymes would be worthwhile since these studies should throw some light on the constancy of the enzyme structure through evolution. Second, comparison of the chemistry of the glycoproteins from the two species might offer a clue as to whether the glycoprotein is in fact an integral component of the enzyme.

As this paper shows, the catalytic subunits from these two enzymes are very similar but not identical since they have different NH2-terminal amino acids. The amino acid compositions are the same within experimental error. With respect to the glycoproteins, no differences in amino acid composition or NH2-terminal amino acid are seen with the enzymes from the two species. The carbohydrate composition of the glycoproteins and the phospholipid composition of the holoenzymes show considerable differences between the two species. Alterations of the latter two parameters might be expected to be more permissive with evolution, since they are unlikely to play a direct role in catalytic activity. The fact that the similar amino acid composition and identical NH2-terminal amino acids are found in the glycoprotein would suggest that the glycoprotein is an integral component of the NaK ATPase and is not a fortuitous contaminant which co-purifies with the NaK ATPase.

**EXPERIMENTAL PROCEDURE**

**Purification and Characterization of NaK ATPase—**Purification of the NaK ATPase from the rectal gland of the dogfish as well as certain other procedures were similar to those previously described (1). This included preparation of the microsomes, Lubrol extraction, zonal centrifugation, ammonium sulfate fractionation, analytical Na dodecyl-SO4 polyacrylamide gel electrophoresis, gel scanning, measurement of phosphorylation of protein with y-32P-ATP, NaK ATPase assay and determination of protein.

The initial steps in the preparation of the electrophoresis tissue were homogenized in a Waring blendor at low speed with 2 volumes of 0.05 M Tris-HCl (pH 7.5) and 1 mM Na7ATP (pH 7.5) for 30 s at 4°. The homogenate was centrifuged at 18,000 X g for 35 min. The fluid of floating material and the sediment were separated from the supernatant, and the insoluble material was gently resuspended in 1 mM Tris-EDTA (pH 7.0) and 1 mM Na7ATP (pH 7.0) in a final volume equal to the initial tissue volume. This suspension was centrifuged at 78,500 X g for 60 min. The supernatant was discarded and the pellets stored overnight at 0°. Pellets prepared by this procedure from 500 g of electrophoresis tissue were homogenized in 125 ml of 3.2% Lubrol and 2 mM Na7ATP (pH 7.0). The extract was clarified by three centrifugations at 105,000 X g for 30, 90, and 60 min. Top floating material with included in the final extract. When this method was used, 500 g of electrophoresis tissue routinely yielded 90 mg of protein at the final stage of purification with a specific activity of 1900 nmol of P, per mg of protein per hour. With the exception of these modifications the purification and characterization procedures for the electric organ NaK ATPase were similar to those previously described (2).

**Determination of Maximal Ouabain Binding—**The binding of [3H]ouabain was determined as described by Lane et al. (13) with slight modifications. To determine total ouabain binding 50 to 300 µg of ammonium sulfate enzyme was incubated at 37° for 10 min in a final volume of 1.0 ml in a medium which contained 1.25 mM ATP, 1.25 mM MgCl2, 50 mM Tris chloride (pH 7.4) and 10-4 M [3H]ouabain obtained from New England Nuclear (specific activity, 2 X 106 dpm per µg). Non-specific binding was estimated by either a 15-min preincubation with 10-4 M ouabain or by omitting ATP from the reaction medium. Specific ouabain binding refers to total binding minus non-specific binding. The reaction was terminated by placing the tubes in an ice bath and centrifuging at 100,000 X g for 60 min. The supernatants were discarded and the tubes were wiped clean of liquid. The resulting pellets were solubilized in 0.20 ml of 0.3 N NaOH. Aliquots (0.02 and 0.05 ml) were taken for determinations of protein and radioactivity, respectively.

**Purification by Preparative Disc Gel Electrophoresis of Two Polypeptide Components of NaK ATPase—**For separation of the catalytic subunit and glycoprotein components of the NaK ATPase a preparative polyacrylamide gel electrophoresis apparatus as described by W. LeStourgeon was employed. The separating gel (15% in volume) contained 8.75% acrylamide, 0.2% N,N'-bisethylene acrylamide, 0.1% tetramethylenediamine, 0.1% ammonium persulfate, 0.37% Tris chloride (pH 8.8), and 0.1% Na dodecyl-SO4. Stacking gel (3 ml) was used containing 3% acrylamide, 0.08% N,N'-bisethylene acrylamide, 0.1% tetramethylenediamine, 0.00% ammonium persulfate, 0.12 µM Tris chloride (pH 8.8), and 0.1% Na dodecyl-SO4. The gel column dimensions were 2 X 14 cm. The gel was overlayed with 0.1% Na dodecyl-SO4 during polymerization. The separating gel was polymerized 10 min and the stacking gel, 30 min at room temperature.

Purified NaK ATPase (5 mg) in 0.5 ml of 1 mM Tris-EDTA (pH 7.0) was solubilized by the addition of 0.25 ml of sample buffer containing 10% glycerol, 5% mercaptoethanol, 3% Na dodecyl-SO4, and 0.02% Tris chloride (pH 8.3) to 500 µl aliquots from alternate fractions. Then 100 µl aliquots of the mixtures were applied to 8.75% analytical polyacrylamide gels and electrophoresis was carried out as described previously (1).

After measurement of absorbance at 280 nm and determination of protein the same gels were dried, mounted, and permanganate-stained and made up to 70 µl with distilled water. The pooled fractions were transferred to a Bio-Fiber 50 Beaker Dialyzer (Bio-Rad) and the Na dodecyl-SO4 removed by passage of cold deionized water through hollow fibers at a flow rate of approximately 150 ml per min. Detergent removal was monitored by adding [38S]Na dodecyl-SO4 (New England Nuclear) to the pooled fractions prior to transfer to the hollow fiber dialyzer. After 48 hours, at least 98.5% of the initial Na dodecyl-SO4 was removed by this technique. The material inside the beaker dialyzer was concentrated to approximately 30 ml by applying a vacuum to one fiber port using a water aspirator and capping the other fiber port. The polyglycoproteins were further concentrated by lyophilization and finally suspended in 10 mM NaClO4 (pH 7.4). The total neutral amino and acid components were determined on a Beckman Spinco Model 120C amino acid analyzer by the method of Spackman et al. (16). Protein samples were hydrolyzed with constant boiling HCl at 110° for 22, 46, and 70 hours in sealed evacuated tubes. Half-cystine and methionine were determined as cysteic acid and methionine sulfone, respectively, after performic acid oxidation (17). Tryptophan was determined by the spectrophotometric method of Edelhoch (18). Amino sugars were measured after mild hydrolysis in 4 X HCl at 100° for 4, 8, and 12 hours (19). Total hexosamine was determined on the short column of the amino acid analyzer and glucosamine on the long column, as described by Spackman et al. (16).

NH2-terminal amino acids were determined by the method of Hartley (20) as modified by Weiner et al. (21). Total neutral sugars were measured by the method of Francois et al. (22) using the orcinol-sulfuric acid reaction. Individual neutral sugars were identified by gas-liquid chromatography by the method of ClAmy et al. (23). Samples of glycoprotein (1 mg) were hydrolyzed with 0.5 ml of 1.5 M methanolic HCl at 90° for 20 to 22 hours in sealed evacuated tubes with mannitol as an internal standard. After the hydrolysates were neutralized with solid Ag2CO3, 0.1 ml of acetic anhydride was added and the tubes were allowed to stand at room temperature for 6 hours. The tubes were centrifuged, the supernatant and two washes of the precipitate were combined and reduced in volume with a stream of nitrogen.

1 W. LeStourgeon, personal communication.
in reaction vials and placed in a vacuum desiccator over PdO overnight. The samples were then derivatized with 100-μl aliquots of trimethylsilylimidazole (Tri-Sil S, Pierce Chemical Co.). After 5 min, 0.5-μl aliquots were injected into a Perkin-Elmer Gas Chromatography Model 880. The columns (6,250 × 4.0 feet) consisted of 2% DECB coated on Gas-chrom Q (Applied Sciences Labs). Nitrogen was used as the carrier at a flow rate of 30 ml per min. The column temperature was constant at 160°C and the injection temperature was 260°C. The instrument contained a flame ionization detector and the output was fed into a CBS 10A Infratronics digital integrator with a chart recorder attached.

Sialic acid was determined by the thiobarbituric acid method of Warren (24).

Phospholipid analysis of the purified NaK ATPases was performed as previously described (25) with the following modification. After phospholipid extraction, 25 μg of lipid phosphorus was submitted to two-dimensional thin layer chromatography using solvent systems a, b and c for the separation of polar lipids (25).

The resulting supernatant was assayed for neuraminidase-sensitive sialic acid by the thiobarbituric acid method of Rouser et al. (26).

NaK ATPase was treated with neuraminidase from Clostridium perfringens as described by Rouser et al. (26). The instrument contained a flame ionization detector and the output was fed into a CR8-110A Infatronics digital integrator with a chart recorder attached.

RESULTS

Stoichiometry of Na+-dependent Phosphorylation to Ouabain Binding—Table I shows the ouabain bound and the phosphorylation of the two purified NaK ATPases. Ouabain binding and phosphorylation for both enzymes ranged from 4000 to 4300 pmol per mg of protein. The level of phosphorylation agrees with that reported earlier for the rectal gland enzyme (1). The ouabain binding to the purified enzyme agrees with that reported for the NaK ATPase from dog kidney (13) and rabbit kidney (27).

Stoichiometries other than 1:1 reported by others (8, 27, 28) could be due to species differences, less pure or partially denatured enzyme preparations, or to different methodologies. The fact that the enzymes from the rectal gland and the electric organ are highly purified and undergo no detectable denaturation during purification and give essentially identical values for ouabain binding and phosphorylation encourages us to believe that there is in fact one ouabain binding site per phosphorylation site. In this connection it is of interest that the large chain which is phosphorylated also carries the ouabain binding site (29).

Molar Ratios of Catalytic Subunit of Glycoprotein of Purified NaK ATPase—The percentage composition of the catalytic subunit and the glycoprotein and their molar ratio for the rectal gland and the electric organ enzymes are shown in Table II. Ninety to 95% of the protein could be accounted for by the catalytic subunit and the glycoprotein. The percentage composition of the two polypeptides from the two enzyme sources is very similar. The molar ratios of catalytic subunit to glycoprotein for NaK ATPase from rectal gland and electric organ were 2.16 and 1.96, respectively, indicating that in these two highly purified enzymes there are two catalytic subunits per glycoprotein.

TABLE I

|        | [3H]Ouabain binding | Phosphorylation by [γ-32P]ATP | [3H]Ouabain bound/TP incorporated |
|--------|---------------------|-----------------------------|---------------------------------|
| Rectal gland | 4007 | 4254 | 0.94 |
| Electric organ | 4298 | 4556 | 0.98 |

TABLE II

| Source          | Catalytic subunit | Glycoprotein | Protein at tracking dye position | Catalytic subunit/glycoprotein |
|-----------------|------------------|--------------|---------------------------------|-------------------------------|
| Rectal gland    | 72.5             | 19.0         | 8.5                             | 2.16                          |
| Electric organ  | 72.5             | 20.3         | 4.5                             | 1.96                          |

Na dodecyl-SO4 polyacrylamide gel electrophoresis—We previously purified the two polypeptide components associated with the NaK ATPase from rectal gland and electric organ by gel filtration on Sephadex G-150 in the presence of 0.1% Na dodecyl-SO4 (1, 2). The yield of purified subunit was 70%, whereas the yield of purified glycoprotein was only 50% because of contamination of the glycoprotein peak by catalytic subunit. Since the glycoprotein of the purified enzyme was only 20% of the total protein (Table II), development of an alternative technique for the isolation of the polypeptide components was desirable. Two commercial preparative polyacrylamide gel electrophoresis apparatuses were found to be rather unsatisfactory, and the apparatus of LeStourgeois (23) was constructed. Fig. 1A shows the elution profile of material absorbing at 280 nm after continuous separation and elution by electrophoresis on an Na dodecyl-SO4-polyacrylamide gel column after solubilization of 5 mg of rectal gland enzyme with Na dodecyl-SO4 as described under "Experimental Procedure." Fractions from Peaks 1 and 2 were each pooled and concentrated without removal of Na dodecyl-SO4. Fractions from Peaks 3 and 4 were pooled separately, Na dodecyl-SO4 was removed and the fractions were concentrated as described under "Experimental Procedure." Photographs of analytical Na dodecyl-SO4-polyacrylamide gels are shown above each peak in Fig. 1A. They show that the glycoprotein peak (No. 3) was cleanly separated from the catalytic subunit (No. 4). Routinely, the glycoprotein could be isolated in 90 to 100% yield and the catalytic subunit in 45 to 65% yield. Photographs of the analytical electrophoretograms also indicated that the protein running at the tracking dye position on analytical Na dodecyl-SO4-polyacrylamide gels, which accounted for 5 to 10% of the total enzyme protein, was present exclusively in Peak 2. Glycolipid, which appeared as a white band below the tracking dye position on analytical gels and stained with periodic acid-Schiff reagent was found in both Peaks 1 and 2 (1, 2). Fig. 1B shows the optical density profile of a preparative gel column developed
shows the gas-liquid chromatograms for a set of standard tri-
gland and electric organ. The most striking difference was the
tyrosine content of the glycoprotein which was twice that found
in the catalytic subunit.

The amino acid compositions of the catalytic subunits from the rectal
was found for each of the purified polypeptides of the NaK
the NH2-terminal amino acid found for the glycoprotein from
alanine and for that from the electric organ, serine. Alanine was
ATPases, attesting to their purity. The NH2-terminal amino
sources were also quite similar but were clearly different from the
subunit from rectal gland and electric organ were quite similar.

The amino acid compositions of the purified polypeptides are
the electric organ NaK ATPase gave results which were quite similar
exception of fucose. The sum of the neutral sugars as measured by
fucose, mannose, galactose, and glucose. In addition, there is an
unknown compound running near galactose and one running between
glucose and mannitol. It is not known whether these compo-
s are sugars. Table IV shows the carbohydrate composition of the glycoproteins isolated from the rectal gland enzyme and
the electric organ enzyme. Most strikingly, the carbohydrate composition of the glycoprotein of the NaK ATPase from the
rectal gland and from the electric organ is quite different. This
difference is reflected in the values for the amino sugars, glucos-
amine, sialic acid and the individual neutral sugars with the ex-
ception of fucose. The sum of the neutral sugars as measured by
gas liquid chromatography equaled the total neutral sugar value
as determined by the orcinol-sulfuric acid method. All neutral
sugars gave reproducible values on repeated runs with the ex-
ception of glucose. Since glycoproteins do not generally contain glu-
cose (30, 31), it may be a contaminant from the sucrose used
during the zonal centrifugation step during purification of the
NaK ATPase, although one would have thought that ammonium
sulfate fractionation followed by preparative Na dodecyl-SO4-
gel electrophoresis would have removed all of the sucrose. The
presence of fructose cannot be of help in resolving this matter
since it was decomposed in our gas liquid chromatography
system.

Phospholipid Composition of Purified NaK ATPases—Table V
shows the total phospholipid and the phospholipid composition of
the NaK ATPases from rectal gland and electric organ. Compari-
sion of the individual phospholipids in three thin layer chroma-
Fig. 2. Gas-liquid chromatography of the neutral sugars of the purified glycoprotein from the rectal gland NaK ATPase. Methanalysis with mannitol as an internal standard and gas liquid chromatography were carried out as described under "Experimental Procedure." A, chromatogram of a set of standard trimethylsilylated neutral sugars. The identity of the peaks are as follows: fucose 1, 2, 3; mannose 4, 6; galactose 5, 7, 8; glucose 9, 10; and mannitol 11. B, gas liquid chromatogram of the trimethylsilylated neutral sugars from the purified glycoprotein from the rectal gland NaK ATPase. Peaks A and B are artifacts.

Table IV

Carbohydrate composition of purified glycoprotein of NaK ATPase from rectal gland and electric organ

Analysis of carbohydrates was performed as described under "Experimental Procedure." The values for amino sugars and glucosamine are an average of two experiments with two separate glycoprotein preparations after hydrolysis with 4 M HCl for 4, 8, and 12 hours at 100°C. The values for sialic acid are an average of three experiments with three separate glycoprotein preparations. The values for total neutral sugars and individual neutral sugars are from a representative experiment.

| Carbohydrate       | Rectal gland | Electric organ |
|--------------------|--------------|---------------|
|                    | mol/100 mol amino acid | mol/100 mol amino acid |
| Amino sugars       | 8.1          | 2.0           |
| Glucosamine        | 7.0          | 1.5           |
| Sialic acid        | 1.3          | 4.2           |
| Total neutral sugars| 16.4        | 16.2          |
| Fucose             | 1.00         | 1.5           |
| Galactose          | 8.8          | 2.9           |
| Mannose            | 4.1          | 2.2           |
| Glucose            | 2.2          | 9.2           |
| Total carbohydrate | 23.8         | 22.4          |

Table V

Phospholipid composition of purified NaK ATPase from rectal gland and electric organ

Phospholipid analysis was performed as described under "Experimental Procedure." The values are averages of three separate NaK ATPase preparations. The phospholipids of each enzyme preparation were separated using different solvent systems.

| Phospholipid       | Rectal gland | Electric organ |
|--------------------|--------------|---------------|
|                    | µg P/mg protein | % of total | µg P/mg protein | % of total |
| Sphingomyelin      | 22           | 5.7          | 1             | 0.5        |
| Lecithin           | 196          | 50.4         | 86            | 39.2       |
| Phosphatidylserine | 31           | 8.4          | 31            | 14.8       |
| Phosphatidylethanolamine | 138     | 35.5         | 90            | 43.0       |
| Phosphatidylinositol| 2           | 0.5          | 1             | 0.5        |
| Total phospholipid | 389          | 100.1        | 209           | 98.0       |

Fig. 3. Effect of neuraminidase digestion on NaK ATPase activity of purified rectal gland enzyme. Neuraminidase treatment, sialic acid determination, and assay of NaK ATPase activity were carried out as described under "Experimental Procedure." Percent of total sialic acid released: no neuraminidase, O--O; with neuraminidase, O--O NaK ATPase activity: not treated, O--O; neuraminidase-treated, O--O.
acid. Addition of fresh neuraminidase (40 μg per ml) to the incubation mixture after 1 hour did not result in a further release of sialic acid. There was no loss of enzyme activity relative to the control after 2 hours digestion with neuraminidase.

Table VI shows that in the case of the electric organ enzyme all of the sialic acid was released by neuraminidase digestion after 1 hour with no effect on NaK ATPase activity. The time course of sialic acid release (not shown) indicated that the neuraminidase digestion was complete after 1 hour. Analysis of the sialic acid content of the isolated glycoproteins from both enzymes showed that all of the sialic acid in the holoenzyme could be accounted for by that in the glycoprotein. Confirming this, no sialic acid was found in the lipid extract of the holoenzyme, indicating that none was present in the glycolipids. The fact that only 40% of the sialic acid was released from the dogfish enzyme while 100% was released from the eel enzyme suggests differences in accessibility of the neuraminidase to the two enzymes or an effect of different oligosaccharide structure to attack by neuraminidase.

### DISCUSSION

In the present study two highly purified preparations of NaK ATPase give ratios of ouabain bound to active site phosphorylation of 1:1. The levels of phosphorylation and ouabain binding are as high or higher than with any previous preparation (7-9, 13, 28), with the possible exception of a recent preparation reported by Jorgensen (27). In this latter preparation the level of ouabain binding is somewhat less than we report here, but the level of phosphorylation is 7300 pmol per mg of protein as compared to approximately 4300 pmol per mg of protein that we report. Our modified electroplax preparation reported here gave as high a specific activity as Jorgensen reported for his preparation (14). Our ratio of catalytic subunit to glycoprotein appears to be alanine in all three species (see also (14)). This along with its rather constant amino acid composition provides some support for the view that the glycoprotein is indeed an integral component of the NaK ATPase. It appears that there are small but not major differences in amino acid sequences for the catalytic subunit and the glycoprotein through vertebrate evolution. As might be expected differences in amino acid composition between the catalytic subunit and the glycoprotein are more evident, as exemplified by the two fish enzymes studied here. For example, the tyrosine content of the glycoprotein is twice that of the catalytic subunit, and the sulfur-containing amino acids are about half as great. Examination of the data in Table III shows other less striking differences.

Even though the NaK ATPase is a highly intrinsic membrane enzyme, requiring detergents for its solubilization, the protein chains are not particularly apolar. Using the criteria of Hatch and Bruce (38) each chain contains about 30% apolar amino acids. This value is within the range for typical soluble proteins.

Detailed quantitative analysis of the carbohydrates in the glycoprotein has not been performed before although amino sugars, sialic acid, and total neutral sugar content have been reported for the enzyme from dog kidney (14). We have found four neutral sugars by gas-liquid chromatography which account for the total neutral sugar content of the enzyme, namely fucose, galactose, mannose, and possibly glucose. Glucosamine accounted for almost all of the amino sugar content. With the exception of fucose all of the sugars in the glycoproteins from the electric organ and rectal gland enzymes differed in amounts, although both glycoproteins contained the same sugars. It is perhaps not surprising that there has been considerable permissiveness in sugar composition of the glycoprotein of NaK ATPases during evolution, since the oligosaccharide components probably do not play a role in catalysis. Oligosaccharides have been cleaved from several glycoprotein enzymes without loss of enzyme activity (36). Perhaps the oligosaccharides play a general orientating or structural role in the enzyme rather than a catalytic role, and this type of function probably would not require a precise oligosaccharide structure. As shown here one can remove all of the sialic acid from the 

| Enzyme source | Total sialic acid | Sialic acid released by neuraminidase | NaK ATPase activity remaining after neuraminidase |
|---------------|------------------|--------------------------------------|-----------------------------------------------|
| Rectal gland  | 85 g/mol/mg      | 79 g/mol/mg (93)                     | 99 %                                           |
| Electric organ| 85 g/mol/mg      | 79 g/mol/mg (93)                     | 99 %                                           |
electric organ enzyme without affecting catalytic activity. The effect of removal of other sugars on catalytic activity is now under investigation.

The total phospholipid content and the composition of the individual phospholipids also showed considerable difference between the rectal gland and the electric organ enzymes. The only phospholipid which was present in the same amount (based on protein) was phosphatidylserine. It is of interest that this phospholipid became enriched with respect to the other phospholipids on purification of beef brain enzyme (25). Some (37-40) but not others (25, 41-43) have claimed that phosphatidylserine is uniquely required for enzyme activity. The fact that it is not uniquely required (43) but critical phosphatidylserine molecules representing a small percentage of the total may have escaped decarboxylation.

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