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THE LARGE SUBUNIT OF (SODIUM + POTASSIUM)-ACTIVATED ADENOSINE TRIPHOSPHATASE FROM THE ELECTROPLAX OF ELECTROPHORUS ELECTRICUS IS A GLYCOPROTEIN

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SUMMARY: The large subunit of Na,K-ATPase purified from eel electroplax was found to contain amino sugars, neutral sugars and sialic acid. The concentration of these carbohydrates in the large subunit was 5-10% of that found in the smaller subunit and in total accounts for 2.6% of the mass of the large subunit. The periodic acid-Schiff's stain for glycoproteins on polyacrylamide gels is apparently not sufficiently sensitive to detect glycoproteins with these low levels of carbohydrates.

Previously, carbohydrates have not been detected in the large subunit of Na,K-ATPase isolated from dog kidney (1), shark rectal gland (2) and eel electroplax (3) using the periodic acid-Schiff's stain for glycoproteins in polyacrylamide gels. Recently, carbohydrates were detected in the large subunit of brine shrimp Na,K-ATPase using the periodic acid-Schiff's stain (4). These data prompted an analysis of the carbohydrate content of the large subunit of Na,K-ATPase purified from the electroplax of Electrophorus electricus using more sensitive assays and direct measurements of amino sugars, neutral sugars and sialic acid.

MATERIALS AND METHODS

The Na,K-ATPase used in these experiments was prepared from electroplax tissue of Electrophorus electricus by the method of Dixon and Hokin (3,5). The specific activity averaged 1158 micromoles P_i/mg protein/h. The large subunit was isolated from eel Na,K-ATPase by a new method utilizing a column of Bio-Gel A 1.5 m (200-400 mesh) as previously described (6).

Total amino sugars were determined on the short column of a Beckman Spinco Model 120C amino acid analyzer by the method of Spackman *et al.* (7). The amino sugars were measured after mild hydrolysis in 4 N HCl at 100°C for 4 h (8). The concentration of amino sugars relative to total amino acids (9) was determined utilizing lysine as the internal standard. Neutral sugars were determined by the orcinol-sulfuric acid method of Francois *et al.* (10). Sialic acid was determined by the thiobarbituric acid method of Warren (11). Protein concentration was measured by the Lowry method as modified by Peterson (12).

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RESULTS AND DISCUSSION

The large subunit isolated from eel electroplax tissue has been previously documented to be homogeneous on SDS-polyacrylamide gels (3,5) by N-terminal amino acid analyses (9) and isoelectric focusing (6).

Table I shows the amounts of amino sugars, neutral sugars and sialic acid found in the large subunit from eel electroplax. The amounts represent 2-3 amino sugar residues, 10-12 neutral sugar residues and 1 sialic acid residue per 1 large subunit chain. In comparison with the small subunit, the concentration of amino and neutral sugars in the large subunit is about one-tenth of that in the small subunit. For sialic acid, the concentration in the large subunit is about one-twentieth of that in the small subunit. Thus, carbohydrates account for 2.6% of the mass of the large subunit and 23% of the mass of the small subunit.

The inability of the periodic acid-Schiff's stain to detect carbohydrates in the large subunit of Na,K-ATPase is undoubtedly due to the low level of carbohydrates present in this subunit. Even with the smaller subunit, large amounts of protein were necessary for a detectable positive stain. Thus a negative reaction for a protein band on periodic acid-Schiff's stained gels is insufficient evidence for the absence of carbohydrates.

TABLE I

Carbohydrate Content of Large Subunit from Eel Electroplax Na,K-ATPase

Carbohydrate[a]	Moles carbohydrate/100 moles amino acid	
	Large subunit[b]	Small subunit[c]
Amino sugars	0.30 ± 0.05 (n=5)	2.0
Neutral sugars	1.34 ± 0.12 (n=5)	16.2
Sialic acid	0.09 ± 0.02 (n=4)	1.7

[a]The carbohydrate analyses were determined as described under Materials and Methods.

[b]Values given are means ± S.E. for the number of determinations shown in parentheses.

[c]Data from Marshall and Hokin (6) and Perrone *et al.* (9).

Considerable species variation in the carbohydrate composition is observed in the small subunit of the Na,K-ATPase (1,9). This species variation is probably also the case for the large subunit. The positive periodic acid-Schiff's stain for the large subunit of Na,K-ATPase from brine shrimp nauplii (4) suggests that the carbohydrate composition of the large subunit may be higher in this species than in eel. Periodate oxidation and [^{3}H]-sodium borohydride reduction of sialic acid residues was shown by Giotta (13) to result in little or no detectable sialic acid residues in the dog kidney Na,K-ATPase large subunit. One and even 0.5 sialic acid residues per large chain would be within the detectable level by this method according to the data of Giotta (13).

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