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THE α AND β SUBUNITS OF LAMB KIDNEY Na,K-ATPase ARE BOTH GLYCOPROTEINS

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Summary: We have determined the carbohydrate compositions of the protein components of lamb kidney Na,K-ATPase. The α subunit contains a total of about 16 monosaccharide residues per mol of protein, while the β subunit contains about 36 residues per mol. The γ protein, a proteolipid associated with the Na,K-ATPase, contains only traces of carbohydrate. A comparison of our results with those of others shows considerable variability in the carbohydrate compositions of α and β subunits from different species.

Introduction: Na,K-ATPase is the enzyme that catalyzes active Na^+ and K^+ transport across cell membranes. It is specifically inhibited by ouabain and other cardiac glycosides and is generally believed to be the pharmacological receptor for these drugs (1). The enzyme contains an α subunit of Mr∿100,000, a β subunit of Mr∿50,000 and possibly a small (Mr∿12,000) γ component, or proteolipid (2,3). Until recently, only the β subunit was considered to be a glycoprotein, since it is the only subunit to give a positive response to the periodic acid - Schiff's stain for glycoproteins on polyacrylamide gels. Recently, however, carbohydrate has been found to be present in the α subunit of brine shrimp (4) and eel electroplax (5) Na,K-ATPases. The question of whether mammalian kidney α subunits are also glycoproteins is still not settled. Dog kidney α seems to contain no

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Abbreviations: (Na,K)-ATPase, sodium plus potassium stimulated adenosine triphosphatase (EC 3.6.1.3.); SDS, sodium dodecylsulfate.

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TABLE I

Carbohydrate Compositions of the α and β Subunits of Na,K-ATPase[a]

	Lamb		Rabbit Kidney[b]		Eel Electroplax[c]		Brine Shrimp[d]	
	α	β	α	β	α	β	α	β
Total Hexosamine					0.30	2.0	0.66	1.06
galactosamine:	e	e	e	0.3				
glucosamine:	0.86	3.03	2.0	10.1				
Total Neutral Sugars:	0.62	3.46			1.34	16.2	2.9	5.7
glucose:	f	f	0.9	2.0				
galactose:	0.39	3.09	0.9	5.5				
mannose:	0.25	0.83	0.3	2.5				
fucose:	0.02	0.14	g	g				
Sialic Acid:	0.09	0.85	0.35	3.2	0.09	1.7	0	0
Total Carbohydrate:	1.6	7.9	4.4	20.1	1.7	19.9	3.6	6.8

[a]Expressed as mol of carbohydrate per 100 mol of amino acid.

[b]From Peters, et al,. (7).

[c]From Churchill, et al., (5); values for individual hexosamines and neutral sugars not reported.

[d]From Peterson and Hokin, (4); values for individual hexosamines and neutral sugars not reported.

[e]Not detectable.

[f]Small amounts may be present.

[g]Not reported.

carbohydrate (6), whereas rabbit kidney α contains quite a lot, despite the fact that it does not stain with the periodic acid - Schiff's stain (7). This controversy prompted us to investigate the carbohydrate content of the protein components of lamb kidney Na,K-ATPase.

Experimental Procedures: The lamb kidney Na,K-ATPase was prepared as described previously (8). The α , β and γ components were separated by chromatography on a Sepharose CL-6B column in the presence of 0.1% SDS (3), and then lyophilized. The α and β subunits were redissolved in a small volume of 0.01% sodium azide and dialyzed vs. 1ℓ of 0.01% sodium azide for 5 hours. Each was then dialyzed overnight vs. 1ℓ of 0.01% sodium azide, adjusted to pH 8, in which a large excess of BioRad AG1-X2 was suspended. This procedure removed 92-96% of the SDS. The samples were then divided into aliquots suitable for carbohydrate analysis, and lyophilized in screw-cap test tubes in a vacuum centrifuge. The samples were extracted with acetone prior to analysis in order to remove remaining SDS. The γ sample was freed of SDS and other low molecular weight material by chromatography on Sephadex LH-60 as described previously (9). The sample was dried in a vacuum centrifuge prior to analysis.

Individual neutral sugars were measured by gas-liquid chromatography as alditol acetate, following hydrolysis in 2.5N trifluroacetic acid at 100° for

4 hours (10). Total neutral sugars were measured by the anthrone procedure (11). Amino sugars were measured by amino acid analysis after hydrolysis with 4N HCl at 100° for 6 hours. Sialic acid was measured by the Warren procedure (12). Protein was determined by amino acid analysis (3).

Results and Discussion: Table I lists the results of carbohydrate analysis of the α and β subunits of lamb kidney Na,K-ATPase, and compares them with those of other species in which α has also been found to contain carbohydrate. As can be seen from the Table, there appears to be considerable species variation. The amount of carbohydrate in lamb kidney α represents about 3 mannose, 4 galactose, 8 glucosamine and 1 sialic acid per mol. The lamb kidney β contains approximately 1 fucose, 4 mannose, 13 galactose, 14 glucosamine and 4 sialic acid per mol. These values are based on assumed polypeptide molecular weights of 95,000 for α and 43,800 for β (8). The γ component of lamb kidney Na,K-ATPase contained only traces of carbohydrate, amounting to no more than about 0.1 residues per mol.

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