THE CARBOHYDRATE COMPOSITION OF SUBMITOCHONDRIAL FRACTIONS FROM RAT LIVER*

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1. Introduction

Liver mitochondria contain certain amounts of carbohydrates which are normally components of glycoproteins [1,2]. We have recently isolated and characterized glycopeptides from rat liver mitochondria [3]. The glycopeptides could be classified into two types based on carbohydrate composition; neutral one consisting of only mannose and *N*-acetylglucosamine and acidic one consisting of fucose, galactose and sialic acid in addition to mannose and *N*-acetylglucosamine. To obtain a clue to understand biological significance of the glycoproteins, localization within mitochondria of the carbohydrates has been investigated with the results described in the present paper.

The intermembrane space and the outer and inner membranes contain mannose, glucosamine, fucose, galactose, sialic acid and galactosamine in similar proportions. The intermembrane space contains more than half of the total carbohydrates and the remainder is distributed nearly equally between the outer and inner membranes. The carbohydrate content, μmoles/g protein, of the outer membrane is about 5 times higher than that of the inner membrane. The matrix is virtually free of the carbohydrates.

2. Materials and methods

Mitochondria were isolated from the livers of male rats, Wistar strain, weighing 110–130 g by the method described previously [3]. The mitochondria were subfractionated into outer membrane (OM), intermembrane space (IMS), inner membrane (IM) and matrix (MAT) by a combination of the method of Schnaitman and Greenawalt [4] and that of Hoppel and Cooper [5]. To check the purity of the isolated mitochondria and the submitochondrial fractions, the following marker enzymes were assayed. Glucose 6-phosphatase [EC 3.1.3.9], a marker for microsomes, was assayed by the method of Nordlie and Arion [6], and acid phosphatase [EC 3.1.3.2], a marker for lysosomes, by the method of De Duve et al. [7]. Cytochrome oxidase [EC 1.9.3.1], a marker for IM, was assayed polarographically according to Schnaitman and Greenawalt [4], malate dehydrogenase [EC 1.1.1.37], a marker for MAT, spectrophotometrically according to Ochoa [8], and kynurenine-3-mono-oxygenase [EC 1.14.1.2], a marker for OM, spectrophotometrically according to Okamoto [9].

The mitochondria and the submitochondrial fractions were treated with 1% phosphotungstic acid in 5% trichloroacetic acid, and the precipitates obtained were collected by centrifugation and washed successively by resuspending and centrifuging in 1% phosphotungstic acid in 5% trichloroacetic acid, ethanol, chloroform–methanol (2:1, v/v) and acetone. The dried samples were used for chemical analysis. To determine protein content, the samples were dissolved in 1 N NaOH.

Hexosamines were determined in a Hitachi KLA-3B amino acid analyzer after hydrolysis in 2 N HCl at 100°C for 16 hr, followed by the Boas' procedures, as described previously [10]. Neutral sugars were separated and estimated by gas-liquid chromatography of trimethylsilyl derivatives of methylglycosides with

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xylose as the internal standard, as described previously [10]. Sialic acid was determined by the method of Warren [11] after hydrolysis in 2.5% trichloroacetic acid at 80°C for 60 min.

Protein was determined by the method of Lowry et al. [12] with bovine serum albumin as standard.

3. Results and discussion

3.1. *Distribution of protein and marker enzymes in submitochondrial fractions*

From the activities of glucose 6-phosphatase and acid phosphatase, contaminations of the isolated mitochondria by microsomes and lysosomes were estimated to be each about 2% on the protein basis.

The distribution of protein and the marker enzymes in the submitochondrial fractions is shown in table 1. The values were comparable with those reported by the investigators who had studied of systematic mitochondrial subfractionation [4,5,9,13,14].

3.2. *Carbohydrate compositions of mitochondria and submitochondrial fractions*

It should be noted that the values for the carbohydrate composition of whole mitochondria, shown in table 2, are markedly lower than those reported previously [3]. This is due to the difference in the pretreatment of mitochondria prior to the analysis. In a previous study [3], mitochondria were dialysed extensively before analysis which resulted in loss of some carbohydrate-free peptides, whereas mitochondria were precipitated with phosphotungstic acid in trichloroacetic acid in the present study. De Bernard et al. [15] who analyzed for hexosamines and sialic acid on the samples which had been purified by gel filtration reported still higher values. It is, however, uncertain if this can be ascribed solely to their pretreatment of the samples.

It should also be noted that glucose may not be a component of glycoproteins, and that some of the galactosamine may be a component of mucopolysaccharides, as described previously [3]. The components other than these two sugars were shown to constitute glycoproteins in which most of the carbohydrate moieties are linked to polypeptide through *N*-glycosidic linkages [3].

Table 2 shows that IMS, OM and IM contain mannose, glucosamine, fucose, galactose, sialic acid and galactosamine in similar proportions, which suggests that glycoproteins with similar carbohydrate compositions and mucopolysaccharides occur in these submitochondrial fractions. IMS had the largest distribution values for every carbohydrate component, comprising about 60% of the total of the respective component except for glucose. OM and IM showed similar distribution values, but the carbohydrate content of OM on the protein basis was about 5 times higher than that of IM. MAT was virtually free of the carbohydrates. Our values for the distribution of

Table 1
Protein, cytochrome oxidase, malate dehydrogenase and kynurenine-3-monooxygenase of mitochondrial subfractions from rat liver

Fraction	Protein	Cytochrome oxidase		Malate dehydrogenase	Kynurenine-3-mono oxygenase		
	(%)	U*/mg protein	(%)	U/mg protein	(%)	mU/mg protein	(%)
Whole mitochondria	100	0.96	100	1.41	100	2.7	100
OM	6.9	1.25	9.0	0.77	3.0	21.0	54.7
IMS	22.1	0.05	1.0	0.56	12.4	0.5	4.9
IM	32.6	2.45	82.5	0.62	14.5	1.3	17.4
MAT	38.4	0.19	7.5	2.63	70.0	1.6	22.9

* One unit of activity is the amount of enzyme which will catalyze the change of one μmole of substrate per min.

Table 2
Carbohydrate compositions of mitochondria and submitochondrial fractions

Fraction	Glucosamine	Galactosamine	Sialic acid	Mannose	Galactose	Fucose	Glucose
Whole mitochondria	3.66* (100)**	0.68 (100)	1.49 (100)	4.35 (100)	1.71 (100)	0.45 (100)	1.31 (100)
OM	9.22 (17.0)	2.28 (23.4)	4.40 (22.7)	9.16 (15.1)	3.80 (17.3)	0.80 (13.5)	10.8 (43.8)
IMS	10.5 (65.2)	1.32 (50.3)	3.40 (57.0)	12.7 (69.6)	4.13 (63.1)	1.20 (68.8)	3.28 (44.6)
IM	1.69 (13.0)	0.55 (23.5)	0.80 (17.1)	1.88 (12.8)	0.88 (16.8)	0.22 (15.3)	0.40 (6.7)
MAT	0.42 (4.8)	0.04 (2.8)	0.08 (3.2)	0.25 (2.5)	0.10 (2.8)	0.02 (2.4)	0.19 (4.8)

* μmoles/g protein;
** % distribution

hexosamines and sialic acid are somewhat different from those of De Bernard et al. [15] and of Sottocasa et al. [16] who reported that about 80% each of hexosamines and sialic acid were contained in IMS. Further, our values for the carbohydrate contents of OM and IM are markedly different from those of De Bernard et al. [15].

The occurrence of glycoproteins in OM and IM may be relevant to the behaviour of these membranes towards various treatments. For instance, the mannose residues of the glycoproteins in OM and IM may be responsible to the binding of these membranes to concanavalin A [17,18]. We found in preliminary studies that a lectin from *Ricinus communis* which is galactose specific agglutinated rat liver mitochondria even more effectively than concanavalin A. This implies that some of the galactose residues in OM are exposed to the outside.

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