Subcellular localisation of cerebral fucosyltransferase

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GDP-fucose: asialofetuin fucosyltransferase from sheep brain was fractionated on a sucrose gradient into two activity peaks. Using purification on Ficoll adapted from the proposed method [(1980) J. Neurochem. 35, 281–296], double localisation of cerebral fucosyltransferase was confirmed and the subcellular active fractions identified as light microsomes and mitochondria.

Fucosyltransferase *Cerebral fractionation* *Enzyme marker*

1. INTRODUCTION

We have demonstrated the existence of a GDP-fucose: glycoprotein fucosyltransferase (EC 2.4.1.68) in the central nervous system of the rat [2] and sheep [3]. This enzyme has been solubilized with neutral detergent and purified using hydrophobic chromatography on ethyl-agarose [4]. A study of the initial velocity patterns using asialofetuin as a substrate provided evidence for a bi-bi random mechanism with dead-end inhibition [2]. Substrate requirements were investigated with asialofetuin and purified glycopeptides from ovalbumin [5]. Important and rapid daily variations were observed in the activity of rat brain fucosyltransferase [6], but its relationship to brain function remains unknown. Here, we have studied the subcellular localisation of glycoprotein fucosyltransferase in sheep cerebral hemispheres.

2. MATERIALS AND METHODS

GDP-[^{14}C]fucose was purchased from New England Nuclear. Ficoll was obtained from Pharmacia. Other reagents came from Sigma and Merck. Sheep heads were obtained from a local slaughterhouse and quickly transported in ice to the laboratory where the brains were removed. The cerebral hemispheres were freed of most of the white matter by careful dissection in the cold room, and immediately homogenized in a buffer solution as described above.

Adaptation of the fractionation proposed in [7]: the cerebral hemispheres, cerebellum and brain stem were homogenized in a Potter-Elvehjem homogenizer in 3 vols of 320 mM sucrose, 50 mM Mes (pH 7.0) per g tissue. The homogenate was centrifuged for 10 min at 10000 × *g* in an R30 Beckman rotor, and the supernatant centrifuged again for 20 min at 17000 × *g* in the same rotor. Four ml of the supernatant were layered at the top of a continuous gradient with a total volume of 30 ml, ranging from 0.8 to 1.3 M sucrose in Mes buffer, and a layer of 2 ml of 2 M sucrose at the bottom. The gradient was run for 14 h at 25000 rpm in a Beckman SW27 rotor. After the run, fractions (about 1.5 ml) were collected using a peristaltic pump (LKB) and the enzymatic activity was tested.

Adaptation of the fractionation proposed in [1]: homogenization was performed by 6 up-and-down strokes in a Potter-Elvehjem homogenizer using

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9 vols of buffer to 1 g tissue. The composition of the fractionation buffer was as described [1] (320 mM sucrose containing 1 mM sodium phosphate buffer) except that 0.1 mM EGTA was added instead of 0.1 mM EDTA and the pH was 7.0 instead of 7.5. The homogenate was filtered through cheesecloth and centrifuged at 1000 × *g* for 15 min. The resulting pellet was called the nuclear fraction. The supernatant was then centrifuged at 11500 × *g* for 25 min, the pellet washed with 4 vols homogenizing buffer and centrifuged again twice at 11500 × *g* for 25 min. The final pellet was referred to as crude mitochondria. The pooled supernatants were centrifuged at 30000 × *g* for 25 min giving a pellet (microsomes I) and a supernatant which was centrifuged at 100000 × *g* for 60 min. The resulting pellet was named microsomes II and the supernatant referred to as the final supernatant. Portions of the crude mitochondria resuspended in the homogenizing buffer were poured onto the top of a discontinuous gradient consisting of 9% Ficoll (15 ml), 16% Ficoll (15 ml) and 2 M sucrose (2 ml) in the homogenizing buffer, and centrifuged for 120 min at 25000 rpm in a Beckman SW27 rotor. Three bands were obtained: one floating over the 9% Ficoll and referred to as the A fraction, one at the 9%/16% interface (B fraction) and one at the 16%/2 M interface (C fraction). All the fractions were resuspended or diluted with the homogenizing buffer, pelleted at 100000 × *g* for 60 min, and stored at −20°C until further use.

GDP-fucose:asialofetuin fucosyltransferase (EC 2.4.1.68) was measured as in [2]. Incubation was performed on 200 μl enzymatic suspension in 50 mM Mes buffer (pH 7.0), containing 0.5% Triton X-100 (w/v), 20 μl asialofetuin (400 μg) and 10 μl GDP-fucose (spec. act. 170 Ci/mol, 590 nmol for an assay). After 10 min at 23°C, the incubation was stopped with 2 ml of 20% (w/v) trichloroacetic acid, and radioactivity counted on Whatman GF/B glass fiber filter as in [8].

Acetylcholinesterase (EC 3.1.1.7) was measured as in [9] and cytochrome oxidase (EC 1.9.3.1) as in [10]. The protein assay was performed according to [11] after precipitation by trichloroacetic acid (10%, v/v), using bovine serum albumin as the standard.

3. RESULTS AND DISCUSSION

A preliminary study was performed using a slight modification of the method proposed in [7]. The results in fig.1 regarding sheep brain cerebral hemispheres clearly show two localisations for the cerebral fucosyltransferase, one in the upper part of the gradient (about 0.85–0.9 M sucrose) and the other at the bottom of the gradient, just under the 2 M sucrose layer. For the cerebral stem, which consists mainly of white matter, one single peak was demonstrated near the top of the gradient, while for the cerebellum, activity was mainly recovered at the bottom. Similar results were obtained using phosphate buffer instead of Mes buffer throughout the procedure.

According to authors in [7], the bottom of the gradient contained a mixture of lysosomes, endoplasmic reticulum and probably some mitochondria and synaptosomes, while the top contained a mixture of plasma membranes, myelin and soluble proteins. No activity was detected in the median part of the gradient, where they found ceramide galactosyltransferase and cerebroside sulfotransferase, assumed by these authors to be Golgi-localised. Thus, although this method very clearly demonstrated fucosyltransferase patterns with two different localisations in the cerebral hemispheres, it was not possible to identify them, and a complementary method is necessary.

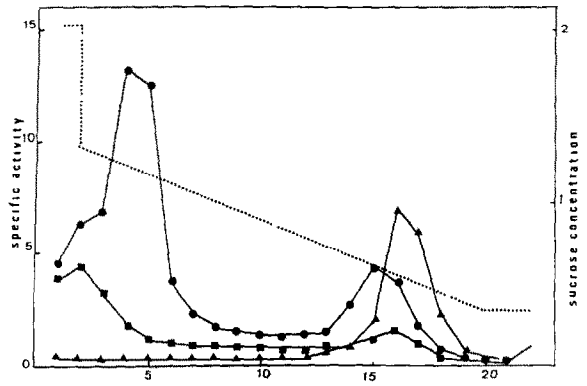

Fig.1. Specific activity of fucosyltransferase in fractions derived from a continuous gradient (0.8–1.3 M sucrose) layered with 17000 × *g* supernatant prepared from cerebral hemispheres (●—●), cerebellum (■—■) and brain stem (▲—▲). Activities are expressed as pmol fucose/min per mg of proteins. (···) Sucrose concentration in the gradient.

The fractionation undertaken in [1] was close to the original procedure proposed in [12], using a Ficoll gradient for the purification of synaptosomes and mitochondria. Our main improvement was the replacement of EDTA by EGTA in the fractionation buffer. Table 1 lists the results obtained from a typical experiment concerning fucosyltransferase distribution between different subcellular fractions. Specific activity was highest in the microsomal II fraction. However, purification of the crude mitochondria gave a very good enhancement of the specific activity in the C fraction obtained after the Ficoll gradient stage. This was corroborated by results concerning total activity distribution: the best percentage was obtained with the microsomes II and crude mitochondria fractions. The activity of the latter fraction was almost totally found in the C fraction. Thus, as far as the procedure in [7] is concerned, it appears clearly that fucosyltransferase activity was located in both a light microsomal fraction and in a heavier fraction.

This fraction was identified by comparison with the results in [1] and by the checking of some enzymatic activities. Acetylcholinesterase, known to be both an endoplasmic reticulum and a plasma membrane enzyme marker, was enhanced in the microsomes I and microsomes II fractions (table 2) but with a specific activity lower than that found for fucosyltransferase. On the other hand, from the crude mitochondria fraction, acetylcholinesterase was enhanced 3-times in the B fraction which according to authors in [1] contained mostly synaptosomes, while its enhancement in the C fraction remained lower. In contrast, cytochrome oxidase was mainly recovered in the crude mitochondria. Purification of this fraction on a Ficoll gradient led to an important enhancement of the specific activity of this enzyme in the C fraction. According to [1], this C fraction would mainly contain mitochondria.

A comparison of fractionation in the buffer initially used in [1] and our buffer containing EGTA instead of EDTA gave roughly similar results in terms of distribution but fucosyltransferase recovery was much lower (less than 10%) and enrichment of the microsomes II and C fraction was no more than 3- and 5-fold respectively. Such striking results could be correlated with the purification of rat brain myelin undertaken in [13] and axolemma in [14] which involved EGTA shock as an essential step in purification, and might explain the relatively low sialyltransferase activity enhancements reported in [1].

From our results, it appeared that if a significant part of the fucosyltransferase activity is located in the light membranes which are assumed to be endoplasmic, another important part is also located in the fraction possessing the greatest enrichment in mitochondria. When considering this activity enhancement, strong contamination of the C fraction by light microsomes is unlikely, and in comparison to acetylcholinesterase and cytochrome oxidase distribution, it clearly demonstrates a

Table 1

Subcellular distribution of sheep cerebral hemispheres fucosyltransferase

Fraction	Total protein content (g)	Specific activity (pmol/min per mg)	Total activity (pmol/min)
Homogenate	11360	4.3	48840 (100 %)
Crude mitochondria	1087	16.8 (× 3.9)	18270 (37.4%)
Microsomes I	318	27.3 (× 6.3)	8680 (17.8%)
Microsomes II	76	170.4 (×39.6)	13010 (26.6%)
A fraction	304	4.7 (× 1.1)	1430 (2.9%)
B fraction	44	27.1 (× 6.3)	1190 (2.4%)
C fraction	116	83.1 (×19.3)	9620 (19.6%)

Relative specific activities (in brackets) were related to homogenate, the activity of which was taken as equal to 1. Percentages of total activity (in brackets) were calculated as follows: (total activity of the fraction/total activity of the homogenate) × 100

Table 2

Subcellular repartition of acetylcholinesterase and cytochrome oxidase in sheep brain cerebral hemispheres

Fraction	Acetylcholinesterase		Cytochrome oxidase	
	Specific activity	Total activity	Specific activity	Total activity
Homogenate	0.033	375.0 (100 %)	1.63	18520 (100 %)
Crude mitochondria	0.035 (1.1)	38.0 (10.1%)	1.99 (1.2)	2160 (11.6%)
Microsomes I	0.134 (4.1)	42.6 (11.3%)	2.43 (1.5)	772 (4.1%)
Microsomes II	0.180 (5.4)	13.7 (3.6%)	n.d.	n.d.
A fraction	0.028 (0.8)	8.5 (2.2%)	0.86 (0.5)	261 (1.4%)
B fraction	0.096 (2.9)	4.2 (1.1%)	9.19 (5.6)	402 (2.2%)
C fraction	0.042 (1.3)	4.9 (1.3%)	18.99 (11.6)	2215 (11.9%)

Specific activities are DO/min per mg of proteins in the enzymatic incubation conditions. Total activities are the product of specific activities by protein content of the corresponding fraction. Relative activities are expressed as in table 1. n.d., not detected

mitochondrial rather than a synaptosomal localisation. We have reported on the transfer of mannose onto glycoprotein in rat brain [15] and rat liver [16] mitochondria, and others have demonstrated the synthesis of mannosylphosphoryldolichol [17], *N*-acetylglucosaminylpyrophosphoryldolichol [18] and glucosylphosphoryldolichol [19] in the outer membrane of mouse liver mitochondria. Thus, results regarding mitochondrial glycosyltransferase localisation provided in the above-mentioned studies may extend to the asialofetuin fucosyltransferase in rat brain cerebral hemispheres.

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