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OCCURRENCE OF α2-8 LINKED POLYSIALOSYL UNITS IN A NEURAL CELL ADHESION MOLECULE

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Summary A brain cell surface protein (BSP-2) was isolated from mice of different ages by affinity chromatography using a monoclonal antibody. Analysis of glycopeptides obtained after pronase digestion revealed that the embryonal and neonatal forms of the antigen contained an unusually high proportion of sialic acid, which decreased during development. Methylation analysis of native and neuraminidase treated glycopeptides indicated that the sialic acid occurred as α2-8 bound polysialosyl units, similar to those of the recently described developmentally regulated polysialosyl glycopeptides of rat brain. Furthermore, the carbohydrate and amino acid composition, and electrophoretic mobility of BSP-2 antigen correspond to those reported for a neural cell adhesion molecule (N-CAM).

Cell surface carbohydrates are thought to play an important role during the development of the nervous system by mediating intercellular recognition and adhesion (1-3). We have recently identified in brain tissue a novel class of developmentally regulated carbohydrate structures, polysialosyl glycopeptides (4). Similar compounds have not been found in other tissues. For the study of the biological significance of the polysialosyl glycopeptides it would be important to identify the glycoproteins which are carriers of these carbohydrate chains.

A brain cell surface protein, BSP-2, has recently been reported to display developmental changes which seem to be due to differences in glycosylation (5). A similar change, caused by differences in sialylation, has also been reported to occur in a neural cell adhesion molecule, N-CAM (6), but the precise structural basis for this modulation is not known. We now provide additional evidence for the structural similarity of BSP-2 and N-CAM and demonstrate that the carbohydrate chains of BSP-2 contain unique α2-8 linked

Abbreviations: BSP-2, brain cell surface protein-2;
N-CAM, neural cell adhesion molecule

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polysialosyl units similar to those previously described as polysialosyl glycopeptides. The developmental changes in the carbohydrate structures of BSP-2 are found to consist mainly in modifications of the number and average length of these polysialosyl units.

METHODS BSP-2 antigen was isolated from the forebrains of mice of different ages as described previously (5). The detergent was removed by precipitation of the antigen with acetone (4 vol, at -20 °C), followed by centrifugation (1000xg, 20 min). After gel electrophoresis (7) scanning of the Coomasie Blue stained gels showed that no single contaminant peak represented more than 2.5 % of the total. Protein concentration was estimated by a modified Lowry procedure (8). The recovery of antigen activity was determined by an absorption assay. Serial dilutions of membrane suspensions, detergent extracts or purified antigen were incubated (14 h at 4 °C) with an aliquot of anti-BSP-2 monoclonal antibody diluted so as to be limiting in the enzyme-linked solid phase assay. Residual antibody activity was estimated by the peroxidase-linked immunoassay of Hawkes et al. (9) using purified BSP-2 as solid phase antigen. Virtually identical results were obtained whether embryonic or adult antigen was used as target in the immunoassay. One unit of BSP-2 activity was defined as the amount of antigen required for 50 % inhibition of antibody binding. Amino acid analysis was performed on a Beckman 121M amino acid analyzer after hydrolysis in 6 M HCl at 110 °C for 24, 48 and 72 h.

Aliquots of 200-300 µg of BSP-2 were dissolved in 250 µl of 0.1 M Tris-HCl buffer, pH 8.0, containing 1 mM $CaCl_2$, and 25 µl of a preincubated solution of pronase (5 mg/ml) was added (10). Digestion was carried out at 50 °C for 48 h; 25 µl of fresh pronase solution was added at 24 h. After lyophilization the digest was dissolved in 100 µl of water and the glycopeptides were desalted by centrifugation through columns of Bio-Gel P-2 (11). The sugar compositions were determined after methanolysis by gas-liquid chromatography (12). Permethylation was carried out using potassium t-butoxide (13) and the linkage of neuraminic acid in the native and neuraminidase treated glycopeptides was determined using mass fragmentographic detection (4).

RESULTS Extraction of the crude membrane fraction of brain tissue results in virtually complete solubilization of the BSP-2 antigen (14). As shown in Table 1, a good recovery of the antigenic activity was obtained from mouse

TABLE 1 Recovery of BSP-2 from the brain of mice of different ages

	E 18-20[a]	P 1-3	P 10-15	P 28-36
Yield of antigen activity (%)[b]	72	64	74	78
mg BSP-2/g membrane protein	9.4	10.0	5.8	3.4
mg BSP-2/g fresh weight of brain	0.173	0.150	0.210	0.073

[a] E, embryonic age in days; P, postnatal age in days

[b] calculated by taking the amount of antigen in crude membranes as 100 %

TABLE 2 Sugar composition and linkage of neuraminic acid in glycopeptides of BSP-2 from mice of different ages

Component	E 18-20	P 1-3	P 10-15	P 28-36
	nmol/100 µg protein[a]			
Fucose	3.6	4.7	4.4	4.5
Mannose	12.8	11.4	14.1	11.3
Galactose	7.5	8.3	10.4	8.6
N-acetylglucosamine	12.7	14.1	16.5	12.9
N-acetylneuraminic acid	32.7	36.5	30.2	12.9
terminal	6.4	4.7	6.4	3.4
8-O-linked	26.3	31.8	23.8	9.5

[a]Not corrected for losses (10-20 %) during isolation of glycopeptides

forebrains of different ages after isolation by affinity chromatography using a monoclonal antibody to BSP-2. The adult mice (P28-36) contained a 2 to 3 times lower amount of antigen as compared to the embryonal or early postnatal stage.

The sugar composition of the different samples of BSP-2 is shown in Table 2. As compared to glycopeptides isolated from other sources, an unusually high proportion of neuraminic acid was observed. Whereas the proportions of other sugars showed little age-dependent variation, there was a nearly 3-fold decrease in the proportion of neuraminic acid during development.

As seen in Fig. 1, most of the neuraminic acid in the embryonal form of BSP-2 was found to be substituted at C-8. Since this substituent was cleaved by neuraminidase treatment (Fig. 1C), it was identified as another residue of neuraminic acid. This indicated the presence of α2-8 linked neuraminic acid units in the embryonal form of BSP-2. The results of the methylation analysis of neuraminic acid are summarized in Table 2. Although the adult form of BSP-2 (P28-36) still contained internal, 8-O-substituted neuraminic acid, its relative amount had decreased approximately 3-fold during development. This was also reflected in the calculated average chain length of the polysialosyl units, which decreased from a value of nearly 8 in the neonatal brain to less than 4 in the adult brain.

Since the sugar composition, electrophoretic migration, and structural changes during development of the neural cell adhesion molecule N-CAM isolated from mouse brain are very similar to those of BSP-2 (6,15,16), an amino acid

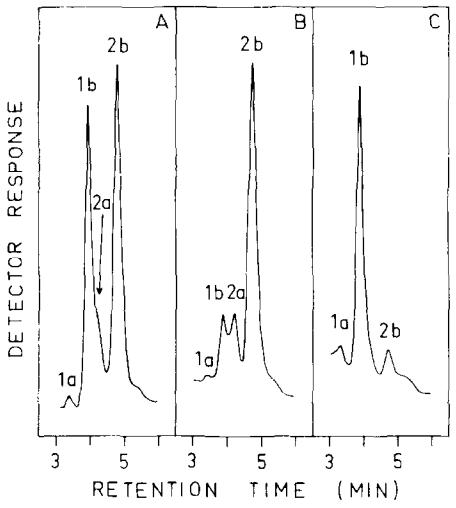

Fig. 1 Gas-liquid chromatography of methylated neuraminic acids. A, disialosyl lactose; B, glycopeptides from BSP-2 (t 18-20); C, B after incubation with neuraminidase. Peaks 1a and 1b, derivatives of terminal N-acetylneuraminic acid; peaks 2a and 2b, derivatives of internal 8-O-substituted N-acetylneuraminic acid (20). Conditions: 4 % OV-101, 246 °C. Detection by mass fragmentography at m/e 201.

analysis was also performed. As seen in Table 3, the amino acid composition of BSP-2 was found to be nearly identical with those previously reported for two different preparations of N-CAM.

DISCUSSION The sugar composition and the results of the methylation analysis of BSP-2 indicate that this glycoprotein bears unique polysialosyl chains containing α2-8 linked neuraminic acid residues. Similar glycans were recently isolated as glycopeptides after pronase digestion from developing rat brain (4). The 2 to 3-fold decrease in the amount of BSP-2 during development together with a 3-fold decrease in the proportion of neuraminic acid accounts for a 6- to 9-fold decrease in the BSP-2 bound polysialosyl units at the postnatal age of 28-36 days. This correlates well with the previously observed decrease in the amount of polysialosyl glycopeptides in rat (4) and human (17) brain during development. As calculated from the concentration of BSP-2 and its sialic acid content, there is approximately 55-85 nmol of BSP-2 bound sialic acid per gram of developing mouse brain. The total amount of sialic acid in polysialosyl glycopeptides of developing rat brain is approximately 80 to 120 nmol and of human brain 60 to 90 nmol per gram of tissue (4,17). These data suggest that the BSP-2 molecule may be a major carrier of the polysialosyl chains in developing brain tissue. However, the presence of other molecules containing these glycans cannot be excluded.

TABLE 3 Amino acid composition of mouse BSP-2 and N-CAM

Amino acid	BSP-2[a]	N-CAM(15G8)[b]	N-CAM(9E11)[b]
		mol / 100 mol	
Asx	9.8	10.2	10.4
Thr	6.6	7.4	7.2
Ser	9.3	8.4	8.9
Glx	14.2	12.1	13.6
Pro	5.8	6.3	6.9
Cys	ND[c]	ND	ND
Gly	8.6	7.6	7.6
Ala	7.6	8.5	8.4
Val	7.1	9.9	7.3
Met	1.2	1.5	1.5
Ile	5.9	4.7	5.3
Leu	6.6	7.4	6.5
Tyr	2.3	2.3	2.5
Phe	3.1	3.0	2.9
His	1.6	1.4	1.4
Lys	6.5	6.1	7.0
Arg	3.4	3.1	3.2
Trp	ND	ND	ND

[a]Purified from P10-15 brain

[b]Data from ref. 15

[c]ND, not determined

As indicated by similar electrophoretic mobilities and similar developmental changes (5,14,16), BSP-2 has properties similar to those of N-CAM. This similarity is further supported by the findings of the present study showing similar sugar and amino acid compositions as well. That BSP-2 is also functionally related to N-CAM is indicated by the recent findings that purified BSP-2 binds to neural cells and that polyclonal anti-BSP-2 antibodies inhibit the formation of cellular aggregates (18). The brain antigen D2 (19) has also been reported to have properties similar to those of BSP-2 and N-CAM, and it is possible that all three antigens may correspond to the same molecular entity. The modulation of the sialic acid structure during development suggests the involvement of carbohydrate in the biological properties of this cell adhesion molecule. The identification of the unique α2-8 linked polysialosyl units not so far found in other glycoproteins of mammalian tissues gives further support to this hypothesis.

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