Preliminary Notes

PN 1208

Preparation of sialomucopolysaccharides from brain mitochondrial fractions

Although the brain is a rich source of glycolipids containing sialic acid, it has been pointed out[1] that brain contains an equally large concentration of protein-bound sialic acid. The presence of sialomucopolysaccharides in brain is of special interest in view of the demonstration by MCILWAIN[2] that gangliosides from brain and a sialomucopolysaccharide from ovarian-cyst fluid[3] restored responses of cerebral-tissue slices to electrical pulses after the normal response was lost by standing in a cold medium.

A sialomucopolysaccharide, or a mixture of sialomucopolysaccharides, has been extensively purified in this laboratory using rat- or dog-brain "mitochondrial" fractions as the source material. The purified material was shown to be free of nucleic acids and protein, was non-dialyzable, and was not precipitated by cetyl pyridinium chloride.

The rat-brain mitochondrial fraction was prepared as previously described[4]. This fraction contains nerve endings, myelin fragments, and synaptic vesicles in addition to the mitochondria[5]. Alternatively, dog-brain mitochondrial fraction was prepared by centrifuging a sucrose homogenate of whole dog brain (8 ml of 0.25 M sucrose per g tissue) at 800 × *g* for 10 min to remove the nuclei, red blood cells, and the cell debris. The residue was resuspended in one-fourth the original volume of the homogenate and the centrifugation was repeated. The supernatants were combined and centrifuged at 14000 × *g* for 15 min to obtain the "mitochondrial fraction". Mitochondria from both rat and dog brain were suspended in water, homogenized, and centrifuged at 46000 × *g* for 20 min; the supernatant, which contains about 20 % of the mitochondrial fraction protein, was discarded. The water-washed mitochondrial fraction was taken up in a minimal amount of water and extracted with 24 vol. of chloroform – methanol (2:1, v/v)[6] to remove the gangliosides. An amount of dried residue, representing 1 g of original brain tissue was suspended in 4 ml of 0.1 M sodium acetate buffer (pH 4.5) containing 0.005 M cysteine and 0.005 M EDTA. After incubation for 24 h at 60° with crystalline papain (0.035 mg/ml suspension), the mixture was centrifuged and the precipitate discarded. The supernatant was now dialyzed after concentration to a small volume. A precipitate which formed during dialysis was removed by centrifugation. Approx. 50 % of the hexosamine solubilized by the papain treatment is dialyzable and represents glycopeptide fragments derived from the glycoproteins. The non-dialyzable material (3.5 mg of hexosamine) was now percolated through a 1 × 8-cm Dowex-1 X2 chloride column, 200–400 mesh. 50 ml of water were used to wash the column. The sialomucopolysaccharides, which are adsorbed on the column, are now eluted with 4 M NaCl and subsequently dialyzed against water. Nucleic acids and glucuronic acid containing mucopolysaccharides are now precipitated with cetyl pyridinium chloride, as described by SCOTT[7]. After centrifugation, the supernatant is treated with amyl alcohol to remove excess cetyl pyridinium chloride[7].

Rat-brain mitochondrial fraction yielded 20–60 μg of sialomucopolysaccharide hexosamine per g of rat brain; the yield from dog brain was substantially lower (7 μg). The dog-brain material was analyzed further. Sialic acid was removed from the sialomucopolysaccharide (s) by hydrolysis in 0.1 N H_2SO_4 for 1 h at 80° and subsequent adsorption on Dowex-1 X10 acetate, 200–400 mesh as described by WHITEHOUSE AND ZILLIKEN[8]. The hexose-containing residue, not retained by the ionic exchanger, was hydrolyzed in 1.5 M HCl for 6 h at 100°. Paper chromatography of the hydrolyzate (*n*-butanol – pyridine – water; 45:25:40, v/v)[9] revealed the presence of galactose, fucose, rhamnose, and mannose. The hexosamine was identified as glucosamine by means of paper chromatography of the pentose derivatives[10].

An estimate was made of the quantitative distribution of the sugars using the primary and secondary cysteine–H_2SO_4 reactions of DISCHE[11] for hexoses and methylpentoses, the thiobarbiturate method of WARREN[12] for sialic acid, and the BOAS[13] modification of the ELSON–MORGAN[14] reaction for hexosamine. The ratio of the various carbohydrate constituents compared to the value of methylpentose was 1, 0.9, 3.1, 4.1, and 2.2 for methylpentose, mannose, galactose, glucosamine and neuraminic acid respectively. These ratios represent the average for three preparations, which agree within 8 %. All of the nitrogen present assayed as amino nitrogen in the ninhydrin test. 2 preparations yielded, respectively, 10 and 16 μmoles of nitrogen per molecule of methylpentose in excess of that expected on the basis of sialic acid and hexosamine content, indicating the presence of a residual peptide group. The napthol test for total sugars[15] gave a value for total carbohydrates 90 % of that expected on the basis of the spectrophotometric methods used to determine the individual sugars.

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[1] L. SVENNERHOLM, *Acta Chem. Scand.*, 10 (1956) 694.
[2] H. MCILWAIN, *Biochem. J.*, 78 (1961) 24.
[3] A. PUSZTAI AND W. T. J. MORGAN, *Biochem. J.*, 78 (1961) 135.
[4] E. G. BRUNNGRABER AND V. AGUILAR, *J. Neurochem.*, 9 (1962) 451.
[5] A. DE ROBERTIS, A. PELLEGRINO DE IRALDI, G. RODRIQUEZ DE LORES ARNAIZ AND L. SALGANICOFF, *J. Neurochem.*, 9 (1962) 23.
[6] J. FOLCH-PI, M. LEES AND G. H. SLOAN-STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
[7] J. E. SCOTT, *Methods Biochem. Anal.*, 8 (1960) 145.
[8] M. W. WHITEHOUSE AND F. ZILLIKEN, *Methods Biochem. Anal.*, 8 (1960) 199.
[9] R. E. GLEGG AND D. EIDINGER, *Anal. Chem*, 26 (1954) 1365.
[10] P. J. STOFFYN AND R. W. JEANLOZ, *Arch. Biochem. Biophys.*, 52 (1954) 373.
[11] Z. DISCHE, L. B. SHETTLESS AND M. OSNOS, *Arch. Biochem.*, 22 (1949) 169.
[12] L. WARREN, *J. Biol. Chem.*, 233 (1959) 1971.
[13] N. F. BOAS, *J. Biol. Chem.*, 204 (1953) 553.
[14] L. A. ELSON AND W. T. J. MORGAN, *Biochem. J.*, 27 (1933) 1824.
[15] Z. DISCHE, *Methods Biochem. Anal.*, 2 (1955) 313.

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