Effect of ionic strength of eluting solutions on behavior of sialomucopolysaccharides from rat brain on Sephadex G-200

The isolation of sialomucopolysaccharides from rat whole brain has been reported[1,2]. The N-acetylneuraminic acid (NANA) content of these substances represents about 23 % of the total NANA present in the tissue and accounts for most of the non-gangliosidic NANA of brain. The glycoproteins of brain, the carbohydrate moiety of which are the sialomucopolysaccharides, are difficult to solubilize quantitatively. As a consequence, we have studied the carbohydrate moiety by destroying the protein portion of the molecule by the proteolytic action of papain. The defatted brain tissue, after successive extractions with chloroform–methanol (2:1, v/v and 1:2, v/v) is subjected to digestion with papain. The insoluble residue is removed by centrifugation. The supernatant was dialyzed to remove small molecular weight fragments released by proteolysis. The nondialyzable sialomucopolysaccharides were further purified by precipitation of nucleic acids and glucuronic acid-containing mucopolysaccharides with cetylpyridinium chloride. Excess cetylpyridinium chloride was removed by extraction with pentan-1-ol in the cold.

The sialomucopolysaccharides are a mixture of polysaccharides that differ in the molar ratios of their constituent sugars. It has been postulated[2] that variable amounts of NANA and fucose are linked to a basic hexosamine–hexose repeating structure.

Gel filtration was used to remove an impurity that adsorbs light in the ultra-

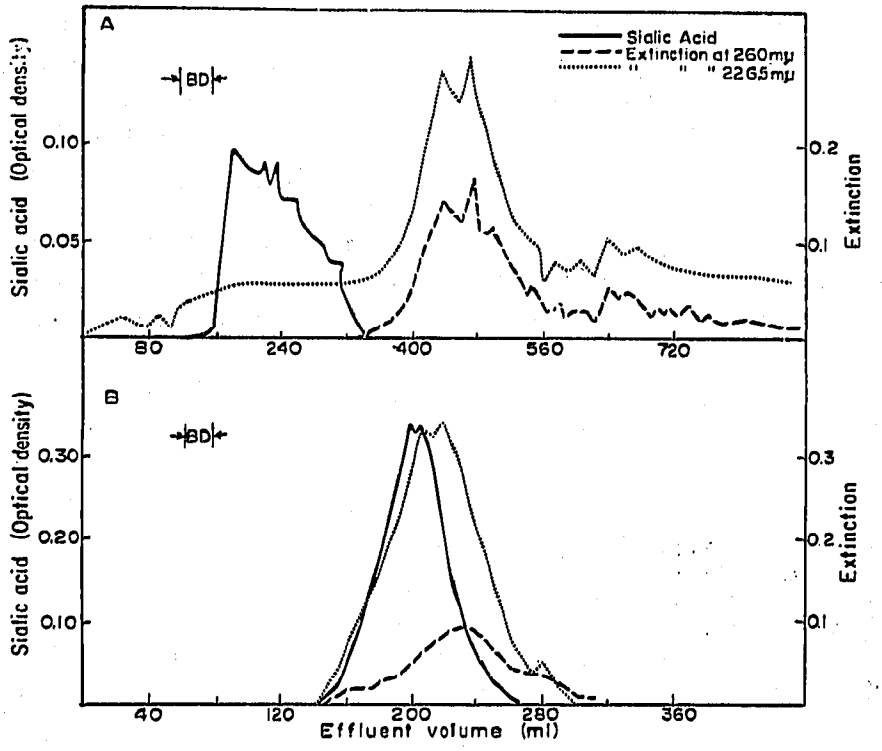

Fig. 1. (A) Chromatogram obtained when a sialomucopolysaccharide preparation was subjected to gel filtration on a column of Sephadex G-200 (34 × 4 cm) with a bed volume of 420 ml using water as eluting agent. (B) Chromatogram obtained when a sialomucopolysaccharide preparation was subjected to gel filtration on a column of Sephadex G-200 (42 × 2.5 cm) with a bed volume of 200 ml using 0.05 *M* Tris–HCl buffer, pH 7.5, containing 0.1 *M* KCl.

violet region (Fig. 1A). The use of water as eluting agent facilitated this purification step since the sialomucopolysaccharide mixture appears in the effluent shortly after the elution of the Blue Dextran 2000. Blue Dextran has an average mol. wt. of 2,000,000 and is completely excluded from the gel matrix. Successful purification by the use of water as eluting agent was undoubtedly due to an aggregation of the sialomucopolysaccharides resulting in an apparent molecular weight in excess of 100,000. Aggregation appears to be dependent upon the content of NANA present in the molecule; it has been shown[2] that the sialomucopolysaccharides eluted between 120 and 240 ml (Fig. 1A) represent those molecules of the sialomucopolysaccharide mixture that contain a higher percentage of NANA.

If gel filtration is carried out in a medium of higher ionic strength, (0.05 *M* Tris–HCl buffer, pH 7.5, in 0.1 *M* KCl), a solvent used by ANDREWS[3] in experiments designed to estimate the molecular weight of proteins by gel filtration on Sephadex 200, the sialomucopolysaccharides are capable of entering the gel matrix and their retardation renders gel filtration impracticable as a purification step (Fig. 1B). In the present experiment, the ratio of elution volume to void volume was 1, 2.65, 2.87, and 3.03 for Dextran Blue, cytochrome c, sialomucopolysaccharides, and sucrose respectively. A preliminary estimate of the molecular weight of the sialomucopolysaccharides, based on this data, is 6–8000.

In these experiments, Sephadex G-200 (140–400 mesh) gel-filtration medium (lot No. T056) was added to distilled water (or buffer) and allowed to swell for at least five days. Flow rates for the columns were approximately 12 ml per h and the columns were operated under a hydrostatic head that never exceeded 15 cm. The gel had been deaerated under reduced pressure before packing into the column. Samples of sialomucopolysaccharides dissolved in water (or buffer) were applied to the top of the column by layering under solution already present. The volumes of the samples applied were 1/80 that of the bed volume of the column. NANA was determined by the method of WARREN[4].

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