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Presence of Activator Proteins for the Enzymic Hydrolysis of G_{M1} and G_{M2} Gangliosides in Normal Human Urine

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SUMMARY

Normal human urine has been found to contain activator proteins that stimulate the enzymic hydrolysis of G_{M1} and G_{M2} gangliosides. These two activators were partially purified by Sephadex G-200 filtration and DEAE-Sephadex A-50 chromatography. The presence of these two activators was assayed by demonstrating the stimulation of the in vitro hydrolysis of G_{M1} and G_{M2} gangliosides. As little as 50 ml of urine is sufficient to detect the presence of these two activators. The crude activator preparation from normal urine was also found to stimulate the hydrolysis of galactosylceramide sulfate by arylsulfatase A.

INTRODUCTION

The discovery of activator proteins that stimulate the enzymic hydrolysis of glycosphingolipids may represent one of the most important developments in the field of glycosphingolipid catabolism. Activator proteins for the enzymic hydrolysis of the following glycosphingolipids have been isolated: galactosylceramide sulfate from human liver [1]; glucosylceramide from Gaucher spleen [2, 3] and bovine spleen [4]; G_{M1} ganglioside from human liver [5]; and G_{M2} ganglioside from human kidney [6], liver [7, 8], and brain [9]. The importance of activator proteins in the catabolism of glycosphingolipids is further substantiated by the occurrence of glycosphingolipid storage diseases caused by the deficiency of the activator protein rather than by the enzyme [9–13]. So far, only such vital organs as kidney, liver, and brain have been used post-mortemly for the analysis of the presence or absence of activator proteins. The detection of activator proteins in human urine will facilitate the diagnosis of glycosphingolipid storage diseases due to the deficiency of activator proteins. We report here the presence of activator

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proteins for the enzymic hydrolysis of G_{M1} ganglioside (G_{M1}-activator) and G_{M2} ganglioside (G_{M2}-activator) in normal human urine.

EXPERIMENTAL PROCEDURE

Materials

β-Galactosidase and β-hexosaminidases A and B were isolated from human liver as described [5, 8]. The method for preparing tritium labeled G_{M1} and G_{M2} gangliosides has also been described [14]. Arylsulfatase A was a gift from Dr. H. Kihara, Lanterman State Hospital, Pomona, California.

Methods

Hydrolysis of G_{M1} ganglioside by human hepatic β-galactosidase in the presence of G_{M1}-activator, and of G_{M2} ganglioside by β-hexosaminidase A in the presence of G_{M2}-activator, was assayed according to reported procedures [5, 8]. Exoglycosidases were assayed using *p*-nitrophenylglycosides as described [15].

Collection and Concentration of Normal Human Urine

Twenty-four-hr urine samples from a normal adult male were collected daily, centrifuged at 10,000 *g* for 15 min, and filtered through Whatman no. 1 filter paper to remove insoluble material. They were then concentrated about 15 times by an Amicon Model DC2 dialyzer/concentrator using an H1P10 hollow fiber cartridge. The concentrates thus obtained were stored in a freezer (−25°C) until used.

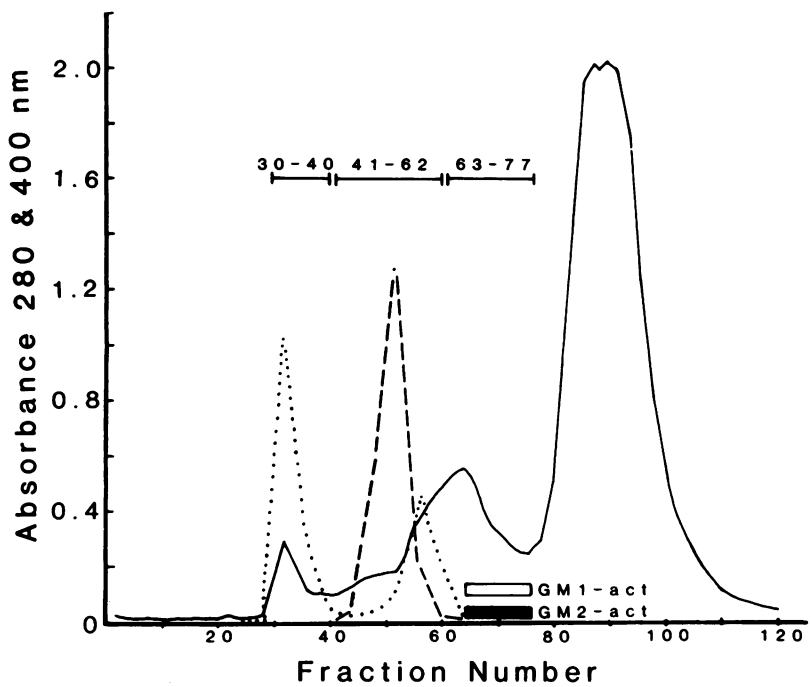

FIG. 1.—Sephadex G-200 filtration of crude urine protein preparation. Fractions indicated by the *horizontal bars* that were found to contain G_{M1}- and G_{M2}-activators were pooled and further purified. (——), E_{280}; (· · ·), E_{400} for β-galactosidase activity; (- - -), E_{400} for β-hexosaminidase activity. For detailed conditions, see EXPERIMENTAL PROCEDURE.

Isolation of G_{M1}- and G_{M2}-Activators from the Concentrated Urine

Unless otherwise indicated, the isolation of activator proteins was carried out at temperatures between 0°–5°C. Frozen concentrates from 30 liters of urine were thawed, pooled, and brought to 30% saturation (176 g/l) with solid $(NH_4)_2SO_4$. After standing for 2 hrs, the precipitated proteins were removed by centrifugation and discarded. The supernatant was then brought to 80% saturation by adding 356 g/l of solid $(NH_4)_2SO_4$. The precipitated proteins were collected the next day and dissolved in 75 ml of 0.05 M sodium phosphate buffer, pH 7.0. A 25-ml aliquot of this preparation was applied to a Sephadex G-200 column (5 × 80 cm) that had been equilibrated with the same buffer. The column was eluted with the same buffer at a flow rate of 40 ml/hr, and 20-ml fractions were collected. The elution profile showing the positions of activator proteins, β-galactosidase, and β-hexosaminidase is illustrated in figure 1. The fractions containing activators were pooled and precipitated by adding solid $(NH_4)_2SO_4$ to 80% saturation. The resulting precipitate was collected and dissolved in a minimum amount of 0.05 M sodium phosphate buffer, pH 7.0. The activator preparations obtained from three Sephadex G-200 columns were pooled, dialyzed against 0.05 M sodium phosphate buffer, pH 7.0, and applied to a DEAE-Sephadex A-50 column (1.6 × 30 cm) that had been equilibrated with the same buffer. After washing the column with the same buffer to remove the unadsorbed proteins, the column was then eluted with 0.05 M sodium citrate buffer, pH 6.0, at a flow rate of 20 ml/hr, and 4-ml fractions were collected. Figure 2 shows the profile of G_{M1}- and G_{M2}-activators eluted from this column. Fractions containing only G_{M1}-activator and G_{M2}-activator were separately pooled, lyophilized, dialyzed against water, and re-lyophilized.

Detection of Activators in a Small Volume of Urine

For the detection of G_{M1}- and G_{M2}-activators in a small volume of urine, a 50-ml aliquot of a random urine specimen, after being centrifuged at 10,000 *g* for 15 min and filtered through Whatman no. 1 filter paper, was dialyzed exhaustively against H_2O, lyophilized,

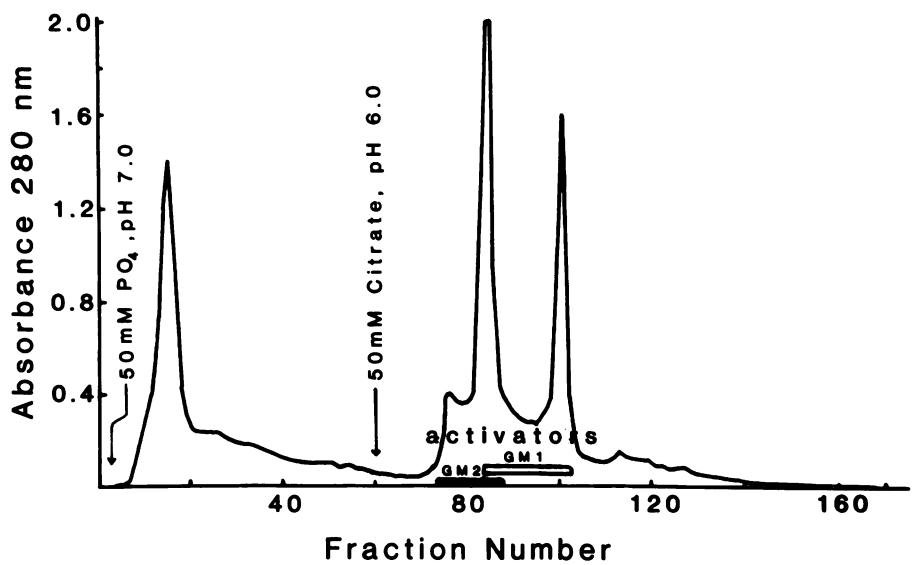

FIG. 2.—Fractionation of crude activator preparations from Sephadex G-200 filtration by DEAE Sephadex A-50 chromatography. Fractions containing only G_{M1}-activator and those containing only G_{M2}-activator were pooled as indicated by the *horizontal bars*. Detailed conditions are described in the EXPERIMENTAL PROCEDURE.

and dissolved in 1 ml H_2O to obtain the crude activator preparation. To check for the stimulation of the in vitro hydrolysis of G_{M1} and G_{M2} gangliosides, the crude activator preparation was further purified by an octyl-Sepharose column as described [16]. By this procedure, about 200–400 μg of protein as determined by Lowry et al. [17] was obtained from 50 ml of urine. One hundred μg of this material was used to assay the stimulatory activity as shown in figure 3.

RESULTS AND DISCUSSION

Using Sephadex G-200 filtration, G_{M1}- and G_{M2}-activators in normal human urine were eluted together but well separated from exo-glycosidases as shown in figure 1. This result is similar to the elution profile for these two activators from human liver [8]. The two activators were subsequently separated from each other by DEAE-Sephadex A-50 chromatography (figure 2). Figure 3 shows the enzymic hydrolysis of G_{M1} and G_{M2} ganglioside in the presence of a crude activator preparation obtained from 50 ml of a random urine specimen. These results were similar to those obtained with the partially purified activators isolated from 30 liters of urine. G_{M1}- and G_{M2}-activators were also found in all 20 specimens of 50 ml normal urine, which is the minimum volume of urine needed to detect the presence of the activator proteins. However, there was considerable variation in the level of activators among different urine specimens. The crude activator preparations from 50 ml normal urine samples were also found to stimulate the hydrolysis of galactosylceramide sulfate catalyzed by arylsulfatase A (results not shown). This suggests that urine may contain activators other than G_{M1}- and G_{M2}-activators.

The discovery of the presence of the activator proteins in normal human urine may have potential diagnostic significance, since it could lead to the development of a method for the detection of lipid storage diseases due to activator deficiencies in the living patient. As one of the variants of type-AB G_{M2} gangliosidosis has been shown to be due to the deficiency of G_{M2}-activator [9–11], an assay for the presence or absence of this activator is required for the definitive diagnosis of this disease. The analysis of G_{M1}-activator in pathological urine specimens may

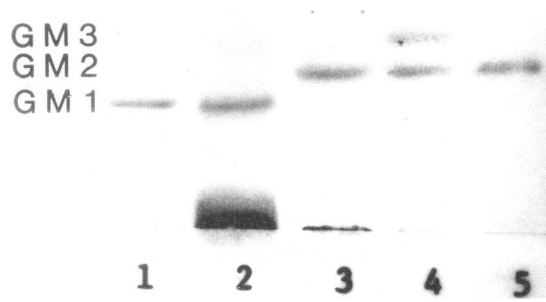

FIG. 3.—Thin-layer chromatogram showing the stimulation of the hydrolysis of G_{M1} and G_{M2} gangliosides by an activator preparation partially purified from 50 ml of urine by an octyl-Sepharose column [16]. One hundred μg each of the nonadsorbed proteins and the adsorbed proteins eluted by octyl-β-glucoside were tested for their stimulatory activities. *1*, G_{M1} + β-galactosidase; *2*, 1 + octyl-Sepharose nonadsorbed fraction; *3*, 1 + octyl-glucoside eluate; *4*, G_{M2} + β-hexosaminidase A + octyl-glucoside eluate; *5*, G_{M2} + β-hexosaminidase A.

reveal a new variant of G_{M1}-gangliosidosis caused by the deficiency of activator rather than that of β-galactosidase. If the presence of other activator proteins can be demonstrated in urine, this finding can also be extended to detect the possible occurrence of other glycosphingolipid storage diseases whose causes reside in the deficiencies of their respective activators. The activators found in urine, however, may represent the modified products of native activator proteins that occur in other organs. So far, the analysis of activator proteins related to the catabolism of glycosphingolipids have been made only on post-mortem tissues. The finding of activator proteins in normal urine should facilitate the routine analysis of the activator in type-AB G_{M2}-gangliosidosis patients and also in other sphingolipidoses due to their respective activator deficiencies. Above all, the advantage of using urine for this purpose is that the analysis can be made in the living patient.

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