Isolation and characterization of an activator protein for the hydrolysis of ganglioside G_{M2} from the roe of striped mullet (*Mugil cephalus*)

Rita DeGASPERI, Yu-Teh LI and Su-Chen LI*

Department of Biochemistry, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, U.S.A.

After the revelation of the presence of ganglioside G_{M2} as the major ganglioside in the roe of striped mullet, *Mugil cephalus* [Li, Hirabayashi, DeGasper, Yu, Ariga, Koerner & Li (1984) J. Biol. Chem. **259**, 8980–8985], we have continued to investigate the catabolism of G_{M2} in this tissue. We have found that mullet roe contains a specific activator protein which stimulates the hydrolysis of G_{M2} carried out by the β -hexosaminidase isolated from the same tissue. This activator has been purified by using conventional procedures including ammonium sulphate fractionation and chromatography on Sepharose 6B, DEAE-Sephadex A-50, octyl-Sepharose and Matrex Gel Blue A columns. This activator protein is also able to stimulate the hydrolysis of G_{M2} carried out by human β -hexosaminidase A. Unlike human G_{M2}-activator, the roe activator protein does not stimulate the hydrolysis of GgOse₃Cer or GbOse₄Cer. The molecular mass (18 kDa) of the roe activator protein was found to be similar to that of human G_{M2}-activator; however, the pI (pH 4.1) was found to be lower than that of human G_{M2}-activator. This is the first report on the presence of a G_{M2}-activator protein in a source other than mammalian tissues.

INTRODUCTION

It is now well established that, in human tissues, the hydrolysis of ganglioside G_{M2} catalysed by β -hexosaminidase A requires the presence of a specific activator protein, G_{M2}-activator (Li & Li, 1984; Conzelmann & Sandhoff, 1986). It is not known, however, whether the involvement of G_{M2}-activator in the enzymic hydrolysis of G_{M2} is universal, since the catabolism of G_{M2} has not been studied in detail in other animal species. Crude boiled extracts from the kidneys of rat, mouse, cattle and pig have been found to stimulate the cleavage of G_{M2} by the autologous β -hexosaminidase (Burg *et al.*, 1983), suggesting that G_{M2}-activators must be present in the kidneys of the above mammals. However, no further isolation or characterization of any of these proteins has been attempted. More recently, the partial purification of a G_{M2}-activator from guinea pig kidney has also been reported (Kasakura *et al.*, 1985). Thus, the activator protein for the hydrolysis of G_{M2} has not been studied in detail from any source other than human tissues (Li & Li, 1984; Conzelmann & Sandhoff, 1986).

We have previously reported that the roe of striped mullet (*Mugil cephalus*) contains G_{M2} as the major ganglioside (Li, Y.-T. *et al.*, 1984). Because of this finding, we extended our studies to investigate the catabolism of G_{M2} in this tissue. Mullet roe β -hexosaminidase was initially found to cleave G_{M2} in the absence of any stimulating factors in buffers of ionic strength lower than 0.02 mol/l. However, the rate of this hydrolysis progressively decreased to zero as the ionic strength was raised to 0.1 mol/l (DeGasper *et al.*, 1988). Later, it was

observed that the activity towards G_{M2} under high ionic strength conditions (> 0.1 mol/l) could be restored by the addition of a low-molecular-mass fraction which was separated from β -hexosaminidase during the gel filtration step. Subsequently, we have isolated, in a highly purified form, a specific activator protein capable of stimulating the mullet roe β -hexosaminidase to cleave G_{M2} under high ionic strength conditions (> 0.1 mol/l).

EXPERIMENTAL

Materials

The roe of *Mugil cephalus*, collected locally during the spawning season, was immediately lyophilized and stored at –70 °C. Ganglioside G_{M2} was isolated from Tay–Sachs brain (Svennerholm, 1972) and mullet roe (Li, Y.-T. *et al.*, 1984). [³H]G_{M2}, labelled at the terminal GalNAc, was prepared as described (Uda *et al.*, 1977). GgOse₃Cer (asialo-G_{M2}) was prepared from G_{M2} by hydrolysis with formic acid (Svennerholm *et al.*, 1973) and G_{M2}-oligosaccharide was derived from G_{M2} by ozonolysis (Esselman *et al.*, 1972). GgOse₄Cer was isolated from human red blood cells (Esselman *et al.*, 1972). G_{M2} which was modified in the carboxyl group of the sialic acid moiety by esterification with methyl group or by reduction to primary alcohol (Handa & Nakamura, 1984) was generously donated by Dr. S. Handa, Tokyo Medical and Dental University, Tokyo, Japan. The following materials were obtained from commercial sources: Sepharose 6B, DEAE-Sephadex A-50, Concanavalin–Sepharose, octyl-Sepharose and Agarose IEF from Pharmacia; Matrex Gel Blue A from Amicon

Abbreviations used: PAGE, polyacrylamide-gel electrophoresis; G_{M2}, II³NeuAc-GgOse₃Cer-; G_{M1}, II³NeuAc-GgOse₄Cer; G_{M3}, II³NeuAc-LacCer; ConA, concanavalin A.

* To whom correspondence should be addressed.

Corp., Lexington, MA, U.S.A.; octylglucoside, *p*-nitrophenyl- β -GlcNAc and methyl- α -mannoside from Sigma; Polygosil 60-2540 C18 from Macherey-Nagel, Düren, Germany; SDS/polyacrylamide gradient gels (3–27%) from Separation Science, Attleboro, MA, U.S.A.; and Aquasol from NEN Research Products.

Analytical methods

The protein concentration was determined using the method of Lowry *et al.* (1951) or the fluorescamine reaction (Castell *et al.*, 1979) using bovine serum albumin as a standard. The isoelectric point was determined by isoelectrofocusing on a flat bed agarose gel using the Ampholite of pH 3–6 according to the instructions from Pharmacia. The purity and the molecular mass of mullet roe G_{M2} -activator was determined by SDS/polyacrylamide-gel electrophoresis (SDS/PAGE) on 3–27% gradient gels (Laemmli, 1970) and the gel was stained with Coomassie Blue.

Isolation of β -hexosaminidase from mullet roe

Mullet roe β -hexosaminidase was isolated by ammonium sulphate precipitation, and then chromatography, successively with Sepharose 6B, DEAE-Sephadex A-50 and ConA-Sephadex and octyl-Sepharose as previously described (DeGasperi *et al.*, 1988). For convenience, the activity was assayed using *p*-nitrophenyl- β -GlcNAc (Li & Li, 1972). One unit of β -hexosaminidase was defined as the amount of enzyme which released 1 μ mol of *p*-nitrophenol per min under the standard assay conditions. Using DEAE-Sephadex chromatography at pH 7.0, three isoenzymes were separated (DeGasperi *et al.*, 1988): β -hexosaminidase I, which was not retained by the column, and β -hexosaminidase II and III which were both retained by the column. Since β -hexosaminidase II was the most abundant form, the routine assay of G_{M2} -activator activity and most of the studies described in this paper were carried out using β -hexosaminidase II.

Assay of G_{M2} -activator activity

The assay mixture contained the following components in 0.2 ml of 50 mM-sodium citrate buffer, pH 4.0: [^{3} H] G_{M2} , 10 nmol (10^4 c.p.m.); mullet roe β -hexosaminidase, 0.5 units and an appropriate amount of activator protein. The mixture was incubated at 37 °C for a preset time. The reaction was terminated by heating the mixture at 100 °C for 3 min followed by the addition of 20 μ l of 1 M-KCl and 100 μ l of a slurry of C_{18} -beads in 0.1 M-KCl (Hirabayashi *et al.*, 1985). The mixture was centrifuged at 7000 *g* for 5 min and the amount of the released [^{3} H]GalNAc in the supernatant was determined by counting an aliquot of the supernatant in a liquid scintillation counter. One unit of the activator protein was defined as the amount which stimulated the hydrolysis of 1 pmol of G_{M2} /unit of β -hexosaminidase II per h under the standard assay conditions, and the specific activity was expressed as units of activator per mg of protein.

When unlabelled G_{M2} , the modified G_{M2} , GbOse₄Cer and GgOse₃Cer were used as substrates, the incubation was performed and treated as described above, except that the glycolipids were extracted from the C_{18} beads with 0.5 ml of methanol followed by 0.5 ml of chloroform/methanol (2:1, v/v). The extracts were combined, dried and analysed by t.l.c. using

chloroform/methanol/water (60:35:8, by vol.) as the developing solvent. Gangliosides and neutral glycosphingolipids were revealed with the resorcinol spray (Svennerholm, 1957) and diphenylamine spray (Harris & McWilliam, 1954) respectively. The extent of the hydrolysis was also quantified by scanning the plate with a Shimadzu CS 930 nm dual-wavelength scanning densitometer at 580 nm (resorcinol spray) or 635 nm (diphenylamine spray). The assay on G_{M2} oligosaccharide was performed as described above except that, after incubation, the mixture was dried and directly analysed by t.l.c., using butanol/acetic acid/water (1:1:1, by vol.) as the developing solvent, and the sialo-oligosaccharides were revealed by the resorcinol spray (Svennerholm, 1957).

Isolation of G_{M2} -activator from mullet roe

All operations were carried out at a temperature of 0–4 °C unless otherwise specified. Centrifugations were routinely performed in a Sorvall RC5C refrigerated centrifuge at 20000–30000 *g* for 30 min. The lyophilized roe (800 g) was first crushed in a mortar and then homogenized with 5 vol. of cold distilled water in a Waring Blendor, and centrifuged. The supernatant was carefully collected to avoid a floating layer of lipid material. The pH of the supernatant was adjusted to 4.4 with a saturated citric acid solution, and the precipitate was removed by centrifugation. The supernatant was then brought to 30% saturation by the addition of solid ammonium sulphate and allowed to stand for 3 h. The precipitated proteins were removed by centrifugation

Fig. 1. Sepharose 6B chromatography of mullet roe G_{M2} -activator

Mullet roe G_{M2} -activator fraction obtained from an ammonium sulphate fractionation step (30 ml) was applied to a Sepharose 6B column (5 cm $\times$ 85 cm) as described in the Experimental section. The activities of β -galactosidase (—), β -hexosaminidase (●), and α -fucosidase (○) were determined by using the appropriate *p*-nitrophenylglycoside. The activator activity was determined from a 1.5 ml aliquot of every third fraction as described in the text. The solid bar represents the fraction with the activator activity and the solid line (no symbols) shows the absorbance at 280 nm.

Fig. 2. DEAE-Sephadex A-50 fractionation of G_{M2} -activator fraction obtained from Sepharose 6B chromatography (a) and the thin layer chromatogram showing the presence of activator activity (b)

(a) The G_{M2} -activator fractions obtained from eight Sepharose 6B columns were divided into two portions and individually applied to a DEAE-Sephadex A-50 column (5 cm $\times$ 40 cm) and eluted as described in the Experimental section. The effluents were divided into 13 fractions as indicated. An aliquot of each fraction (60 μ g of protein) was assayed for the stimulatory activity toward G_{M2} hydrolysis. ●, Absorption at 280 nm; ○, absorption at 400 nm for β -hexosaminidase activity. (b) The hydrolysis of G_{M2} by mullet roe β -hexosaminidase: lane C, the control tube that contained G_{M2} and β -hexosaminidase; lanes 1–13 represent the incubation of G_{M2} , β -hexosaminidase and each of the 13 fractions from the DEAE-Sephadex A-50 column; lane St., standard G_{M3} .

and the supernatant brought to 80% saturation with solid ammonium sulphate. After standing overnight, the precipitate was collected by centrifugation and dissolved in 240 ml of sodium citrate-phosphate buffer, pH 6.0 (14.6 mM-sodium citrate/50 mM-sodium phosphate). Aliquots (30 ml) of this fraction were individually applied to a Sepharose 6B column (5 cm $\times$ 85 cm) which had been equilibrated with the same buffer, pH 6.0. The column was eluted with the same buffer at a flow rate of 50 ml/h and 17 ml fractions were collected. The elution profile of G_{M2} -activator activity was determined as follows. Aliquots (1.5 ml) from every third fraction were dialysed against water, lyophilized and redissolved in 0.5 ml of distilled water. Samples (70 μ l) of each of the above fractions were assayed for the stimulation of G_{M2} hydrolysis. The G_{M2} -activator was found to elute after β -hexosaminidase as indicated by the solid bar (Acts) as shown in Fig. 1. The fractions containing the stimulatory activity were pooled and the protein precipitated by the addition of solid ammonium sulphate to 80% saturation.

DEAE-Sephadex A-50 chromatography

The activator fractions obtained from eight Sepharose 6B columns were pooled, dissolved in 200 ml of 50 mM-

sodium phosphate buffer, pH 7.0, and dialysed exhaustively against the same buffer. This fraction was divided into two aliquots and individually applied to a DEAE-Sephadex A-50 column (5 cm $\times$ 40 cm) which had been equilibrated with 50 mM-sodium phosphate buffer, pH 7.0. After washing with the same buffer to remove the unadsorbed proteins, the column was eluted with 50 mM-sodium citrate buffer, pH 6.0, followed by the same buffer containing 0.5 M-NaCl. Based on the absorbance at 280 nm, the effluents were divided into 13 pooled fractions as indicated in Fig. 2(a). The proteins in these fractions were precipitated by the addition of ammonium sulphate to 80% saturation and the precipitate was dissolved in 20 ml water and dialysed exhaustively against distilled water. An aliquot of each pool fraction containing about 60 μ g of protein was assayed for the stimulatory activity towards G_{M2} hydrolysis. The strongest stimulatory activity was found in the fractions 11 and 12 as shown in Fig. 2(b).

Octyl-Sepharose chromatography

The G_{M2} -activator fractions from the two DEAE-Sephadex A-50 columns (pooled fractions 11 and 12 as shown in Fig. 2) were combined and applied to an

octyl-Sepharose column (1.5 cm × 12 cm) which had been equilibrated with 10 mM-sodium acetate buffer, pH 4.6, at room temperature. The column was washed with the same buffer until no absorbance was detected at 280 nm, and subsequently eluted with 1% octylglucoside dissolved in the same buffer. The fractions containing G_{M2} -activator as shown in Fig. 3 (dashed line) were pooled and lyophilized.

Matrex Gel Blue A chromatography

The G_{M2} -activator fraction from the octyl-Sepharose column (10 mg of protein) was dialysed against 10 mM-sodium acetate buffer, pH 4.6, and applied to a Matrex Gel Blue A column (0.8 cm × 20 cm) which had been equilibrated with the same buffer. After washing the unadsorbed material with this buffer, the column was eluted with a linear gradient generated by 75 ml of 10 mM-sodium acetate buffer, pH 4.6, in the mixing chamber and 25 mM-sodium phosphate buffer, pH 7.0, containing

Fig. 3. Octyl-Sepharose chromatography of mullet roe G_{M2} -activator

The G_{M2} -activator fraction obtained from DEAE-Sephadex A-50 chromatography was applied to an octyl-Sepharose column (1.5 cm × 12 cm) as described in the Experimental section. The G_{M2} -activator was eluted from the column by using 1% octylglucoside in 10 mM-sodium acetate buffer, pH 4.6. The solid line shows the absorbance at 280 nm; the broken line indicates the fractions with the G_{M2} -activator activity.

0.1 M-NaCl, in the reservoir. The elution profile is shown in Fig. 4. The fractions containing G_{M2} -activator were pooled, lyophilized and dialysed against 10 mM-sodium acetate buffer, pH 4.6, and stored at -20°C in the vials which had been coated with silicon and dried. The siliconization prevented the adsorption of the G_{M2} -activator on the glass surface.

A typical purification of G_{M2} -activator from 800 g of dried roe is summarized in Table 1. This table does not list the activator activity in the initial steps, since the activity could be determined only after the Sepharose 6B chromatography step as discussed below.

RESULTS

General properties of mullet roe G_{M2} -activator

The mullet roe β -hexosaminidase in crude extracts was not able to cleave G_{M2} under any given condition. Only

Fig. 4. Matrex Gel Blue A chromatography of mullet roe G_{M2} -activator

The G_{M2} -activator preparation (10 mg) from the octyl-Sepharose column was dialysed against 10 mM-sodium acetate buffer, pH 4.6, and applied to a Matrex Gel Blue A column (0.8 cm × 20 cm) as described in the Experimental section. The G_{M2} -activator was eluted by a pH gradient (upper panel). Solid line, absorbance at 280 nm; broken line, the fractions containing G_{M2} -activator.

Table 1. Purifications and recovery of G_{M2} -activator from 800 g of dried roe

The G_{M2} -activator was purified from the dried mullet roe as described in the Experimental section.

Step	Protein (mg)	$10^{-7} \times$ Total units	Specific activity (units/mg)	Purification (-fold)	Yield (%)
Sepharose 6B	6976	3.6	5.1×10^3	1	100
DEAE-Sephadex	208	4.9	2.3×10^5	45	136
Octyl-Sepharose	23.1	2.8	1.2×10^6	235	77
Matrex Blue Gel	0.16	0.33	2.0×10^7	3921	9

after β -hexosaminidase was partially purified could this enzyme cleave G_{M2} under low ionic strength conditions (< 0.02 mol/l) (DeGasperi *et al.*, 1988). However, when the ionic strength of the buffer was raised to 0.1, this enzyme was not able to cleave G_{M2}. We subsequently found that the addition of a low-molecular-mass fraction, which was separated from β -hexosaminidase by Sepharose 6B column chromatography (see Fig. 1), restored the activity of β -hexosaminidase towards the hydrolysis of G_{M2}. This result suggested the existence of an activator protein in mullet roe. This stimulatory activity could be detected only after the roe extract was fractionated by a Sepharose 6B column, and only by using β -hexosaminidase purified through the ConA-Sepharose step (DeGasperi *et al.*, 1988). It appeared that the crude extract contained some inhibitory materials.

In contrast with human G_{M2}-activator (Li *et al.*, 1979), the mullet roe G_{M2}-activator was tightly bound to DEAE-Sephadex A-50 gel equilibrated with 50 mM-phosphate buffer, pH 7.0. This indicated that mullet roe G_{M2}-activator is more acidic than human G_{M2}-activator. Using flat bed agarose gel electrofocusing, the pI of mullet roe G_{M2}-activator was determined to be about pH 4.1. The hydrophobic nature of mullet roe G_{M2}-activator was indicated by the fact that it was retained by an octyl-Sepharose column.

On SDS/PAGE, using 3–27% gradient gel (Laemmli, 1970), the mullet roe G_{M2}-activator (purified 3900-fold) showed a closely-spaced doublet with molecular mass of about 18 kDa (Fig. 5, lane 3) and a trace of 67 kDa contaminant which was too faint to be picked up by photography. A similar observation in human kidney G_{M2}-activator has been attributed to the heterogeneity of the activator protein (Neuenhofer & Sandhoff, 1985). Due to the low yield and instability of the highly-purified mullet roe G_{M2}-activator, no further purification or separation of the doublet was attempted. The purified G_{M2}-activator lost its activity when heated at 80 °C for 3 min in 50 mM-sodium citrate buffer, pH 4.0. Its stimulatory activity was destroyed by Pronase, confirming the protein nature of mullet roe G_{M2}-activator.

Specificity of mullet roe G_{M2}-activator towards mullet roe β -hexosaminidase isoenzymes and towards human β -hexosaminidase A

Mullet roe was found to contain three β -hexosaminidase isoenzymes. β -Hexosaminidase I was not retained by the DEAE-Sephadex A-50 column at pH 7.0 whereas β -hexosaminidases II and III were both retained by the DEAE-Sephadex A-50 column (DeGasperi *et al.*, 1988). Mullet roe activator stimulated all three isoenzymes to cleave G_{M2}. Under identical conditions, β -hexosaminidase II cleaved G_{M2} at about one-half the rate of β -hexosaminidase I, and β -hexosaminidase III, the most acidic form, cleaved G_{M2} at about one-tenth rate of β -hexosaminidase I. This aspect of mullet roe β -hexosaminidase is quite different from the human enzymes, because only human β -hexosaminidase A, but not β -hexosaminidase B, can cleave G_{M2} in the presence of the activator protein (Li *et al.*, 1981).

Mullet roe G_{M2}-activator could also stimulate human β -hexosaminidase A to cleave G_{M2} at a rate close to the stimulation produced by human G_{M2}-activator. However, human G_{M2}-activator did not stimulate roe β -

Fig. 5. Analysis of mullet roe G_{M2}-activator by SDS/PAGE

The mullet roe G_{M2}-activator (lane 3, 5 μ g) was analysed by SDS/PAGE in a 3–27% gradient gel. Two human G_{M2}-activators from liver (lane 1, 10 μ g) and kidney (lane 2, 5 μ g) were included for comparison. Lane 4 shows the protein markers of various molecular masses.

Fig. 6. Cross-reactivity of mullet roe G_{M2}-activator with human β -hexosaminidase A

Mullet roe G_{M2} (10 nmol) was incubated with 0.5 units of β -hexosaminidase (either human kidney β -hexosaminidase A or mullet roe β -hexosaminidase II) in the presence of 10^3 units of G_{M2}-activator (either human kidney or mullet roe) at 37 °C for 3 h. The % hydrolysis of G_{M2} in each incubation was determined by quantitative t.l.c. as described in the Experimental section. The bars represent the ranges in three experiments.

hexosaminidase II as effectively as did the roe G_{M2}-activator protein (Fig. 6). A similar species specificity among the activator fractions and β -hexosaminidase from cattle, pig, rat, mouse and human has been described (Burg *et al.*, 1983). Immunologically, mullet roe G_{M2}-activator did not cross-react with the polyclonal antibodies raised against human G_{M2}-activator. It is known that human β -hexosaminidase A does not cleave G_{M2} in the absence of an activator protein. Therefore, the ability

of the roe activator protein to stimulate human β -hexosaminidase A in cleaving G_{M2} strongly indicates that the protein isolated from mullet roe is indeed a specific activator protein for the hydrolysis of G_{M2} .

Substrate specificity of mullet roe G_{M2} -activator

The highly-purified mullet roe G_{M2} -activator was very potent. Under the standard assay conditions, 1 μ g of the G_{M2} -activator obtained from the Matrex Gel Blue A step stimulated β -hexosaminidase I to hydrolyse 1.5 nmol of G_{M2} in 10 min. For convenience, G_{M2} isolated from Tay-Sachs brain was routinely used for the assays, even though G_{M2} isolated from mullet roe is the true natural substrate for mullet roe β -hexosaminidase. G_{M2} from mullet roe differs from that isolated from Tay-Sachs brain in the ceramide portion which is more polar and contains phytosphingosine and α -hydroxy monounsaturated fatty acids (Li, Y. T. *et al.*, 1984). Under identical conditions, Tay-Sachs G_{M2} was cleaved at a slightly faster rate than mullet roe G_{M2} by roe β -hexosaminidase II in the presence of roe activator protein. Therefore, it seems that changes in the nature of the ceramide portion can influence the interaction between G_{M2} , β -hexosaminidase, and activator protein.

When the ceramide was removed from G_{M2} , the resulted G_{M2} -oligosaccharide could be hydrolysed by roe β -hexosaminidase in the absence of the activator protein and regardless of the ionic strength of the buffer (DeGasperi *et al.*, 1988). The activator protein had no effect on the hydrolysis of this hydrophilic substrate.

G_{M2} derivatives in which the carboxyl group of sialic acid has been reduced to a primary alcohol (OH- G_{M2}) or esterified with a methyl group (Me- G_{M2}) (Handa & Nakamura, 1984) have been found to be resistant to human β -hexosaminidase A in the presence of human G_{M2} -activator; however, they were hydrolysed by the same enzyme in the presence of sodium taurodeoxycholate (Li, S. C. *et al.*, 1984). Likewise, both OH- G_{M2} and Me- G_{M2} were not cleaved by roe β -hexosaminidase in the presence of roe G_{M2} -activator. Unlike human β -hexosaminidase A, mullet roe β -hexosaminidase was not able to cleave the modified G_{M2} even in the presence of sodium taurodeoxycholate under the low-ionic-strength conditions.

In contrast with human β -hexosaminidase A, the hydrolysis of neutral glycolipids containing terminal β -GalNAc, namely GbOse₄Cer and GgOse₃Cer, by mullet roe β -hexosaminidase was not stimulated by the roe G_{M2} -activator. As reported previously (DeGasperi *et al.*, 1988), these substrates were not cleaved by roe β -hexosaminidase under any conditions. Therefore, these results may simply reflect the substrate specificity of the roe β -hexosaminidase.

Roe G_{M2} -activator did not stimulate the hydrolysis of G_{M1} by human hepatic β -galactosidase or by roe β -galactosidase, nor did it stimulate the hydrolysis of sulphatide by human hepatic arylsulphatase A. As expected, cleavage of the water-soluble synthetic substrates *p*-nitrophenyl- β -D-*N*-acetylglucosaminide and -galactosaminide was also not stimulated by the roe G_{M2} -activator.

As stated previously, human β -hexosaminidase is more sensitive than roe β -hexosaminidase I to the ionic strength of the incubation mixture. The effect of substrate (G_{M2}) concentration on the hydrolysis of G_{M2} in the presence of constant amounts of β -hexosaminidase (0.5 unit) and the

activator protein (1.5 units) was examined in 25 mM-citrate buffer, pH 4.0, ionic strength 0.1 mol/l. The K_m was determined from the double-reciprocal plot (Dixon & Webb, 1979) to be 100 μ M- G_{M2} for both human and roe enzymes. The V_{max} values for roe β -hexosaminidase I and human β -hexosaminidase were calculated to be 10 and 12.5 nmol/h per 0.25 unit of enzyme respectively. These results suggest that the roe activator protein is very similar to the human activator in spite of the different levels of purity.

DISCUSSION

This is the first report on the isolation and characterization of a G_{M2} -activator from a source other than mammalian tissues. The detection and subsequent isolation of this protein from mullet roe enabled us to extend the concept of activator protein for the enzymic hydrolysis of G_{M2} from human to a non-mammalian vertebrate species. Like human G_{M2} -activator (Li *et al.*, 1981), mullet roe G_{M2} -activator was also found to be an acidic hydrophobic protein of low molecular mass. Although both human tissues and mullet roe contain the specific G_{M2} -activators, our studies indicate that there are some basic differences between the proteins from the two species. The requirement of G_{M2} -activator for human β -hexosaminidase A to cleave G_{M2} is absolute, since no hydrolysis can take place in its absence (Li *et al.*, 1981). Mullet roe β -hexosaminidase, on the other hand, has the intrinsic ability to cleave G_{M2} , provided that the ionic strength of the buffer is lower than 0.02 mol/l (DeGasperi *et al.*, 1988). The roe activator enhances the reaction when the ionic strength is above 0.02 mol/l (DeGasperi *et al.*, 1988). Therefore it seems that the function of mullet roe G_{M2} -activator is to assist the interaction between the enzyme and the glycolipid substrate. This interaction may be greatly inhibited by the high ionic strength.

Human G_{M2} -activator stimulates β -hexosaminidase A, but not β -hexosaminidase B to cleave G_{M2} (Li *et al.*, 1981). In mullet roe, all three β -hexosaminidase isoenzymes were stimulated by the roe G_{M2} -activator to carry out the hydrolysis of G_{M2} . Moreover, the roe β -hexosaminidase I cleaved G_{M2} much faster than β -hexosaminidases II and III. Since β -hexosaminidase B from other mammals has also been reported to hydrolyse G_{M2} in the presence of G_{M2} -activator (Burg *et al.*, 1983; Ishikawa *et al.*, 1987) the distinct substrate specificity of human β -hexosaminidase A and B may not apply to other animal species.

In spite of the differences discussed above, it can be concluded that humans and mullet, though phylogenetically distant, share the same activator-dependent catabolic pathway for G_{M2} .

We thank Dr. P. Siegel of Tulane University for his careful reading of this manuscript and for valuable discussion. This work was supported in part by grant DMB-8617033 from the National Science Foundation and grant NS-09626 from the National Institutes of Health.

REFERENCES

- Burg, J., Banerjee, A., Conzelmann, E. & Sandhoff, K. (1983) Hoppe Seyler's Z. Physiol. Chem. **364**, 821-829

- Castell, J., Cervera, M. & Marco, R. (1979) *Anal. Biochem.* **99**, 379–391
- Conzelmann, E. & Sandhoff, K. (1986) *Methods Biochem. Anal.* **32**, 1–23
- DeGasperi, R., Li, S.-C. & Li, Y.-T. (1988) *J. Biol. Chem.* **263**, 1325–1328
- Dixon, M. & Webb, E. C. (1979) in *Enzymes*, pp. 60–68, Academic Press, New York
- Esselman, W. J., Laine, R. A. & Sweeley, C. C. (1972) *Methods Enzymol.* **28**, 140–156
- Handa, S. & Nakamura, K. (1984) *J. Biochem. (Tokyo)* **95**, 1323–1329
- Harris, G. & McWilliam, I. C. (1954) *Chem. Ind. (London)* p. 249
- Hirabayashi, Y., Li, Y.-T. & Li, S.-C. (1985) *J. Neurochem.* **40**, 168–175
- Ishikawa, Y., Li, S.-C., Wood, P. A. & Li, Y.-T. (1987) *J. Neurochem.* **48**, 860–864
- Kasakura, H., Hirabayashi, Y. & Matsumoto, M. (1985) in *Glycoconjugates: Proceedings of the VIIIth International Symposium on Glycoconjugates*, Houston, Texas (Davidson, E. A., Williams, J. C. & DiFerrante, N. eds.), vol. 2, p. 600, Praeger Publishers, New York
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Li, S.-C., Nakamura, T., Ogamo, A. & Li, Y.-T. (1979) *J. Biol. Chem.* **254**, 10592–10595
- Li, S.-C., Hirabayashi, Y. & Li, Y.-T. (1981) *J. Biol. Chem.* **256**, 6234–6240
- Li, S.-C., Serizawa, S., Li, Y.-T., Nakamura, K. & Handa, S. (1984) *J. Biol. Chem.* **259**, 5409–5410
- Li, Y.-T. & Li, S.-C. (1972) *Methods Enzymol.* **28**, 702–713
- Li, Y.-T. & Li, S.-C. (1984) in *Molecular Basis of Lysosome Storage Disorders* (Barranger, J. A. & Brady, R. O., eds.), pp. 79–91, Academic Press, New York
- Li, Y.-T., Hirabayashi, Y., DeGasperi, R., Yu, R. K., Ariga, T., Koerner, T. A. W. & Li, S.-C. (1984) *J. Biol. Chem.* **259**, 8980–8985
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, J. R. (1951) *J. Biol. Chem.* **193**, 265–275
- Neuenhofer, S. & Sandhoff, K. (1985) *FEBS Lett.* **185**, 112–114
- Svennerholm, L. (1957) *Biochim. Biophys. Acta* **24**, 604–611
- Svennerholm, L. (1972) *Methods Carbohydr. Chem. Biochem.* **6**, 464–474
- Svennerholm, L., Mansson, J. E. & Li, Y.-T. (1973) *J. Biol. Chem.* **248**, 740–742
- Uda, Y., Li, S.-C., Li, Y.-T. & McKibbin, J. M. (1977) *J. Biol. Chem.* **252**, 5194–5200

Received 11 November 1988/30 January 1989; accepted 10 February 1989