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PROPERTIES OF A PROTEIN ACTIVATOR OF GLYCOSPHINGOLIPID HYDROLYSIS ISOLATED FROM THE LIVER OF A PATIENT WITH GM1 GANGLIOSIDOSIS, TYPE 1

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SUMMARY: The heat stable protein activator of GM1 ganglioside hydrolysis was isolated from the liver of a patient with GM1 gangliosidosis, Type 1. It was found to be present at a level about 35 times that found in a liver sample from an age matched control. This activator protein was demonstrated to stimulate the hydrolysis of GM1 ganglioside and GA1 (asialo-GM1 ganglioside) in the presence of purified GM1 ganglioside β-galactosidase without the need for bile salt detergents. It could not stimulate the hydrolysis of two other galactosphingolipids, galactosylceramide and lactosylceramide, in the presence of the same enzyme. Lactosylceramide was a good substrate for this enzyme when sodium glycodeoxycholate was included in the assay. This activator protein had two isoelectric points pH 4.1 and 4.6, and it had an apparent molecular weight of 27,000 by gel filtration.

INTRODUCTION: In 1974 Li and coworkers (1) isolated a protein from control human liver which stimulated the hydrolysis of GM1 ganglioside, trihexosylceramide and GM2 ganglioside in the presence of partially purified human liver β-galactosidase, α-galactosidase and β-hexosaminidase A, respectively. This heat stable protein could activate the hydrolysis of these sphingolipid substrates without the need for detergents such as bile salts. Further studies by Li and Li (2) determined that the molecular weight was 21,000 and that the isoelectric point was 4.1. It was then demonstrated that control human liver contains two immunologically distinct activators, one for GM1 ganglioside degradation and one for GM2 ganglioside degradation (3). In addition to these activators, other heat stable proteins have been isolated from spleen, liver and brain from humans which activate cerebroside sulfate sulfatase (4), glucosylceramide β-glucosidase (5), galactosylceramide β-galactosidase (6) and possibly sphingomyelinase (7). The specificity of these activators has not been defined. In addition, the examination of tissues for activator proteins

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from patients with a deficiency of a specific sphingolipid hydrolase has not been carried out. To better define the specificity of the activator of GM1 ganglioside β-galactosidase (EC 3.2.1.23), and to determine its activity in patients with a genetic deficiency of GM1 ganglioside β-galactosidase activity we purified the heat stable protein from liver of a patient with GM1 gangliosidosis, Type 1.

MATERIALS AND METHODS: [^{3}H]galactose-labeled galactosylceramide and lactosylceramide were prepared in this laboratory as described previously (8). GM1 ganglioside was prepared from mixed bovine brain gangliosides (Sigma Chemical Co., St. Louis, MO) by the action of Clostridium perfringens neuraminidase (Sigma Chemical Co.). Following oxidation with galactose oxidase (Sigma Chemical Co.) the terminal galactosyl moiety was labeled using [^{3}H]$NaBH_4$ (New England Nuclear, Boston, MA) using the method of Suzuki and Suzuki (9). [^{3}H]GA1 was prepared from the labeled GM1 ganglioside by acid hydrolysis in 0.03N HCl for two hr at 80°C followed by silica gel column chromatography (9).

GM1 ganglioside β-galactosidase was purified from control human liver using the method of Miller et al. (10). It had a final specific activity of 4,000 units/mg protein using 4-methylumbelliferyl-β-D-galactopyranoside (4MU-β-gal) as substrate. A unit of enzyme activity is defined as nmoles 4MU-β-gal hydrolyzed per min at 37°C. This enzyme preparation also had high specific activity toward GM1 ganglioside, GA1 and lactosylceramide using the specific in vitro assay conditions using bile salt detergents (11). These assays involve the use of sodium taurodeoxycholate for GM1 ganglioside and GA1. Lactosylceramide hydrolysis was assayed either in the presence of pure sodium taurocholate plus oleic acid (12) or in the presence of sodium glycodeoxycholate (11). The free [^{3}H]galactose released was measured according to our published procedure (8).

To measure the enzymatic hydrolysis of the different beta-linked galactosyl terminal glycosphingolipids by human liver β-galactosidase in the presence of activator protein a modified method was used. Into a dry test tube were added 10 nmoles of [^{3}H]labeled substrate in chloroform-methanol. After evaporation of the solvent with nitrogen, the lipid was suspended in distilled water and sodium acetate buffer (0.05M final concentration containing 0.1M NaCl) by sonication in a 50°C water bath for 10 min. To this mixture were added 1 unit of β-galactosidase activity and 50 to 100 μg activator protein in a final incubation volume of 0.1 ml. After incubation for 20 min (GM1 ganglioside and GA1) or 60 min (galactosylceramide and lactosylceramide) the free [^{3}H]galactose released was measured. A unit of activator protein is defined as the amount which stimulates the hydrolysis of 1 nmole of substrate per min per unit of GM1 β-galactosidase activity.

Activator protein was purified from 200 g of liver from a metabolically normal 3 year old child and from 200 g of liver from a child who died at 2 years of age with typical GM1 gangliosidosis, Type 1. This patient had only 1-2% of normal GM1 ganglioside β-galactosidase activity in leukocytes, fibroblasts, liver and brain. The sialidase activity was normal, and the parents had half normal GM1 β-galactosidase activity in their leukocytes. The purification procedure of Li and Li (2) was used with only minor modifications. The molecular weight was estimated by gel filtration in phosphate buffer (0.05M, pH 7.0) using Sephadex G-75 (superfine) and the standard molecular weight calibration kit (Pharmacia, Piscataway, NJ). Isoelectric focusing was carried out in a 25 ml column using carrier ampholines of pH 3.5-5 (LKB, Stockholm, Sweden). In addition, SDS-polyacrylamide gel electrophoresis was carried out according to the procedure of Shapiro et al. (13) using the pro-

Table 1

Purification of GM1 β-galactosidase activator protein from liver samples* from a control and from a patient with GM1 gangliosidosis, Type 1

Steps	Total protein (mg)		Total activity (units)		Specific activity (units/mg protein)	
	Control	Patient	Control	Patient	Control	Patient
Heat and concentrate	795	1634	55.7	2260	0.07	1.38
DEAE-Sephadex**	113	302	25.9	1020	0.23	3.38
CM-Sephadex	4.07	48	9.0	410	2.21	8.54
Sephadex G-75	0.65	24	6.0	220	9.23	9.17

* Both purifications were started with 200 g liver samples. ** The most active fractions were pooled and concentrated for further purification.

tein molecular weight standards and gel concentrations recommended by Bethesda Research Laboratories, Inc. (Gaithersburg, MD).

RESULTS: The purification of the activator for GM1 β-galactosidase from control and GM1 gangliosidosis livers is summarized in Table 1. Following the nearly identical purification procedures in the two liver samples, we reached the same level of specific activity. However, the yield from the liver of the patient with GM1 gangliosidosis, Type 1 was about 35 times that from the control. Two other control livers gave values like the sample from the young control. Preliminary studies in livers from patients with Niemann-Pick disease, Gaucher disease, and GM1 gangliosidosis, Type 2 (juvenile form) indicate that the activator protein concentrations were higher than controls, but not nearly as high as in the liver from the patient with GM1 gangliosidosis, Type 1. The properties of the activator proteins isolated from the control and GM1 gangliosidosis, Type 1 livers were compared. The molecular weights as determined by gel filtration were similar. They were estimated to be 27,000 daltons. Using SDS-polyacrylamide gel electrophoresis the activator protein from the liver of the patient with GM1 gangliosidosis, Type 1 was estimated to be 10,000 daltons. This could indicate that it is a dimer or trimer in the native state. Insufficient sample from the control was available for a similar study. Isoelectric focusing of the activator protein

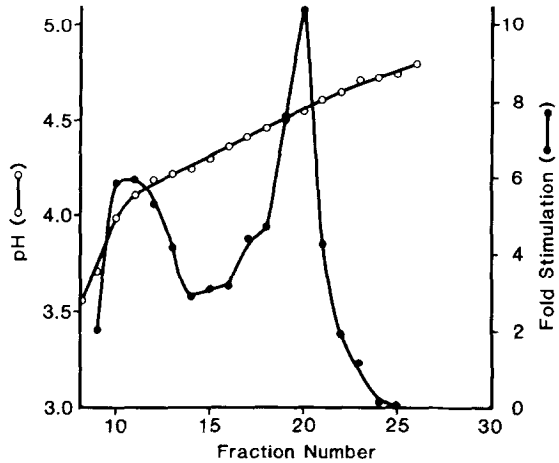

Figure 1: Isoelectric focusing of activator protein from the liver of a patient with GM1 gangliosidosis, Type 1. Active fractions from the Sephadex G-75 column were pooled and concentrated. All fractions (1.14 ml) were assayed using 0.08 ml and 2 units of purified β-galactosidase. Incubations with 10 nmoles of [^{3}H]GM1 ganglioside were carried out as described in the text.

from the liver of the patient with GM1 gangliosidosis, Type 1 revealed two peaks of activating ability (Fig. 1). They had pI's of 4.1 and 4.6 with the ratio of protein being approximately 2 to 1 and the ratio of specific activity being approximately 1 to 4, respectively. Activator protein from control human liver was reported to have a pI of 4.1 by Li and Li (2).

To determine the substrate specificity of the activator protein toward other galactosphingolipids in the presence of GM1 β-galactosidase, similar studies were carried out with GA1, lactosylceramide and galactosylceramide (Table 2). It is clear that this activator could activate also GA1 hydrolysis in the absence of bile salt detergents but not lactosylceramide or galactosylceramide. Using the identical enzyme preparation in the presence of sodium glycodeoxycholate, lactosylceramide could be hydrolyzed at a rate comparable to the hydrolysis of GM1 ganglioside and GA1.

DISCUSSION: Heat stable protein activators of GM1 β-galactosidase were isolated from human liver both from controls and from a patient with GM1 gangliosidosis, Type 1. When the activators are carried to the same stage of purity using similar techniques a 35-fold excess of activator protein was found in the liver from the patient with GM1 gangliosidosis, Type 1. These patients

Table 2

Activity of purified activator protein toward galactosphingolipids in the presence of purified GM1 β-galactosidase

Galactosphingolipid	Activator* (ug protein)	Enzyme (units)	Specific activity (units/mg protein)	Percent of bile salt assay
GM1 ganglioside	65	1	9.17	55%
GA1	81	1	3.54	29%
Lactosylceramide	81	1	not detectable	-
Galactosylceramide	81	1	not detectable	-

* This activator protein was purified from the liver of a patient with GM1 gangliosidosis, Type 1. Nearly identical results were obtained from the activator protein purified from liver from a control.

have a severe deficiency of GM1 ganglioside β-galactosidase activity. The excess activator protein may reflect either the excess activator produced in response to lysosomal storage of galactosyl terminal glycolipids or the activator remaining unused in the degradation of galactolipids because of the genetic mutation causing inactive catalytic protein. Liver samples from patients with other storage diseases such as Niemann-Pick disease, Gaucher disease, and GM1 gangliosidosis, Type 2 (juvenile form with death at 7 years of age) were carried through a similar purification procedure. The amount of activator was elevated only moderately (2-3 fold) above that found in normal livers.

The properties of the activator isolated from the liver of a patient with GM1 gangliosidosis was compared to the activator isolated from the liver of the control. The activator protein from the patient had two isoelectric points, one at pH 4.1 and the other at pH 4.6 , while the control was found to have only one major peak at pH 4.1 (2). Both had a molecular weight estimated by gel filtration to be 27,000 daltons and by SDS-PAGE to be 10,000 daltons.

To determine the specificity of the activator proteins we tested their ability to activate the hydrolysis of the major beta-linked galactosphingolipids in the presence of purified human liver GM1 β-galactosidase. As shown on Table 2 the activator protein could stimulate the hydrolysis of GM1 ganglio-

side and GA1 in the presence of catalytic protein. The activity could reach levels of 29-55% of that obtained with the optimum amount of bile salts. In addition, incubation of [^{3}H]galactose-labeled GM1 ganglioside and GA1 with activator protein followed by disc gel electrophoresis resulted in the binding of these sphingolipids to the activator (unpublished data). Galactosylceramide and lactosylceramide could not be hyrolyzed by the GM1 β-galactosidase in the presence of activator protein. This is in contrast to the reactions using bile salts where this enzyme preparation could hydrolyze lactosylceramide with a specific activity of 24,000 nmoles/mg protein/hr versus specific activities of 30,000 for GM1 ganglioside and 18,000 for GA1. [^{3}H]galactosylceramide and lactosylceramide cannot be demonstrated to bind this activator protein following incubation and disc gel electrophoresis. Another activator protein isolated from control human liver and Gaucher disease spleen will activate the hydrolysis of galactosylceramide and lactosylceramide using galactosylceramide β-galactosidase but not with GM1 ganglioside β-galactosidase (6).

This manuscript describes for the first time the isolation of the activator protein for GM1 β-galactosidase from the liver of a patient with GM1 gangliosidosis, Type 1. Despite the almost total deficiency of the activity for the catalytic protein there is a significantly higher level of the activator protein. These experiments demonstrate that in vitro assays carried out in the presence of relatively large amounts of detergents, such as bile salts may not give us a true picture of the substrate specificity of a given enzyme. Studies in tissues from patients with certain lipid storage diseases may be important if we are to understand the relationship between the biochemical findings and the clinical picture.

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