An activator protein which stimulates the degradation of globotriaosylceramide by human hepatic α-galactosidase (EC 3.2.1.22) was isolated from human liver and purified some 1300-fold. The purified activator was heat stable up to 95 °C, its molecular weight was estimated at 20,000 by gel filtration. Amphotolyte displacement chromatography resolved the activating factor into two fractions with isoelectric points at pH 4.6 and pH 4.8, respectively, with otherwise identical properties. The protein did not stimulate the hydrolysis of the water-soluble 4-methylumbelliferyl-α-galactoside.

The degradation of glycolipids is accomplished in the lysosome by the sequential action of exoglycosidases (1-5). The inherited deficiency of one of the enzymes involved in the degradative pathway of a glycolipid impairs normal degradation and leads to the accumulation of the respective enzyme’s lipid substrates in the lysosome (2-6). In Fabry’s disease, GbOse3Cer, a glycosphingolipid of the globo series, possessing an α-glycosidically bound galactose as nonreducing terminal galactose oxidase/NaB3H4 method according to Suzuki and Suzuki (21). Its specific radioactivity was 6 Ci/mol. Ganglioside GM2 was isolated from postmortem human brain (22) and 3H-labeled in the terminal galactose moiety by the sodium acetylated a-galactosidase of globotriaosylceramide by α-galactosidase.

**MATERIALS AND METHODS**

**Glycolipids**

Globotriaosylceramide was isolated from formalin-fixed Fabry liver (19, 20) and 3H-labeled in the terminal galactose moiety by the galactose oxidase/NaBH₄ method according to Suzuki and Suzuki (21). Its specific radioactivity was 6 Ci/mol. Ganglioside GM₂ was isolated from postmortem human brain (22) and 3H-labeled in the N-acetylgalactosamine moiety (21) to a specific radioactivity of 10 Ci/mol.

**Purification of α-Galactosidase**

α-Galactosidase was partially purified from normal human liver essentially following the method of Dean and Sweetly (23).

**Enzyme Assays**

**Degradation of 4-MU-α-galactoside**—A stock solution of 5 mM 4-MU-α-Gal in 50 mM citrate buffer, pH 4.5, was prepared. 100 µl of this solution were mixed with an aliquot of the enzyme solution to be tested and adjusted to a total volume of 200 µl with distilled water. The reaction was carried out at 37 °C and terminated after 30 min by the addition of 1 ml of a stop buffer (0.2 M glycine, 0.2 M sodium carbonate). Liberated 4-methylumbelliferone was measured fluorimetrically with a Locarte fluorimeter equipped with excitation filters (365 nm) and emission filters (440 nm). One enzyme unit is defined as the amount of enzyme that hydrolyzes 1 µmol of this substrate/min. The α-galactosidase preparation used had a specific activity of approximately 0.23 units/mg.

**Degradation of Globotriaosylceramide**—A stock solution of 1 mM 3H-labeled GbOse3Cer was prepared by dissolving GbOse3Cer with sonication (Branson sonifier B-12, Branson, Danbury, CT) in 200 µl of distilled water. The reaction was carried out at 37 °C for 1 h and terminated by the addition of 0.5 ml of chloroform/methanol (2:1, v/v) and 100 µl of 0.4 mM galactose in 200 mM citrate buffer. The biphasic system was mixed on a Vortex mixer and then centrifuged to resolve the phases. An aliquot of the upper phase containing the liberated 3H-galactose was transferred to a scintillation vial, mixed with 5 ml of scintillation solvent (Aqua Luma, Baker, The Netherlands) and counted in a liquid scintillation counter (Berthold, Wildbad, Federal Republic of Germany).

**Degradation of Ganglioside GM₂**—Ganglioside GM₂ degradation was measured as previously described (15).
Purification of an Activator Protein for the Degradation of GlbOse3Cer

All steps were performed at 4 °C. All buffer solutions used contained 0.01% NaN₃ as an antimicrobial agent.

Extraction—The activator protein was prepared from postmortem human liver stored at -20 °C. 1000 g of normal human liver was minced in three volumes of distilled ice-cold water with an Omni-Mixer (Dupont, Bad Nauheim, Federal Republic of Germany) and homogenized with an Ultra-Turrax (Janke and Kunkel, Staufen im Breisgau, Federal Republic of Germany). The homogenate was centrifuged at 100,000 × g for 30 min. The pellet was resuspended in three volumes of distilled water and again centrifuged. The clear supernatants were combined.

Precipitation by Heat—The crude liver extract was heated to 55-60 °C in a water bath. Precipitates were removed by centrifugation at 10,000 × g for 45 min. The supernatant was filtered through glass wool and dialyzed against 10 mM sodium phosphate buffer, pH 7.0. The dialyzed solution was again centrifuged at 10,000 × g and filtered to remove precipitated material.

Ion Exchange Chromatography—The dialyzed solution was applied to a DEAE-cellulose column (2.6 × 40 cm) that had been equilibrated with 10 mM sodium phosphate buffer, pH 7.0. Bound protein was eluted with a linear gradient of NaCl (600 mL total, 0 to 0.5 M) in sodium phosphate buffer. All fractions were tested for their content of protein and of a factor stimulating the degradation of GlbOse3Cer. Fractions containing the activator were pooled, dialyzed against distilled water, and concentrated by lyophilization.

Gel Filtration—The concentrated solution (15 mL) was filtered to remove precipitated material and applied to a Sephadex G-75 superfine column (2.6 × 80 cm) that had been equilibrated with 10 mM sodium phosphate buffer, pH 7.0. Dextran blue (4 mg) and 5 × 10⁶ cpm of [3H]labeled leucine were added to determine void volume and bed volume, respectively. The proteins were eluted with 10 mM phosphate buffer at a flux rate of 0.9 mL/cm²/h. Fractions of 15 mL were collected and tested for protein and activator content. Fractions containing the activator were pooled and concentrated by ultrafiltration on a UM-2 membrane (Amicon, Witten, Federal Republic of Germany).

Ampholyte Displacement Chromatography—This step was performed on a Pharmacia chromatofocusing set according to the manufacturer's instructions. The concentrated solution (5 mL) was dialyzed against 25 mM imidazole-HCl, pH 7.4, (starting buffer) and applied to a polybuffer exchanger column, PBE 94 (0.9 × 23 cm) previously equilibrated with starting buffer and covered with 5 mm of Sephadex G-10. Following application of the sample, the column was washed with starting buffer. Bound protein was eluted with an elution buffer (Pharmacia polybuffer PB 74 diluted 1:8 with distilled water and adjusted to pH 4.0 with HCl). Fractions of 5 mL were collected and tested for protein and activator content and for pH value. The two activator peaks were pooled separately and further purified individually.

Hydrophobic Chromatography—The activator solution was applied to a small octyl-Sepharose 4 B column (1.5 mL) equilibrated with 10 mM sodium phosphate buffer, pH 6.5. Bound protein was eluted with 15 mL of 1% sodium cholate in phosphate buffer. Fractions (1.5 mL) containing the activator were pooled and dialyzed against distilled water. The activator solution was further purified by a second gel filtration on a Sephadex G-75 superfine column (1.6 × 56 cm) which was carried out as described above. Fractions containing the activator were pooled and concentrated by application to small DEAE-cellulose columns (1 mL) and elution with 3 mL of 300 mM NaCl in 10 mM sodium phosphate buffer, pH 7.0.

Molecular Weight Estimation—The molecular weight of the activator was determined by gel filtration on a Sephadex G-75 superfine column (1.6 × 56 cm), equilibrated with 10 mM sodium phosphate buffer containing 150 mM NaCl, pH 7.0. Dextran blue and [3H]-labeled leucine were used to determine void volume and bed volume. Horse heart cytochrome c, chicken ovalbumin, and bovine pancreas chymotrypsinogen were used as molecular weight standards.

Determination of the Isoelectric Point—The isoelectric point of the activator was determined by isoelectric focusing on a polyacrylamide flat gel in a Multiphor focusing apparatus (LKB, Bromma, Sweden) according to the manufacturer's instructions.

Prior to application, 36 and 38 pg, respectively, of the two activator preparations were each mixed with 6 nmol of [3H]Glbose3Cer, adjusted to a total volume of 100 µL of 40 mM citrate buffer, pH 4.1, and incubated at 37 °C for 30 min. These mixtures were applied to small (10 × 5 mm) filter papers, which were then placed on the polyacrylamide gel. In a control run, [3H]Glbose3Cer was applied alone. After focusing, the gel was fractionated into 5-mm strips. The pH value of the gel strips was measured with a pH surface electrode (Ingold, Frankfurt/Main, Federal Republic of Germany). The activator content of the gel strips was estimated by measuring the radioactivity of activator-bound [3H]Glbose3Cer.

Digestion of the Activator by Proteases

Trypsin and Chymotrypsin—Aliquots of the activator preparation after ion exchange chromatography (60 µL, 0.7 mg of protein) were mixed with 0.25 mg of trypsin or of chymotrypsin, adjusted to a final volume of 140 µL of 100 mM phosphate buffer, pH 7.6, and incubated at 37 °C. After 4 h, another 0.5 mg of trypsin or chymotrypsin were added. After 24 h, the remaining activator content of the incubation mixtures was assayed. Both trypsin and chymotrypsin are proteolytically active.

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**Fig. 1. Stimulation of the enzymatic degradation of globotriaosylceramide by extracts from human tissues.** Aqueous homogenates from liver, kidney, and spleen in distilled water (25%, w/v) were centrifuged at 100,000 × g for 1 h. Aliquots of the clear supernatants were added to reaction mixtures which contained 15 µL of [3H]globotriaosylceramide and either 3.0 milliunits of α-galactosidase (open bars) or distilled water (hatched bars) in a total volume of 100 µL of 40 mM citrate buffer, pH 4.1. The reaction mixtures were incubated at 37 °C for 5 h. Control assays with purified α-galactosidase were run without tissue extracts (control). Liberated [3H] galactose was determined as described under "Materials and Methods." Broken lines indicate the sums of the control values without purified α-galactosidase and without tissue extracts, respectively.
ically inactive at the pH value of the activator test.

Pronase P—25 μl of the purest available activator preparation were mixed with 0.125 mg of pronase P from *Streptomyces griseus*, adjusted to a final volume of 55 μl of 100 mM phosphate buffer, pH 6.0, and incubated at 37°C. After 60 and 120 min, additional 0.125 mg of pronase were added. After 20 h, the activator content of the incubation mixture was tested. Pronase P is proteolytically inactive at the pH value of the activator test.

**RESULTS**

The degradation of globotriaosylceramide by partially purified \( \alpha \)-galactosidase from postmortem human liver was markedly enhanced by aqueous extracts from human liver or kidney (Fig. 1). This stimulating activity was stable up to 60°C but was rapidly lost at higher temperatures. No activating effect could be demonstrated in crude spleen extracts.

The activating factor from liver was completely adsorbed to DEAE-cellulose at pH 7.0 and could be eluted with NaCl at a concentration of approximately 350 mM (Fig. 2). An additional small peak of stimulating activity was found at a lower salt concentration, coinciding with the bulk of protein. This apparent stimulation could, however, be attributed to the residual \( \alpha \)-galactosidase activity which also eluted in these fractions (data not shown). Further steps of purification were carried out with the major activator peak. Gel filtration on Sephadex G-75 superfine separated the activator, which eluted at a volume corresponding to a molecular weight of 20,000 to 30,000, from the bulk of proteins (Fig. 3).

Subsequent ampholyte displacement chromatography ("chromatofocusing") resolved the activating factor into two fractions with isoelectric points of pH 4.6 and 4.8, respectively (Fig. 4).

Both fractions were further purified and analyzed separately. Except for the difference in their isoelectric points, they were, however, found to behave identically (see below).
**Fig. 4.** Ampholyte displacement chromatography. The partially purified activator solution from Sephadex G-75 superfine chromatography was concentrated by ultrafiltration and applied to a Pharmacia polybuffer exchanger column (14.5 ml), pH 7.4, which had been equilibrated according to the manufacturer's instructions. The proteins were separated by elution with 200 ml of a 1:8 diluted solution of carrier ampholytes, PB 74-HC1, pH 4.0. Fractions of 5 ml were collected. Aliquots of each fraction (25 μl) were analyzed for protein (■■■) and for their ability to stimulate the enzymatic degradation of globotriaosylceramide (○○○), and their pH value was measured (O—O).

### Table I

| Step                                      | Activator | Protein | Specific activity | Purification factor | Yield % |
|-------------------------------------------|-----------|---------|-------------------|---------------------|--------|
| Extract                                   | 960,000   | 25,000  | 39                | 1                   | 100    |
| Heat denaturation                         | 940,000   | 11,800  | 79.7              | 2.1                 | 97.4   |
| DEAE-cellulose                            | 1,140,000 | 621     | 1,840             | 47.4                | 118    |
| Gel filtration on Sephadex G-75 superfine | 790,000   | 59.6    | 13,100            | 338                 | 80.5   |
| Ampholyte displacement chromatography      |           |         |                   |                     |        |
| Peak I, pH 4.8                            | 70,100    | —       | —                 | —                   | 7.2    |
| Peak II, pH 4.6                           | 285,000   | —       | —                 | —                   | 29.5   |
| Peak I                                    | 99,500    | 4.3     | 22,800            | 590                 | 10.2   |
| Octyl-Sepharose                           | 61,500    | —       | —                 | —                   | 6.3    |
| Gel filtration on Sephadex G-75 superfine | 22,700    | 1.57    | 14,200            | 367                 | 2.4    |
| Concentration on DEAE-cellulose           |           |         |                   |                     |        |
| Peak II                                    | 212,700   | 4.66    | 45,600            | 1,170               | 22     |
| Octyl-Sepharose                           | 174,900   | —       | —                 | —                   | 18     |
| Gel filtration on Sephadex G-75 superfine | 101,600   | 2.03    | 50,060            | 1,295               | 10.5   |
| Concentration on DEAE-cellulose           |           |         |                   |                     |        |

*One activator unit is defined as the amount of activator that stimulates the hydrolysis of GbOse3Cer by 1 nmol/h/unit of α-galactosidase.

*Protein could not be determined due to the presence of carrier ampholytes.

*Protein could not be determined due to the addition of cytochrome c.

The activating factors, although being entirely water-soluble, adsorbed to octyl-Sepharose 4B even in 10 mM phosphate buffer and were eluted with 1% (w/v) of sodium cholate in the same buffer. Since at this stage the protein concentration was rather low, 1 mg of cytochrome c/ml was added to minimize unspecific adsorption of the activator onto dialysis tubing, glass surfaces and Sephadex gels. Addition of a trace amount of [3H]cholate (approximately 10^6 dpm) facilitated monitoring of the subsequent removal of the detergent. After extensive dialysis, the activator fractions were subjected to a second gel filtration step on Sephadex G-75 superfine to remove the last traces of the detergent. The activator-containing fractions of each column run were pooled and concentrated by adsorption to a small DEAE-cellulose column and subsequent elution with 300 mM NaCl. This last step also served to separate the activator fractions from the cytochrome c. The purification data are summarized in Table I.

In contrast to the heat lability observed with the crude extract, the purified activator preparations were found to be heat-stable at 95 °C for 30 min.

Both activator preparations were remarkably resistant against the action of trypsin and chymotrypsin but lost 90% of their activity after 20 h of incubation with the bacterial protease pronase P. Aliquots of both fractions were subjected to flat gel isoelectric focusing, together with [3H]GbOse3Cer, as detailed under "Materials and Methods." Radioactivity of transported [3H]GbOse3Cer was found to concentrate in sharp bands, at pH values of 4.7 and 4.8, respectively (not shown).

The molecular weight of both globotriaosylceramide activator fractions was estimated to be at 20,000 from analytical gel filtration on Sephadex G-75 superfine. The pH profile of GbOse3Cer degradation in the presence of activator was mark-
edly different from that of the reaction in the presence of the anionic detergent taurocholate (Fig. 5). The latter reaction proceeded optimally at pH 5, whereas the activator-mediated glycolipid hydrolysis displayed a more acidic pH optimum (pH 4.1). The degradation of the artificial water-soluble substrate 4-MU-α-D-galactoside showed a broader pH profile with an optimum at an intermediate value (pH 4.5).

The time course of the degradation of globotriaosylceramide by α-galactosidase, in the presence of either activator preparation, was not linear (Fig. 6); the reaction rate slowed down with time, presumably due to the instability of the α-galactosidase A under acidic conditions (25). With increasing concentration of either activator preparation, the GbOseCer...

**Fig. 5.** The pH profiles of the enzymatic hydrolysis of 4-MU-α-Gal and of globotriaosylceramide in the presence of activator protein or of sodium taurocholate. Reaction mixtures (total volume, 50 μl) contained 5 nmol of globotriaosylceramide, 2.4 milliunits of α-galactosidase, 2 μmol of citrate buffer of the pH values indicated and 5.8 μg of activator protein, pH 4.7, (○—○) or 3.6 μg of activator protein, pH 4.8; (△—△), or 0.25 μmol of crude taurocholate (■—■), respectively. The reaction mixtures were incubated at 37 °C for 1 h. Liberated [3H]galactose was determined as described under “Materials and Methods.” The pH values are indicated above. After 30 min, the reaction was stopped, and liberated 4-MU was measured fluorometrically as described under “Materials and Methods” (■—■).

**Fig. 6.** Time course of the enzymatic degradation of globotriaosylceramide in the presence of activator proteins. 15 nmoles of globotriaosylceramide were incubated with 2.4 milliunits of α-galactosidase and either 7.6 μg of activator protein, pI 4.8, (■—■) or 11.6 μg of activator protein, pI 4.6, (○—○) in 50 μl of 40 mM citrate buffer, pH 4.1, at 37 °C. The reaction was stopped after different times as indicated. Liberated [3H]galactose was determined as described in the inset, double reciprocal plot of the data. In control incubations (broken lines), the activator protein was substituted by bovine serum albumin.

**Fig. 7.** Dependence of the enzymatic globotriaosylceramide hydrolysis on the amount of activator protein. Assays with GbOseCer (15 nmol of substrate, 1.2 milliunits of α-galactosidase, 2 μmol of citrate buffer, pH 4.1, in a total volume of 50 μl) were incubated with increasing amounts of the activator protein (pI = 4.6 for the case shown) as indicated. After 1 h, the reaction was stopped. Control incubations were lacking α-galactosidase (broken lines). Inset, double reciprocal plot of the data.
Substrate dependence of the enzymatic globotriaosylceramide hydrolysis. Assays containing 1.2 milliunits of α-galactosidase, 5.8 μg of the activator protein (pI = 4.6 in the case shown), 2 μmol of citrate buffer, pH 4.1, and GbOse₃Cer in increasing concentrations as indicated above, in a total volume of 50 μl, were incubated for 1 h at 37 °C. Control assays were run without activator protein and subtracted. Inset, double reciprocal plot of the values.

The effect of the activator protein on the degradation of water-soluble substrate by α-galactosidase. Standard assays with 4-MU-α-Gal (2.5 mM 4-MU-α-Gal, 0.14 milliunits of α-galactosidase, 25 mM citrate buffer, pH 4.5, total volume 200 μl) were incubated in the presence of the activator protein at 37 °C. The reaction was stopped after different times as indicated. Liberated 4-MU was measured fluorometrically as described under "Materials and Methods." ○, 3.6 μg of activator protein; ■, 1.8 μg of activator protein; O, no activator protein.

Comparison of the activator stimulating the degradation of globotriaosylceramide with the activator protein for ganglioside GM₂ degradation. For degradation of GbOse₃Cer, incubation mixtures contained 15 nmol of GbOse₃Cer, 3.0 milliunits of α-galactosidase and 10 μg of the activator protein to be tested in a total volume of 100 μl of 40 mM citrate buffer, pH 4.1. The assays were incubated at 37 °C for 3 h. For degradation of ganglioside GM₂, reaction mixtures contained 10 nmol of [³H]ganglioside GM₂, 20 μg of bovine serum albumin, 4 μg of the activator to be tested, and 0.1 unit of β-hexosaminidase A in a total volume of 40 μl. The assays were carried out in 100 mM citrate buffer, pH 4.0, at 37 °C for 1 h. GbOse₃Cer activator, purest preparation available of the activator protein for the degradation of globotriaosylceramide by α-galactosidase (mixture of the fractions with pI = 4.6 and pI = 4.8). GM₂ activator, electrophoretically pure activator for the degradation of ganglioside GM₂ by β-hexosaminidase A.
degradation increased in a hyperbolic fashion, as would be expected from the model proposed (i.e. the activator-lipid complex is the true substrate of the reaction) (shown for one of the fractions in Fig. 7). A double reciprocal plot of the data yields a straight line which intersects the ordinate at an apparent \( V_{max} \) of 3.3 nmol/h x enzyme unit (Fig. 7, inset).

In the presence of a constant amount of activator, the reaction rate initially increased with the GbOse\(_2\)Cer concentration in the way expected for substrate saturation kinetics but reached a plateau at concentrations above 1 nm (Fig. 8).

The partially purified activator preparation apparently stimulated also the degradation of the water-soluble substrate 4-MU-\( \alpha \)-galactoside. However, an analysis of the time course of this reaction revealed that the activator does not really enhance the reaction velocity but rather serves to stabilize the enzyme (Fig. 9). The degradation of ganglioside GM\(_1\) by \( \beta \)-hexosaminidase A was not significantly stimulated by the GbOse\(_2\)Cer activator studied here. Conversely, a highly purified ganglioside GM\(_{2}\) activator did enhance the breakdown of globotriaosylceramide by \( \alpha \)-galactosidase to some extent (Fig. 10). Although at the moment it is still unclear whether this stimulation is due to an additional specificity of the latter activator for \( \alpha \)-galactosidase or to a contamination of the preparation employed with a specific \( \alpha \)-galactosidase activator, the results clearly demonstrate that the activator described in this work for the degradation of globotriaosylceramide by \( \alpha \)-galactosidase is not identical with the GM\(_2\) activator.

**DISCUSSION**

Since the initial observation by Mehl and Jatzkewitz (10), that a low molecular weight nonenzymic protein cofactor is required for the degradation of sulfatides by purified arylsulfatase A *in vitro* in the absence of detergents, some attention has been focused upon the mechanism by which water-soluble lysosomal hydrolases interact with their membrane-bound lipid substrates. In the course of these studies, two similar “activator proteins” have been isolated, one specific for the hydrolysis of ganglioside GM\(_1\) by \( \beta \)-galactosidase (12), the other one for the cleavage of ganglioside GM\(_{2}\) by \( \beta \)-hexosaminidase A (15, 26). The physiological importance of these cofactors is emphasized by the occurrence of an enzymic variant (variant AB) of infantile GM\(_1\) gangliosidosis that is caused by the deficiency of the activator protein for ganglioside GM\(_1\) degradation (13, 17, 27).

In the present work, we report on the identification and partial purification of an apparently analogous activator protein for the hydrolysis of globotriaosylceramide by \( \alpha \)-galactosidase. The protein was purified some 1300-fold by conventional techniques, including ion exchange chromatography, gel filtration, ampholyte displacement chromatography and hydrophobic chromatography. A minor peak of stimulation of globotriaosylceramide degradation, which on ion exchange chromatography eluted ahead of the major activator peak, together with the bulk of protein, could be shown to arise from residual \( \alpha \)-galactosidase activity which elutes in these fractions. In contrast, ampholyte displacement chromatography resolved the activator into two distinct fractions with slightly different isoelectric points. The molecular basis of this apparent heterogeneity is not yet known.

However, it seems unlikely that two “isoactivators” with different amino acid sequences exist, since both preparations behaved essentially identically in all other experiments. Presumably, both proteins have the same structure and differ only with respect to some secondary modification, e.g. glycosylation or phosphorylation. A similar observation has recently been reported for a preparation of ganglioside GM\(_1\) activator isolated from the liver of a patient with GM\(_1\) gangliosidosis. Isoelectric focusing resolved this factor into two fractions with isoelectric points of pH 4.1 and 4.6, respectively (28). The ganglioside GM\(_1\) activator from normal human liver was found to focus at pH 4.1 only (12).

The purified globotriaosylceramide activator was heat stable at 95 °C for at least 30 min, which is in contrast to its heat lability above 60 °C in the crude extract. An explanation for this difference may be that in the crude extract the activator protein is not really denatured but coprecipitates with other, denatured proteins.

The protein nature of the activator is indicated by its sensitivity to digestion with the bacterial protease pronase F, whereas trypsin and chymotrypsin were nearly without effect. Similar heat stabilities as well as resistance against tryptic digestion have been reported for the sulfatide activator (18) and for the ganglioside GM\(_2\) activator (12).

The mechanism by which this activator protein stimulates the enzymic breakdown of globotriaosylceramide appears to be similar to the one postulated for the action of the sulfatide activator and of the ganglioside GM\(_2\) activator (11, 15, 16). According to this model, the activator, which is water-soluble, binds to the hydrophilic headgroup of the glycolipid, extracts the lipid from the membrane, and forms a water-soluble activator-lipid complex which is the true substrate of the reaction.

The globotriaosylceramide activator presented in this work did not stimulate the hydrolysis of the water-soluble substrate 4-methylumbelliferyl-\( \alpha \)-d-galactoside, suggesting that it does not “activate” the enzyme. (The small apparent activation observed could be shown to arise from stabilization of the enzyme and not from activation (Fig. 9).) Also, the fact that the activator protein, although being entirely water-soluble, adsorbed to octyl-Sepharose even at low salt concentrations indicates that this protein must possess some kind of lipid binding domain. The saturation kinetics observed with increasing concentrations either of activator protein (Fig. 7) or of lipid substrate (Fig. 8) are likewise compatible with the proposed mechanism, although the reason for the plateau of the reaction velocity at higher lipid concentrations is not yet known.

The strongly nonlinear time course of globotriaosylceramidase cleavage in the presence of the activator (Fig. 6) can be explained by the lability of \( \alpha \)-galactosidase A under incubation conditions (25), the partially purified \( \alpha \)-galactosidase preparation employed in the experiments described lost some 55% of its activity towards 4-MU-\( \alpha \)-galactoside after 3 h of preincubation at pH 4.1, the remaining 45% being stable (not shown). The finding that after the same incubation time the degradation of globotriaosylceramide in the presence of the activator ceased completely would suggest that the activator interacts nearly exclusively with \( \alpha \)-galactosidase A but not with the B isoenzyme. Such an assumption would also be in accordance with the observation that in patients with Fabry’s disease the deficiency of \( \alpha \)-galactosidase A leads to the accumulation of globotriaosylceramide in spite of normal levels of \( \alpha \)-galactosidase B (30, 31). A similar isoenzyme specificity has already been demonstrated for the degradation of ganglioside GM\(_2\) in the presence of the respective activator protein, this reaction is catalyzed only by \( \beta \)-hexosaminidase A but not by the closely related hexosaminidase B (15). The isoenzyme specificity of the ganglioside GM\(_2\) activator explains the accumulation of ganglioside GM\(_{2}\) and its asialo derivative, glycolipid GA\(_2\), in the neuronal tissues of patients with Tay-Sachs disease (variant B of GM\(_2\) gangliosidosis) who lack only hexosaminidase A but retain normal or even elevated levels of the B isoenzyme. By the same line of reasoning, a similar
specificity may be predicted for the sulfatide activator. The study of the isoenzyme specificity of the globotriaosylceramide activator has to be one of the subjects of future investigations.

The pH profile of the α-galactosidase-catalyzed globotriaosylceramide degradation in the presence of the activator is shifted towards more acidic pH values with an optimum at pH 4.1, as compared to the reaction measured with taurocholate, which proceeds optimally at pH 5. This difference additionally supports the notion that the interaction between activator and enzyme is highly specific. The degradation of the water-soluble 4-MU-α-galactoside shows a broader pH profile with an optimum at pH 4.5. The fact that all assays were performed at the respective pH optimum may explain why the α-galactosidase activity was stabilized by the activator protein when the 4-MU glycoside was used as substrate (assayed at pH 4.5) but not in the case of globotriaosylceramide (which was incubated at pH 4.1).

The activator for the degradation of ganglioside GM₃ by β-galactosidase was initially reported to stimulate also the cleavage of globotriaosylceramide by α-galactosidase (12). Meanwhile, it has become clear that the latter activation represented a quantitatively negligible unspecific side effect of this protein (32). In contrast, 10 µg of the partially purified activator preparation described in this work for globotriaosylceramide degradation enhanced the cleavage of the glycolipid up to 100-fold, which exceeds the stimulation that can be achieved with detergents.

Direct comparison of the globotriaosylceramide activator with the ganglioside GM₂ activator protein demonstrated clearly that the factor described here is not identical with the ganglioside GM₂ activator, although it could not be ruled out that the latter activator may to some extent facilitate also globotriaosylceramide catabolism. The sample of ganglioside GM₂ activator has to be one of the subjects of future investigations.

To clarify the relation between the GbOse₃Cer activator described in this work and the ganglioside GM₂ activator, a similar comparison of these two proteins would be desirable. From the different isolectric points of the two activators of pH 4.1 (GM₂ activator (12)) and pH 4.7 (GbOse₃Cer activator), respectively, it might be expected that both prove to be different proteins. More detailed studies on this subject are under way.

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