Biochimica et Biophysica Acta, 481 (1977) 561—572

BBA 68103

THE ACTIVATOR OF CEREBROSIDE SULPHATASE

BINDING STUDIES WITH ENZYME AND SUBSTRATE DEMONSTRATING THE DETERGENT FUNCTION OF THE ACTIVATOR PROTEIN

G. FISCHER and H. JATZKEWITZ

Max-Planck-Institut fur Psychiatrie, Neurochemische Abteilung, Kraeplinstr. 2, D-8000 Munchen 40 (G.F.R.)

(Received October 18th, 1976)

Summary

1. Sulphatase A (cerebroside sulphatase) (EC 3.1.6.1.) and a 12-fold excess of its physiological activator protein were chromatographed together on Sephadex G-75. The elution buffer was the same as that used in the enzymic degradation of sulphatides. The two proteins were eluted in different peaks indicating that no stable complex was formed.

2. Activator protein was incubated with sulphatides under conditions used favouring the sulphatase activity. Incubation solutions were then examined by electrophoresis on a polyacrylamide gel gradient. An one-to-one complex between activator and sulphatides was observed. Half maximal binding occurred with 2.5 nmol of sulphatides together with 1 or 2 nmol of activator in 100 μl.

3. Cerebrosides as the enzymic degradation products of sulphatides, bind also to the activator protein. A ratio of one-to-one could possibly be obtained at high cerebroside concentrations. The binding to cerebrosides is less specific than that to sulphatides. A 7-fold excess of cerebrosides was necessary for half maximal binding.

4. In a mixture of sulphatides and cerebrosides the formation of the complex with the activator protein is partly inhibited. The total amount of bound lipids changed as the composition of the lipid mixture was varied. In a one-to-one mixture of the two lipids 60% of the total bound lipids are sulphatides and 40% are cerebrosides.

Introduction

Sulphatase A (EC 3.1.6.1) degrades sulphatides (cerebroside sulphates) in buffers of low ionic concentrations (<0.1 M; with an optimum at 0.02 M) [1]

which are presumably unphysiological. In buffers with ionic concentrations in the physiological range (~0.3 M) the addition of detergents (e.g. taurodeoxycholate) [2—4] or a physiological activator [5] is necessary. This activator has been purified and identified as a low molecular weight protein (M_r = 21 500) [5].

Since it could be shown that the activator, together with the enzyme, is localized in lysosomes [6] it may be assumed that it is also necessary for the in vivo degradation of sulphatides by sulphatase A. The enzymic activity of other sphingolipid-hydrolysing enzymes such as cerebroside galactosidase, cerebroside glucosidase, β-*N*-acetylgalactosaminidase, and sphingomyelinase, which are all lysosomal in origin, could also be increased by addition of a low molecular weight protein fraction isolated from lysosomes [7]. However, the mechanism of this activation is unclear. On the one hand, in the case of glucocerebrosidase, a strong binding between the enzyme and its activator could be shown, but only in the presence of phospholipids [8,9]. It was suggested that it was the complex which was enzymically active. On the other hand, in the case of β-galactosidase, a stoichiometric relationship between the activator and the substrate ganglioside G_{M1} (nomenclature according to Svennerholm) was assumed based on kinetic data [10], thus favouring binding of the activator to the substrate.

In this paper it is demonstrated that the activator of sulphatase A strongly binds sulphatides and, to a lesser extent, their enzymic degradation products (cerebrosides) in an one-to-one complex indicating that it acts on the substrate rather than on the enzyme.

Materials and Methods

Chemicals

Nitrocatechol sulphate was purchased from Sigma Chem. Co. (St. Louis, U.S.A.). Sulphatides, cerebrosides, bovine serum albumin, as well as all the chemicals necessary for polyacrylamide gel electrophoresis were purchased from Serva (Heidelberg, G.F.R.). Sephadex was from Pharmacia (Uppsala, Sweden). Tritium gas was supplied by The Radiochemical Centre, (Amersham, U.K.). All reagents of analytical grade were obtained from Merck (Darmstadt, G.F.R.). Tissue solubilizer TS-1 and Unisolve were from Koch-Light Lab. (Colnbrook, U.K.). Tritium-labelled sulphatides and cerebrosides were prepared as previously described [1].

Preparation of sulphatase A and assay of arylsulphatase activity

Sulphatase A was prepared from human liver according to Stinshoff [11]. The preparation had a specific activity of 30 units per mg, when the arylsulphatase activity was determined according to Baum et al. [12], one unit being defined as the amount of enzyme which degrades 1 μmol of nitrocatechol sulphate (2-hydroxy-5-nitrophenyl sulphate) per min at 37° C.

Assay of cerebroside sulphatase activity

The incubation mixtures contained in a total volume of 100 μl, 20 μmol of sodium acetate buffer (pH 4.8), 20 nmol of sulphatides and various amounts

of enzyme and activator solutions. The incubations and the calculation of product formed were performed as previously described [5].

Preparation of activator

The activator was prepared from human liver and desalted by chromatography on Sephadex G-10 (1.6 × 32 cm column) [5]. 1 μg of this preparation stimulated the degradation by 0.1 unit of arylsulphatase A of 1 nmol of sulphatides in 4 h at 37°C in a mixture containing, in 100 μl, 20 nmol of sulphatides and 20 μmol of sodium acetate buffer, pH 4.8.

Protein determination

Protein was measured by the method of Lowry et al. [13]. In the eluates obtained after gel chromatography absorption at 280 nm was measured. Bovine serum albumin was used as a standard in both methods.

The binding of sulphatase A and activator

A mixture of 60 μg of sulphatase A and 160 μg of activator in 600 μl of 0.2 M sodium acetate buffer, pH 4.8, was applied to a column (0.9 × 20 cm) of Sephadex G-75 superfine which was equilibrated and eluted with the same buffer. Chromatography was performed at 20°C. The fractions (500 μl/6 min) were examined for arylsulphatase activity [12] using 20 μl in an incubation volume of 400 μl. Further, each fraction was examined for cerebroside sulphatase activity, using 50 μl with and without addition of 5.5 μg of activator, under the incubation conditions described above. The activator effect was tested under the same incubation conditions using 50 μl of each fraction with addition of 2 μg of sulphatase A. In both cases the incubation time was 14 h.

Electrophoresis and staining methods

Electrophoresis (apparatus: Desaga Flat-bed electrophoresis 146 200 Desaga, Heidelberg, G.F.R.) was performed according to Williams and Reisfeld [14] using a discontinuous gel gradient consisting of equal amounts of 5, 7.5, 10 and 15% polyacrylamide gel polymerized in 7 mM Tris · HCl buffer, pH 8.0, together with a spacer gel. The electrode buffer was 0.03 M diethylbarbituric acid/Tris, pH 7.0. To prepare the lipid solutions appropriate amounts of lipid in benzene/ethanol were evaporated to dryness in the incubation vessel. The residue was dispersed in 50 μl of 0.05 M ammonium acetate buffer, pH 4.8, by treatment with a sonifier, type B-12 (Branson Sonic Power Comp., Danbury, Conn., U.S.A.) Cup-Horn, 2 × 10 s, 100 W.

To these lipid solutions were added the protein solutions. After an incubation period of 1 h at 37°C, the solution was made to 10% (v/v) with glycerol containing 1% bromphenol blue as a front marker. These solutions were applied to the gel on five lanes of 1 cm. The distance between two lanes was 0.5 cm. The separation was carried out at 45 mA, 300 V. Electrophoresis was stopped when the front marker had reached the end of the gel.

Staining methods

(a) Protein staining. After electrophoresis the proteins were stained with Coomassie Brilliant Blue R-250 (0.25% in 7% acetic acid, 2 h, without

preceding fixation) [15]. The gel was finally washed with 7% acetic acid.

(b) Lipid staining. The lipids were stained with Sudan Black B according to Prat et al. [16].

Determination of radioactivity

After electrophoresis the gel was cut into pieces corresponding to the different lanes. Each piece was cut into nine sections, one for the spacer gel and two for each gradient step (see Fig. 2). Each section was placed in a vial for scintillation counting. Deionised water (100 μl) and 1.5 ml of tissue solubilizer TS-1 were added and the vials incubated for 1 h at 55° C. After shaking for 20 min at room temperature 60 μl of 4% ascorbic acid and 10 ml of Unisolve were added [17]. After further shaking for 15 min the radioactivity was determined in a liquid scintillation counter Mark II (Nuclear Chicago, U.S.A.). To calculate the amount of lipids in each section the recovered radioactivity was related to the total radioactivity applied in the experiment (corresponding to the total amount of lipids applied).

Results

Binding studies of sulphatase A and activator

A mixture of 8 nmol of activator and 0.6 nmol of sulphatase A was chromatographed on Sephadex G-75. The column was equilibrated and eluted with the same buffer as used for the degradation of sulphatides with an activator-stimulated sulphatase A. In the eluate two distinct protein peaks were seen (Fig. 1). The first peak showed arylsulphatase A activity (86% of the total enzyme activity employed) but no cerebroside sulphatase activity unless activator was added. This is characteristic for the pure enzyme. The second peak had a very low enzyme activity (9% of the total enzyme activity employed) due to the slight contamination with enzyme from peak one. All of the activator activity was localized in this peak. When sulphatase A is chromatographed alone under the same conditions a symmetrical peak of enzyme activity is obtained. In a mixture of sulphatase A and activator the enzyme is eluted in an asymmetrical peak with trailing on the activator side (see Fig. 1).

Binding studies of activator and sulphatides

Activator protein (1.75 nmol) and 40 nmol of sulphatides or mixtures of both were incubated under the conditions previously described (see Materials and Methods). After electrophoresis of the incubation mixtures on a discontinuous polyacrylamide gel gradient the gel was cut and stained for lipids and proteins.

After staining for lipids with Sudan Black it was observed that in the presence of activator more sulphatides migrate in the separation gel forming two new bands, one in section 4, the other in the region of section 8. The latter co-migrates with the activator as shown by protein staining with Coomassie Brilliant Blue. (The activator itself is not stained by Sudan Black) (Fig. 2).

Binding capacity of the activator for sulphatides. To examine the stoichiometric relationship between sulphatides and activator during electrophoresis,

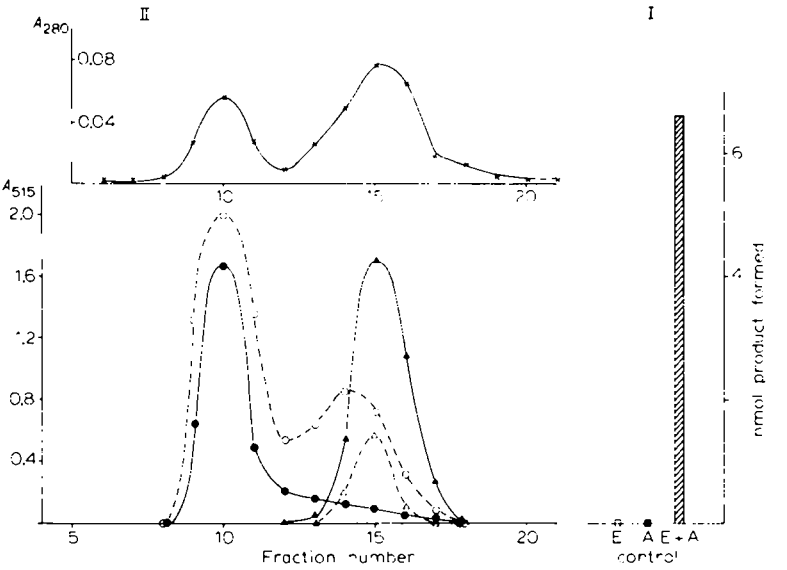

Fig. 1. Chromatography of a mixture of sulphatase A and its activator protein on Sephadex G-75. The protein pattern as well as enzyme and activator activities were determined as previously described (see Materials and Methods). (I) Cerebroside sulphatase activities of 2 μg of enzyme (E □), of 5.5 μg of activator protein (A ■), or a mixture of both (E + A ▣). The cerebroside sulphatase activities are expressed in nmol product formed under the incubation conditions described. (II) Protein expressed as A_{280} (2 cm) (X———X). Assay for arylsulphatase A activity (A_{515}) (●———●). Assay of cerebroside sulphatase activities of the different fractions without additions (△- - - - -△); with addition of activator (○- - - - -○), with addition of enzyme (▲———▲).

radioactively labelled sulphatides (415 000 dpm per incubation) were used in the following experiments.

20 nmol of sulphatides were incubated with 0.5—5 nmol of activator under the conditions described. After electrophoretic separation on the gel gradient the gel was cut into nine sections (see Fig. 2) and the radioactivity in each section determined (see Materials and Methods). Fig. 3a shows the distribution of the labelled sulphatides in the absence or presence of 1.2 or 4.8 nmol of activator. Without any activator protein added, more than 90% of the total radioactivity on the gel was localized in the first two sections. Sections 5—9 had a constant low background of less than 0.5%, indicating that no significant amounts of micellar sulphatides were migrating in these sections. By the addition of activator protein this distribution of radioactivity changed. More sulphatides penetrated the separation gel forming two new bands in agreement with the staining in Fig. 2. Only the radioactivity transported by the activator protein to section 8 (which shows the activator protein staining band) was proportional to the amount of activator added. The high recovery of radioactivity in the gel (up to 90%) allowed the amount of sulphatides transported by the activator to be calculated from the radioactivity in section 8 expressed as a percentage of the total sulphatide activity applied (corresponding to 20 nmol). The ratio between the amount of activator added and sulphatides transported is one-to-one in the activator-sulphatide complex (Fig. 3b).

Saturation curve of the binding in the activator-sulphatide complex. In the

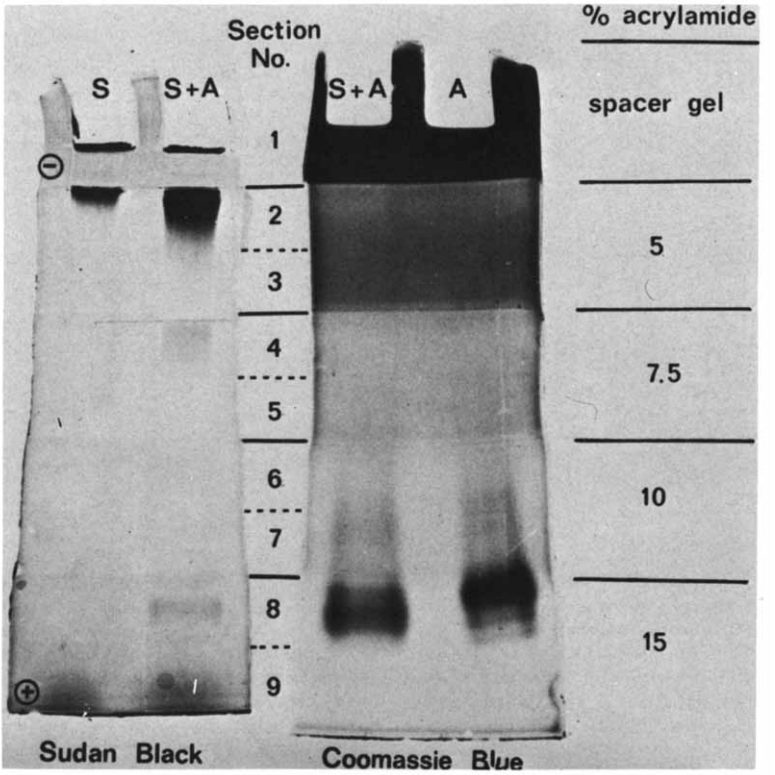

Fig. 2. Electrophoresis on a polyacrylamide gel gradient of sulphatides, activator protein, or mixtures of both. 1.75 nmol of activator protein (A), 40 nmol of sulphatides (S), or mixtures of both (S + A) were incubated in a total volume of 100 μl and separated electrophoretically as previously described (see Materials and Methods). The gel was cut and one part stained for lipids (Sudan Black), the other for proteins (Coomassie Brilliant Blue). The sections in which radioactivity was determined in the following experiments are designated 1—9.

following experiments a constant amount of 1 or 2 nmol of activator was incubated with 1—40 nmol of sulphatides. Electrophoresis and calculation of the sulphatides bound in the activator-lipid complex were carried out as outlined in Fig. 3a. Even at the lowest lipid concentration used, which is perhaps in the range of the critical micellar concentration, the sulphatides alone were unable to penetrate the polyacrylamide gel unless the activator protein is added. With the addition of increasing amounts of sulphatides to the activator solution (1 or 2 nmol) more and more sulphatides were bound until the saturation was reached, again demonstrating the one-to-one ratio of activator protein to sulphatides (Fig. 4). Half maximal saturation of this binding was obtained at a concentration of 2.5 nmol of sulphatides per 100 μl of incubation volume in both cases (1 or 2 nmol of activator).

To examine whether the activator-sulphatide complex dissociates due to dilution of the incubation volume the following experiments were carried out. Activator (2 nmol) was incubated with 10 nmol of sulphatides for 60 min at 37°C in a total volume of 50 or 100 μl (Materials and Methods). Although the sulphatides were dispersed by sonication in different volumes the amount of

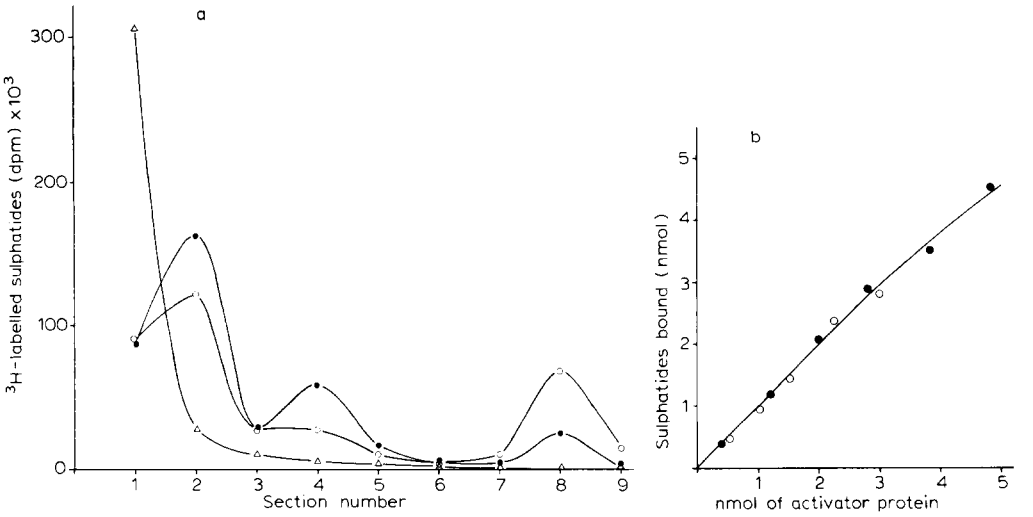

Fig. 3. (a) Separation of the activator-sulphatide complex by electrophoresis. Distribution of ^{3}H-labelled sulphatides on the gel. Various mixtures of activator protein and sulphatides were incubated in a total volume of 100 µl and separated electrophoretically as previously described (see Materials and Methods). △———△, 20 nmol sulphatides; without activator protein ●———●, 20 nmol sulphatides + 1.2 nmol activator protein; ○———○, 20 nmol sulphatides + 4.8 nmol activator protein. The amount of ^{3}H-labelled sulphatides was determined in each section of the gel gradient (see Fig. 2 and Materials and Methods). The activator-sulphatide complex is localized in section No. 8. (b) Stoichiometrical relationship in the activator-sulphatide complex. The activator-bound sulphatides in section 8 (Fig. 3a) from the total amount of 20 nmol were determined as previously described (see Materials and Methods). They are expressed in nmol and are related to the total amount of activator protein incubated with the sulphatides. Two series of experiments were performed (○———○ and ●———●).

activator-bound sulphatides did not change (Table I). It decreased, however, when the incubation solution was diluted 2-fold after 60 min followed by incubation for another 10 min.

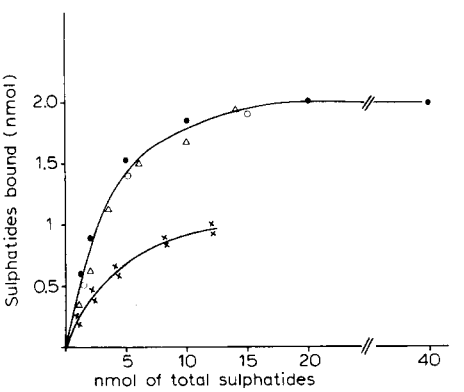

Fig. 4. Binding curve of activator protein and sulphatides. 1 or 2 nmol of activator protein were incubated with increasing amounts of ^{3}H-labelled sulphatides in a total volume of 100 µl. The incubation solutions were then separated electrophoretically. The amount of activator-bound sulphatides was determined as previously described (Fig. 3a) and is expressed in nmol on the ordinate. Three series of experiments were performed (○———○, ●———● and △———△) with 2 nmol of activator and two series (X———X) with 1 nmol of activator.

TABLE I

PARTIAL DISSOCIATION OF THE ACTIVATOR-SULPHATIDE COMPLEX BY DILUTION

Activator (2 nmol) was incubated with 10 nmol of sulphatides under standard conditions (60 min at 37°C in 100 µl of 0.05 M ammonium acetate, pH 5.0). After 60 min of incubation in a volume of 50 µl the solutions were diluted 2-fold followed by incubation for another 10 min. The solutions were then subjected to electrophoresis and the activator-bound sulphatides determined as previously described. The values for the activator-bound sulphatides agreed within 10% in two determinations.

Incubation time (min)	Dispersion volume for sulphatides (µl)	Incubation volume (µl)	nmol of sulphatides	nmol of activator	nmol of activator-bound sulphatides
60	50	100	10	2	1.8
60	35	50	10	2	1.8
60	35	50	10	2	
after dilution		diluter with buffer			
10		50			1.5

Binding of activator and cerebrosides

To study the binding properties between activator and cerebrosides analogous experiments were performed. After the incubation, the precipitate was suspended by vigorous shaking since the cerebrosides (in contrast to the sulphatides) were not totally dispersable by sonification. Some cerebrosides remained at the wall of the incubation vessel and could only be eluted by using organic solvents. Nevertheless the suspension was then used in the electrophoretic experiments (see Materials and Methods).

As a consequence the recovery of radioactivity was low (about 40%) compared to that with labelled sulphatides (90%) parallel experiments were in good agreement (S.E. $\leqslant$10%). The intensity of the protein band after staining was identical when activator was electrophoresed with or without addition of cerebrosides (analogous to Fig. 2), indicating that the activator protein was transfered nearly quantitatively to the gel. In Fig. 5a 0.5 or 3 nmol of activator protein were incubated with 30 nmol of ^{3}H-labelled cerebrosides (1 200 000 dpm) and their migration behaviour compared with that of the cerebrosides alone under the conditions described. From the total radioactivity obtained on the gel without addition of activator protein more than 95% remained in the spacer gel (section 1). With the addition of activator variable amounts of cerebrosides were transported to sections 7 and 8 depending on the amount of activator protein. The relationship between activator added and cerebrosides transported was evaluated by two series of experiments, using 20 or 30 nmol of cerebrosides. The amount of cerebrosides transported was calculated as previously described. Fig. 5b shows the relation on a molar basis of activator protein and cerebrosides transported into sections 7 and 8. A ratio of 1 : 1 was not obtained under the conditions used but it is conceivable that such a relationship might be obtained at higher concentrations of cerebrosides.

To determine the binding curve of the complex formation between activator protein and cerebrosides, various amounts of cerebrosides (3—35 nmol) were added to a constant amount (2 nmol) of activator protein. Incubations, electro-

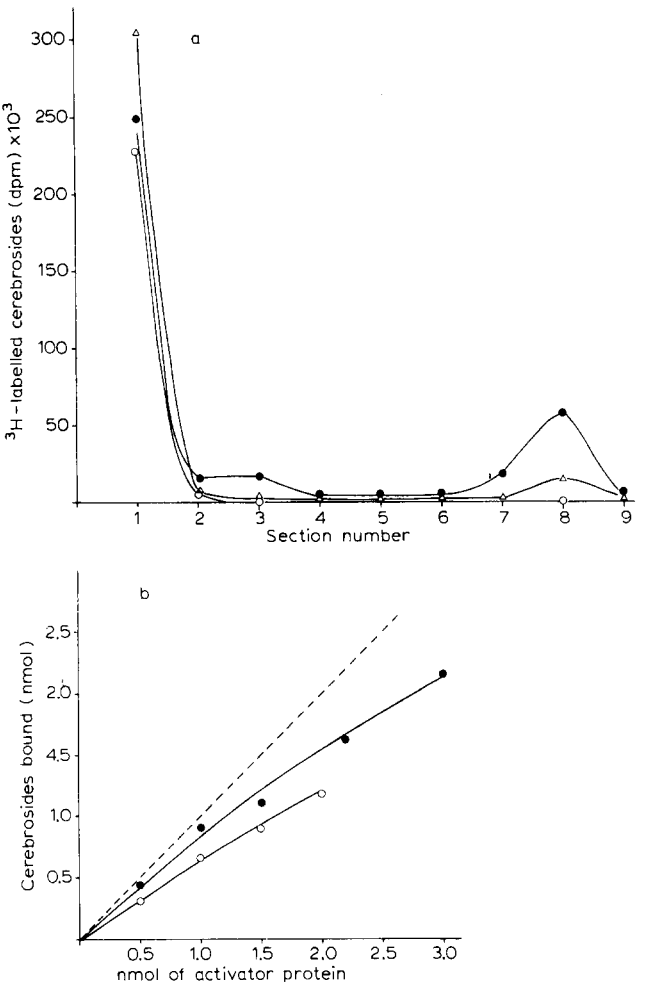

Fig. 5. (a) Separation of an activator-cerebroside complex on the gel. Distribution of ^{3}H-labelled cerebrosides. Various mixtures of activator protein and cerebrosides were incubated in a total volume of 100 µl and separated by electrophoresis as previously described (see Materials and Methods). ○———○, 30 nmol cerebroside without activator protein; △———△, 30 nmol cerebrosides + 0.5 nmol activator protein; ●———●, 30 nmol cerebrosides + 3 nmol activator protein. The amount of ^{3}H-labelled cerebrosides was determined in each section of the gel gradient (see Fig. 2 and Materials and Methods). The activator-cerebroside complex is localized in sections 7 and 8. (b) Stoichiometrical relationship in the activator-cerebroside complex. The activator-bound cerebrosides in the corresponding sections (Fig. 5a) from the total amount of 20 or 30 nmol were determined as previously described (see Materials and Methods). They are expressed in nmol and are related to the total amount of activator protein incubated with the cerebrosides (in a total volume of 100 µl). ●———●, 30 nmol cerebrosides; ○———○, 20 nmol cerebrosides. Each point represents the average of two experiments. The dotted line represents the theoretical one-to-one ratio.

phoresis, and calculation of cerebrosides transported were carried out as already described. When increasing amounts of cerebrosides were added to a constant amount of activator more and more cerebrosides were bound until a plateau was reached corresponding to a binding capacity of 1.5 nmol of cerebrosides per 2 nmol of activator under these conditions. When a 7-fold

excess of cerebrosides was employed half of the activator bound lipid (see Fig. 7).

Binding studies of activator protein with mixtures of sulphatides and cerebrosides

(a) Variable mixtures of sulphatides and cerebrosides. Since the activator protein binds sulphatides as well as cerebrosides binding studies with mixtures of both lipids and the activator protein were carried out. Various amounts of cerebrosides (3—60 nmol) were added to 20 nmol of sulphatides. The lipids were dispersed together, incubated with 2 nmol of activator protein and electrophoresis performed as previously described. In these experiments the recovery of both lipids on the gel was consistently high (80—90%). To distinguish between sulphatides and cerebrosides two analogous series of experiments were carried out in which either sulphatides or cerebrosides were ^{3}H-labelled. With increasing amounts of cerebrosides added the amount of sulphatides bound by the activator decreases (Fig. 6). Simultaneously the amount of cerebrosides bound increases. The total amount of both lipids bound by the activator changes, however, with the relation of the two components of the lipid mixture.

(b) A one-to-one mixture of sulphatides and cerebrosides. To decide whether the binding properties found with mixtures of sulphatides and cerebrosides are concentration dependent or not various concentrations of a one-to-one mixture of both lipids were used. 3—60 nmol of each lipid component were dispersed

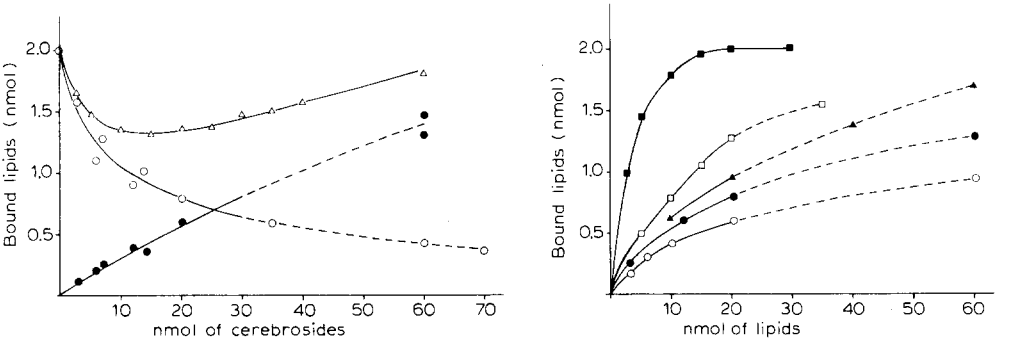

Fig. 6. Binding studies with the activator and mixtures of sulphatides and cerebrosides. A constant amount (2 nmol) of activator protein and 20 nmol of sulphatides were incubated with various amounts of cerebrosides in a total volume of 100 µl. The incubation solutions were then separated by electrophoresis. The activator-bound lipids (sulphatides or cerebrosides) were determined as previously described. △———△, total amount of bound lipids (sulphatides + cerebrosides); ○———○, bound sulphatides; ●———●, bound cerebrosides. The dotted lines indicate where the lipids were not completely dispersable by sonification. Each point represents the average of two experiments.

Fig. 7. Binding curve of activator and a one-to-one mixture of sulphatides and cerebrosides compared with binding curves for each lipid component in the mixture and each lipid component alone. 2 nmol of activator protein were incubated with various amounts of lipids in a total volume of 100 µl. The amount of activator-bound lipids after electrophoresis is expressed in nmol. ■———■, sulphatides; □———□, cerebrosides; ●———●, sulphatides in the one-to-one mixture; ○———○, cerebrosides in the one-to-one mixture; ▲———▲, total amount of bound lipids of the one-to-one mixture. The dotted lines indicate where the lipids were not completely dispersable by sonification. Each point represents the average of at least two experiments.

together and incubated with 2 nmol of activator protein. After electrophoresis the activator-bound lipids were calculated as described previously. There was a constant ratio of sulphatides and cerebrosides (60 : 40) bound by the activator protein over the whole concentration range examined (Fig. 7).

In Fig. 7 the amounts of each lipid component (from the one-to-one mixture) bound by the activator were added up to give the total amount of bound lipids. The resulting binding curve of the one-to-one mixture is compared with the binding curves for each component alone. Sulphatides were bound more than cerebrosides. In mixtures of both lipids, there was a competition between cerebrosides and sulphatides in their interactions with activator.

Discussion

As mentioned in Introduction it has been shown that glucocerebrosidase and its activator form a complex in the presence of phospholipids which was suggested to be enzymically active. Phospholipids, however, strongly inhibit the degradation of sulphatides (ref. 18, and unpublished results). Therefore experiments to elucidate the mechanism of activation in the case of sulphatase A and its activator were performed without addition of phospholipids.

Sulphatase A was incubated with a 12-fold excess of its activator protein under conditions which are usually used for the enzymic degradation of sulphatides. This mixture was then chromatographed on Sephadex. The eluted enzyme peak did not show activity against sulphatides without addition of activator. This indicates that no activator was present in the enzyme fraction and therefore no stable complex was formed between the enzyme and its activator (Fig. 1). However, the asymmetry of the enzyme peak trailing towards the activator peak indicates that there is a weak bond between these proteins.

To elucidate possible interactions between activator and sulphatides, they were incubated together under conditions similar to those used in degradation experiments and then subjected to polyacrylamide gel gradient electrophoresis. The electrophoretic migration behaviour of sulphatides shows that they form large aggregates and no small micelles in the concentration range used (Figs. 2 and 3a). Mixed micelles of sulphatides and taurodeoxycholate which should be of small size [19] penetrate the gel and migrate to section 8 (unpublished results). Together with the activator sulphatides penetrate the gel forming an one-to-one complex which is stable (Fig. 3b) during electrophoresis. This interaction between activator and sulphatides seems to be very specific since half maximal binding is obtained with about 2.5 nmol of sulphatides together with 1 or 2 nmol of activator in 100 μl (Fig. 4). The observation that half maximal binding occurred at a constant sulphatide concentration despite different activator concentrations could be due to the formation of sulphatide aggregates. Further experiments on the aggregation behaviour of sulphatides are necessary and could provide the basis for the calculation of the apparent association constant of the activator-sulphatide complex.

The activator-sulphatide complex partly dissociates due to dilution of the incubation solution (Table I). The dissociation of the complex seems to be faster than the rearrangement of sulphatides and the subsequent formation of new complex. Further experiments are necessary to determine the time dependence of these reactions, however.

The enzymic degradation products of sulphatides, the cerebrosides, are also bound by the activator but less specifically (Fig. 7). This may partly be due to the fact that they are dispersable by sonification to a smaller extent than sulphatides [20]. It is, however, possible that a one-to-one complex may be obtained at higher cerebroside concentrations (Fig. 5b).

In mixtures of sulphatides and cerebrosides both lipids are bound by the activator. The binding properties indicate that they compete for the same binding site (Fig. 6). This may explain the inhibitory effect of cerebrosides on the enzymic degradation of sulphatides by sulphatase A in the presence of the activator (ref. 18, and unpublished results). Due to the specific binding and kinetic data (unpublished results) it is suggested that only activator-bound sulphatides are degraded in vitro. This may also be the case in vivo since both the enzyme and its activator are localized in lysosomes [6]. The activator may thus work as a detergent-like protein, perhaps also for other sphingolipids, e.g. for the degradation of galactocerebrosides by galactocerebrosidase.

Acknowledgement

The skilful technical assistance of Mrs. V. Böhm is gratefully acknowledged.

References

1 Stinshoff, K. and Jatzkewitz, H. (1975) Biochim. Biophys. Acta 377, 126—138
2 Percy, A.K., Farrell, D.F. and Kaback, M.M. (1972) J. Neurochem. 19, 233—236
3 Porter, M.T., Fluharty, A.L., de la Flor, S.D. and Kihara, H. (1972) Biochim. Biophys. Acta 258, 769—778
4 Jerfy, A. and Roy, A.B. (1973) Biochim. Biophys. Acta 293, 178—190
5 Fischer, G. and Jatzkewitz, H. (1975) Hoppe-Seyler's Z. Physiol. Chem. 356, 605—613
6 Mraz, W., Fischer, G. and Jatzkewitz, H. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1181—1191
7 Mraz, W., Fischer, G. and Jatzkewitz, H. (1976) FEBS Lett. 67, 104—109
8 Ho, M.W. and Light, N.D. (1973) Biochem. J. 136, 821—823
9 Ho, M.W. and Rigby, M. (1975) Biochim. Biophys. Acta 397, 267—273
10 Li, S.-C. and Li. Y.-T. (1976) J. Biol. Chem. 251, 1159—1163
11 Stinshoff, K. (1972) Biochim. Biophys. Acta 276, 475—490
12 Baum, H., Dodgson, K.S. and Spencer, B. (1959) Clin. Chim. Acta 4, 453—455
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
14 Williams, D.E. and Reisfeld, R.A. (1964) Ann. N.Y. Acad. Sci. 121, 373—381
15 Maizel, J.V. (1966) Science 151, 988—990
16 Prat, J.P., Lamy, J.N. and Weill, J.D. (1969) Bull. Soc. Chim. Biol. 51, 1367—1371
17 Pellkofer, R. and Jatzkewitz, H. (1976) J. Neurochem. 27, 351—354
18 Mehl, E. and Jatzkewitz, H. (1964) Hoppe Seyler's Z. Physiol. Chem. 339, 260—275
19 Borgstrom, B. (1965) Biochim. Biophys. Acta 106, 171—183
20 Gammack, D.B., Perrin, J.H. and Saunders, L. (1964) Biochim. Biophys. Acta 84, 576—586