AN ACTIVATOR OF CEREBROSIDE SULPHATASE IN HUMAN NORMAL LIVER AND IN CASES OF CONGENITAL METACHROMATIC LEUKODYSTROPHY*

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1. Introduction

As previously reported, cerebroside sulphates (sulphatides) are the physiological substrates of arylsulphatase A (*EC 3.1.6.1*), *a lysosomal enzyme [1]*. This enzyme is deficient in cases of metachromatic leukodystrophy (ML), a sphingolipidosis following a recessive autosomal mode of inheritance [2]. The cerebroside sulphatase activity of purified arylsulphatase A is enhanced by a heat stable factor, which was first isolated from pig kidneys and described in terms of a complementary fraction of the enzyme [1]. This paper reports its presence not only in liver tissues from normal persons but also in patients suffering from metachromatic leukodystrophy. The factor, therefore, may be regarded now as an activator rather than a complementary fraction. Its enrichment by isoelectric focussing is described.

2. Materials and methods

2.1. *Purification procedures*

The preparation of arylsulphatase A was essentially the same as the one described by Stinshoff [3]. The source of the enzyme, however, was normal human liver.

For the isolation of the activator 15 g human liver, obtained within 24 hr post mortem, were homogenized in a mixture of 45 ml 0.2 M sodium acetate, pH 4.5, and 4.5 ml n-butanol (2—4°), stirred for 24 hr and centrifuged (17,000 *g*, 30 min). In a 4 ml sample of the supernatant the patterns of arylsulphatases A and B were established by isoelectric focussing at 12° after 24 hr of dialysing against 1% glycine solution [4]. The remaining supernatant was fractionated by acetone precipitation at −10°. The fraction precipitating between 33 and 66% (v:v) acetone was centrifuged (40,000 *g*, 10 min), suspended in and dialysed against 1% glycine solution, and again centrifuged. The resulting supernatant was heated for 30 min to 80°, cooled, and the clear supernatant subjected to isoelectric focussing.

The isoelectric focussing was performed in a microanalytical column [5]. The pH of the fractions obtained from the focussing column was measured in a micro-pH-meter (Radiometer, Copenhagen) and their protein content estimated by the A_{280} method (standard: bovine serum albumin). For the enzyme assay the pH of the fractions was adjusted to 4.5 by addition of NaOH or acetic acid.

To assay the cerebroside sulphatase activity and the effect of the activator, a mixture of 27 μg tritiated sulphatide [4, 6], 20 μl 0.5 M sodium acetate buffer, pH 4.5, and 40 μl of either distilled water or the different fractions from the isoelectric focussing of the activator preparation was prepared. The reaction was triggered by the addition of 40 μl of a solution of the purified arylsulphatase A in distilled water (= 1 μg protein). The amount of sulphatide degradation was measured after 1 hr of incubation at 37° as previously described [4].

Arylsulphatase A and B activities were determined with 2-hydroxy-5-nitrophenyl sulphate (Sigma Chem. Comp., St. Louis, Mo., USA) as substrate according to [4].

* Dedicated to Adolf Butenandt on his 70th birthday.

3. Results and discussion

The data presented here are obtained from normal (20 years at death) and ML (6 years at death) liver tissues removed at autopsy. They are similar to data obtained from another normal and two other ML cases (aged 6 and 16 years at death). The arylsulphatase patterns of the liver extracts confirm results previously obtained [2, 4]. In nonpathological tissues, essentially two arylsulphatases can be distinguished: the A enzyme with an IP of pH 4.8 and the nonhomogeneous B enzyme with IP's of pH 8.2 and pH 8.9 (fig. 1a). In the case of conventional ML, arylsulphatase A activity is deficient (fig. 2a).

The presence of n-butanol in the homogenate is required to extract the activator from the tissue. From the homogenate, both the activator and the enzyme are precipitated by the acetone treatment described in the methods section. Heat treatment (table 1) is found to completely destroy enzyme activity but does not affect the activator which can then be further purified by isoelectric focussing. In the absence of activator fractions, 1 μg of protein from the purified enzyme preparation degrades about 6.7 nmoles of sulphatides per hr. In the presence of the different fractions obtained by isoelectric focussing of the heated supernatant two effects are observed (fig. 1b): Fractions with an IP of about pH 4.5 stimulate the cerebroside sulphatase activity of the enzyme, whereas fractions with IP's lower than pH 4 and higher than pH 5.5 inhibit the enzyme activity. This decrease in activity is possibly nonspecific. It may be explained by an inhibition which proteins generally exert on the cerebroside sulphatase activity [8, and unpublished results]. The activating effect of fractions with an IP between 4 and 5 is such, that enzyme activity is increased up to 320% above the level observed in the absence of any additives.

Table 1

Arylsulphatase A activity and protein content from normal liver and liver from ML case (each 15 g).

	Arylsulphatase A activity (mU)		Protein content (mg)	
	Normal	ML	Normal	ML
Extract	1094	< 44	110	109
Acetone precipitate	1171	< 5	46	48
Solution after heat treatment	–	–	15.3	16.2

For details of the preparation see Materials and methods. Arylsulphatase A activity is measured according to [4]. 1 mU is defined as the amount of enzyme which degrades 1 nmole of of substrate per min at 37°. Protein is estimated according to [7].

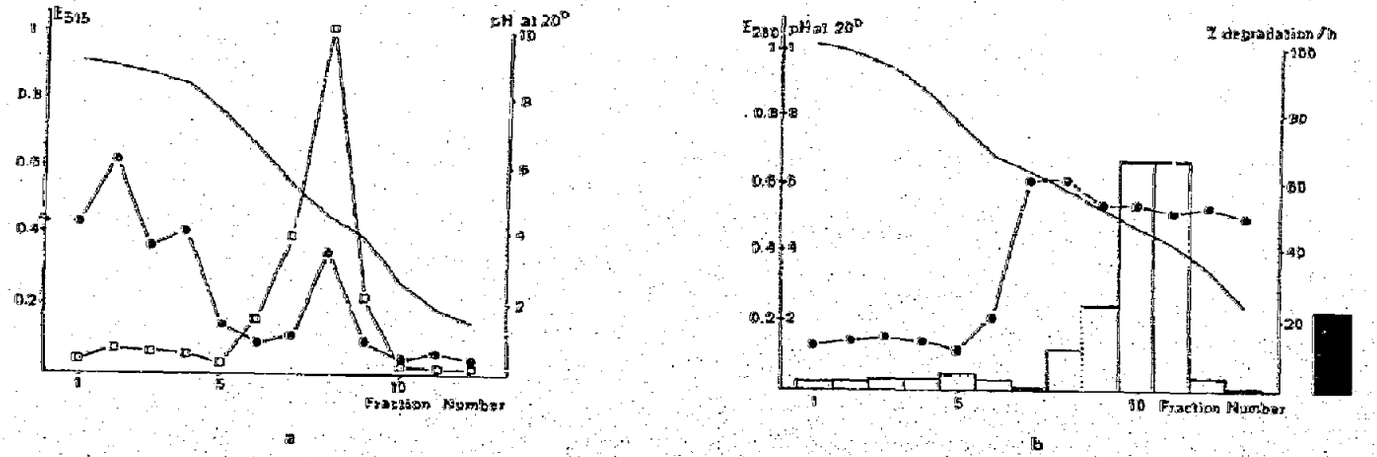

Fig. 1. Arylsulphatase and activator patterns obtained after isoelectric focussing of extracts from normal human liver. a) Arylsulphatase A and B pattern. Enzyme extract equivalent to 1 g of tissue (wet weight). Determination of enzyme activities according to [4]. (●—●—●) Assay of combined sulphatase A and B activities. (□—□—□) Assay of sulphatase A activity alone. Enzyme activity given as extinction of the liberated nitrocatecholate at 515 nm, 0.5 cm cuvette. Incubation time: 30 min. (——) pH-gradient. b) Activator pattern. Liver extract equivalent to 14 g of tissue (wet weight). (——) pH-gradient. (●—●—●) Protein content given as extinction at 280 nm, 0.5 cm cuvette. Light columns represent cerebroside sulphatase activity of a mixture of purified arylsulphatase A and fractions from the isoelectric focussing of the activator preparation, black column represents cerebroside sulphatase activity of arylsulphatase A alone. Assay conditions as outlined under Materials and methods.

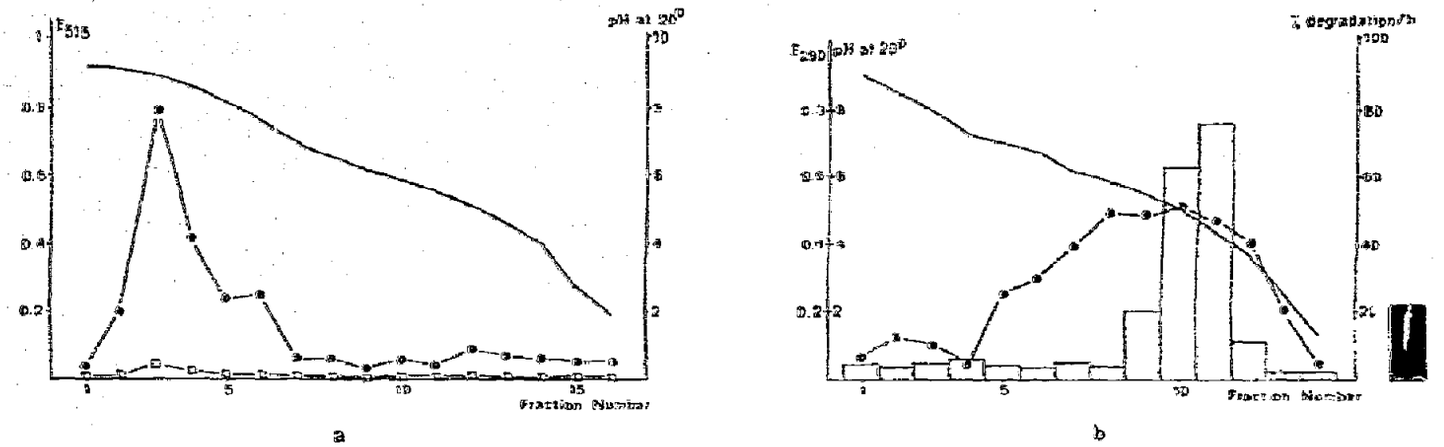

Fig. 2. Arylsulphatase and activator patterns obtained after isoelectric focussing of extracts from liver of a conventional ML case. a) Arylsulphatase pattern. Details as outlined under fig. 1a. b) Activator pattern. Details as outlined under fig. 1b.

Fig. 2b demonstrates the presence of the activator in the tissue of an ML case, which is largely deficient in arylsulphatase A activity. The activator of the ML case differs neither in its IP nor in its quantity from that of the normal case.

In contrast to a prior communication [1] it must, therefore, be assumed that the component in question can no longer be regarded as an integral part of cerebroside sulphatase but rather as an "activator". This assumption is supported by the observation that detergents such as taurodeoxycholate also stimulate the cerebroside sulphatase activity [8–10]. At present, however, there is no evidence that the activator from human tissues belongs to the group of cholates. Thus the nature of the activator still remains obscure.

References

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