ARYLSULPHATASE B (MAROTEAUX-LAMY FACTOR): A PART OF THE ENZYME SYSTEM RESPONSIBLE FOR SULPHATE RELEASE FROM MUCOPOLYSACCHARIDE FRAGMENT

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1. Introduction

Maroteaux-Lamy syndrome (Mucopolysaccharidosis VI) is characterized by excessive deposition in tissues and excretion of dermatan sulphate. Although it has been shown in several laboratories that the activity of arylsulphatase B is diminished in the patient's serum, urine and fibroblasts [1–5], the enzymatic basis of the metabolic defect has not been completely resolved. According to Matalon et al. [6] the marked diminution of sulphate release from [^{35}S]chondroitin-4-SO_4 and [^{35}S]heptasaccharide, which were extracted from fibroblasts with Maroteaux-Lamy syndrome may be due to chondroitin sulphate-*N*-acetylgalactosamine-4-sulphate sulphatase deficiency. The same conclusion was reached by O'Brien et al. [7] on the basis of the chemical structure analysis of a degradation product of dermatan sulphate derived from Maroteaux-Lamy fibroblasts. In a very recent work, Stumpf et al. [8] reported that UDP-*N*-acetylgalactosamine-4-sulphate might serve as substrate for arylsulphatase B.

The results presented in this paper suggest that although arylsulphatase B may be identical to Maroteaux-Lamy corrective factor, it is only a part of an enzyme system required for sulphate release from mucopolysaccharide fragments in vitro.

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2. Materials and methods

2.1. *Materials*

The reagents Δ-4S-disaccharide, Δ-6S-disaccharide and chondroitinase A, B and C (lot 7204) were obtained from Seikagaku Fine Biochem., Japan; Resin AG-1 X 8 (400 mesh Cl^- form) was obtained from Bio-Rad Laboratories, USA; Ampholine (pH 5.0–8.0) was obtained from LKB, Sweden; Nitrocatechol sulphate (dipotassium salt) and glucuronolactone were obtained from Sigma Chemical Corporation, St. Louis, USA, while Dextran Blue 2000. Dextran 10 000, Dextran 20 000, Sephadex G-50 and Sephadex G-10 were from Pharmacia Fine Chemicals, Uppsala, Sweden; Chondroitin-6-sulphate was from Miles, Labs, Elkhart, Indiana, USA; Heparin was from Boots Pure drug Co. Ltd, Nottingham, England and dermatan sulphate (Ro 1-2232/715) was a gift of Dr K. Von Berlepsh from Hoffmann-La Roche Laboratories, Switzerland.

2.2. *Methods*

Digestion of dermatan sulphate by chondroitinase A, B and C. Digestion of 10 mg of dermatan sulphate was carried out with one unit of chondroitinase A, B and C at 37°C for 24 h in 0.5 M Tris-acetate buffer, pH 8.1, in a total volume of 2 ml. Digestion of 10 mg of high molecular weight glycosaminoglycans (prepared according to the procedure of Di Ferrante et al. [9]), from urine of individuals with Maroteaux-

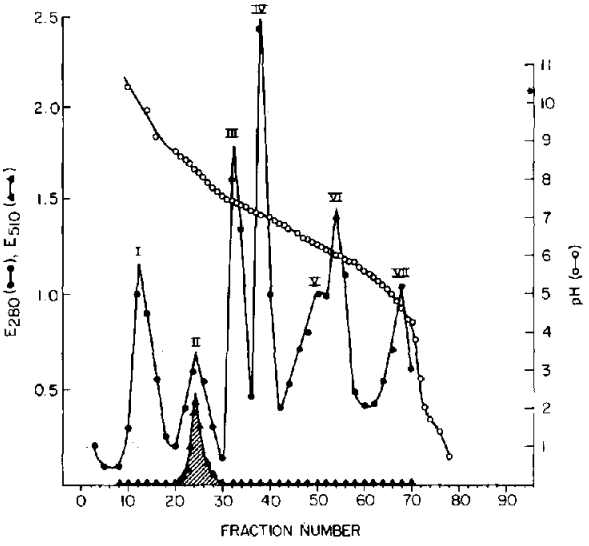

Fig.1. Isoelectric focusing of placental arylsulphatase $B_{\alpha, \beta, \gamma}$. The LKB isoelectric focusing column was filled according to Vesterberg [17]. A total of 20 mg of arylsulphatase $B_{\alpha, \beta, \gamma}$ (specific activity, 6.1 μmol of nitrocatechol converted min^{-1} mg^{-1}) were applied to an isoelectric focusing column (110 ml). Focusing was performed in pH 5.0–8.0 ampholines at 2°C and 500 V with a starting current of 12 mA. At the end of the experiment, 1.5 ml fractions were collected. Arylsulphatase B activity was determined using a 5 μl aliquots of each fraction in a 5 min incubation at 37°C with 10 mM nitrocatechol sulphate in 0.5 M sodium acetate, pH 5.8. Protein concentration was monitored at 280 nm and pH measured with a Corning Model 7 pH meter at 20°C.

Lamy syndrome, was carried out under identical conditions. The degradation products as well as Δ-4S-disaccharides and inorganic sulphate were separated on AG 1 × 8 ion exchanger column as described under fig.3. The primary product of degradation of dermatan sulphate was eluted with 0.9 N NaCl. The two degradation products obtained from urine of Maroteaux-Lamy affected individual were eluted at 0.7 and 1.2 N NaCl. The main degradation product of dermatan sulphate and the two fractions from Maroteaux-Lamy mucopolysaccharide were collected, concentrated and desalted on Sephadex G-10 column (2.8 × 70 cm).

Molecular weight determinations were carried out by gel filtration on Sephadex G-50 (fine); 0.9 × 110 cm column. Molecular weight standards included: nitrocatechol sulphate (mol. wt. 311), Δ-6S-disaccharide (mol. wt. 397), dextran 10 000 and 20 000, heparin (mol. wt. 11 000), dermatan sulphate (mol. wt. 27 000).

Polyacrylamide gel electrophoresis was performed according to the procedure of Weber et al. [10].

Arylsulphatase B activity was measured in 0.5 M acetate buffer, pH 5.8, using 10 mM 2-hydroxy-5-nitrocatechol sulphate as the substrate, according to the procedure of Robinson et al. [11].

Sulphate content was quantitated by the Dodgson procedure [12]. For estimation of total sulphate, the sample was hydrolysed in 0.25 N performic acid (100°C, 12 h) prior to determination. Uronic acid content was determined by the procedure of Wardi et al. [13]. Protein content was estimated by the method of Warburg and Christian [14].

Tissue culture experiments were performed as described by Di Ferrante et al. [15].

Arylsulphatase B from human placenta was prepared by the procedure of Gniot-Szulzycka [16] and subfractionated by isoelectrofocusing [17].

U.v. spectra were recorded on Beckman spectrophotometer; CD spectra for dermatan sulphate, octasaccharide and Δ-4S-disaccharide were measured on Cary 61 spectropolarimeter. The mean residue ellipticity, [Θ], was calculated according to the equation:

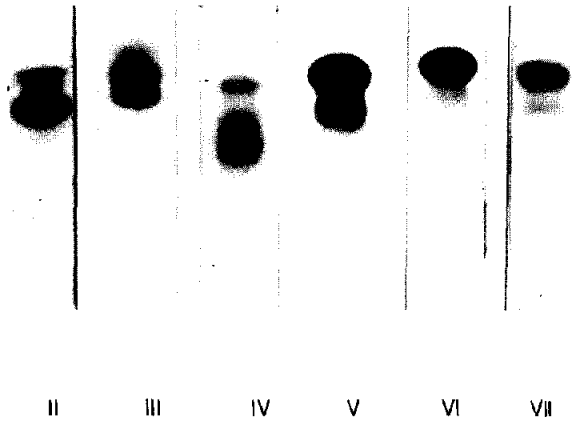

Fig.2. Acrylamide gel electrophoresis pattern of subfractions after isoelectric focusing. Electrophoresis was carried out at pH 7.8 in 5% polyacrylamide gels (8 mA/tube, 6 h), according to the procedure of Weber et al. [10]. 100 μg of protein were applied to each gel. Gels were stained for protein with Comassie brillant blue and destained in 5% acetic acid.

Table 1
Arylsulphatase B activity and corrective activity in tissue culture of different subfractions after isoelectric focusing

Fraction	I	II	III	IV	V	VI	VII	Total
p*I*	–	8.1	7.4	7.0	6.3	6.0	4.6	–
Total arylsulphatase B activity (nM of nitrocatechol per min)	70	8640	308	200	0	0	0	9220
Specific activity (μmoles of nitrocatechol per mg protein per min)	0.04	21.6	13.6	0.8	–	–	–	–
Percent of maximal correction in the presence of 75 μg of protein[a]	0	60	19	14	–	–	–	–

[a] 72 h $^{32}SO_4$ uptake of patient's fibroblasts in the presence of 75 μg of different subfractions after isoelectric focusing was measured.

$$[\Theta] = \frac{\Theta\lambda MRW}{10\,1c}$$

where MRW is the mean residue weight, Θ is the measured ellipticity at wavelength λ, c concentration in g/ml and 1 is the cell path length in cm (18).

4. Results and discussion

Seven different subfractions have been obtained after isoelectrofocusing of human placental arylsulphatase $B_{\alpha,\beta,\gamma}$ (specific activity of 6 μmol of nitrocatechol sulphate converted to nitrocatechol per min per mg of protein). Table 1 shows that in addition to the most active subfraction (II), subfractions I, III and IV revealed arylsulphatase activity after a 1 h incubation of the assay mixture. Fractions V, VI and VII did not exhibit any arylsulphatase B activity. The overall recovery of the enzymatic activity and protein was 50% and 73%, respectively. Two protein bands were present in all but one of the subfractions as demonstrated by polyacrylamide gel electrophoresis.

The most highly purified subfraction (II), specific activity 21 μmol nitrocatechol produced per min per mg protein (both protein bands were active), revealed the highest corrective activity (table 1). These data strongly suggest that the missing factor in Maroteaux-Lamy syndrome is identical to arylsulphatase B.

The highly purified subfraction of arylsulphatase B, contrary to the less purified enzyme, did not hydrolyse the mucopolysaccharide fragments which were obtained by digestion of dermatan sulphate by chondroitinase A, B and C and which eluted from AG 1 X 8 ion exchanger with 0.9 N NaCl (fig.3). The digestion product of chondroitinase A, B and C, according to the literature [19], should contain Δ-4S-disaccharide which should be eluted with 0.4 N NaCl from AG 1 X 8 resin.

However, the degradation product of dermatan sulphate Ro-1-2232/715 by chondroitinase A, B and C respectively gave a product which eluted with 0.9 N NaCl and had a mol. wt. of approx. 1600 (fig.4). The ratio of hexosamine, uronic acid and sulphate was 1.0 : 1.04 : 0.55. These data suggest that the degradation product is an octasaccharide. The CD spectrum for the octasaccharide (fig.5) appeared to be different to that for Δ-4S-disaccharide. Fig.6 shows u.v. spectra for octasaccharide, desulphated octasaccharide and dermatan sulphate.

The ability for sulphate release from this octasaccharide substrate can be restored by combining the subfraction with arylsulphatase B activity (p*I* = 8.1) with fractions containing no arylsulphatase B activity. The most active complementary subfraction had an isoelectric point of 6.0. From the octasaccharide substrate containing 24 μg of sulphate, 75% of the sulphate was released after incubation with 1.85 μg of subfraction II and 4 μg protein of subfrac-

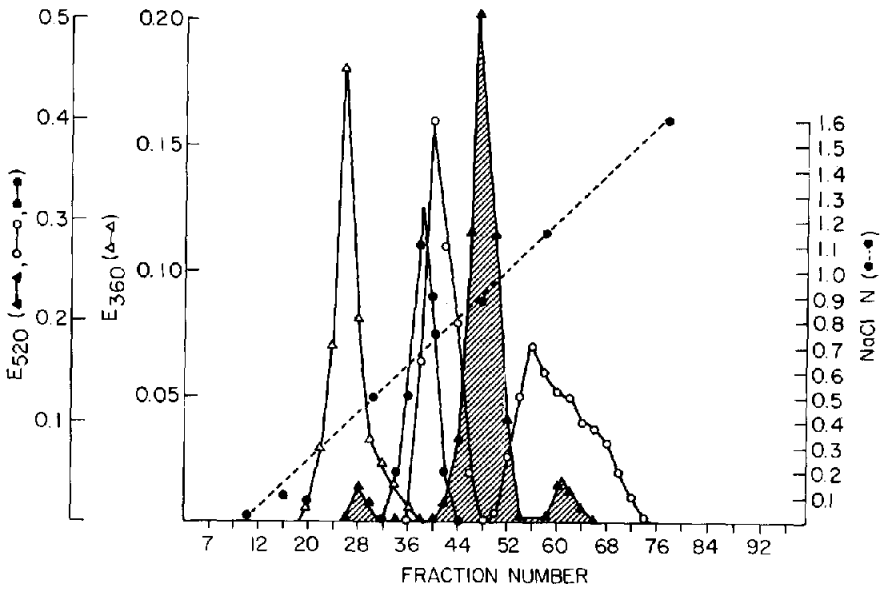

Fig.3. AG 1 X 8 chromatography of mucopolysaccharide fragments after chondroitinase A, B and C digestion of dermatan sulphate Separation was carried out on 15 X 1.5 cm columns of AG 1 X 8 resin. The following samples were diluted and applied to the column: 5 mg of dermatan sulphate digested by chondroitinase A, B and C (–▲–); the degradation products of the high molecular weight fraction of glycosaminoglycans from Maroteaux-Lamy urine (–○–); Δ-4S-disaccharide (–●–); and inorganic sulphate (–△–). Elution of each column was effected with a NaCl gradient consisting of 10% NaCl (120, ml; limit solution) and water (120 ml; receiving solution).

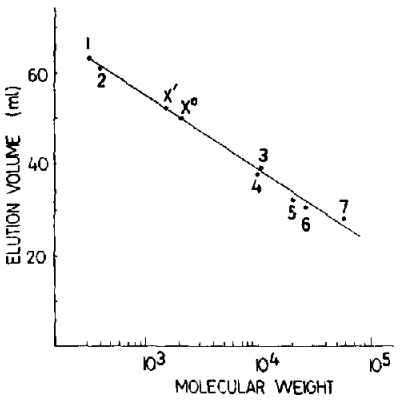

Fig.4. Mol. wt. determination of mucopolysaccharide fragment after chondroitinase A, B and C digestion of dermatan sulphate. The column (0.9 X 110 cm) was packed with Sephadex G-50 (fine) and standardized with: (1) nitrocatechol sulphate; (2) Δ-4S-disaccharide; (3) Dextran G 10 000; (4) Heparin; (5) Dextran G 20 000; (6) Dermatan sulphate; (7) Chondroitin-6-sulphate. X°, Degradation product of dermatan sulphate:, X', desulphated degradation product. Deionized water was used for elution, 1.7 ml fractions were collected.

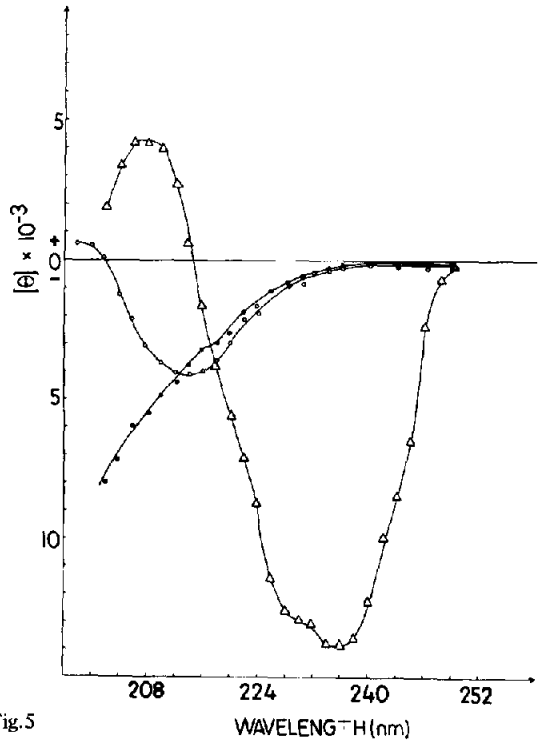

Fig.5

Fig.5. The circular dichroism spectra for dermatan sulphate, octasaccharide and Δ-4S-disaccharide. The circular dichroism (CD) spectra were recorded at room temperature on Cary 61 spectropolarimeter in 0.005 M Tris-HCl buffer pH 7.5. Dermatan sulphate (–●–); octasaccharide (–△–); Δ-4S-disaccharide (–○–).

tion VI. The reaction was carried out for 14 h at 37°C in 0.5 M acetate buffer, pH 5.8. The sulphate release changes either with increasing quantity of arylsulphatase B (p*I* = 8.1) or the subfraction VI (p*I* = 6.0). In addition, the mol. wt. of the octasaccharide modified by arylsulphatase B and subfraction VI remained greater than 1000, indicating that the substrate was not greatly degraded.

Subfraction II and VI, when combined, released only small quantities of sulphate (2–5%) from undegraded dermatan sulphate, Δ-4S-disaccharide, Δ-6S-disaccharide, galactosamine-4-sulphate or galactosamine-6-sulphate. The degradation product of glycosaminoglycans from Maroteaux-Lamy urine which eluted with 1.2 N NaCl did not serve as substrate, while that eluted with 0.7 N NaCl was hydrolysed (35% of the sulphate was released, table 2).

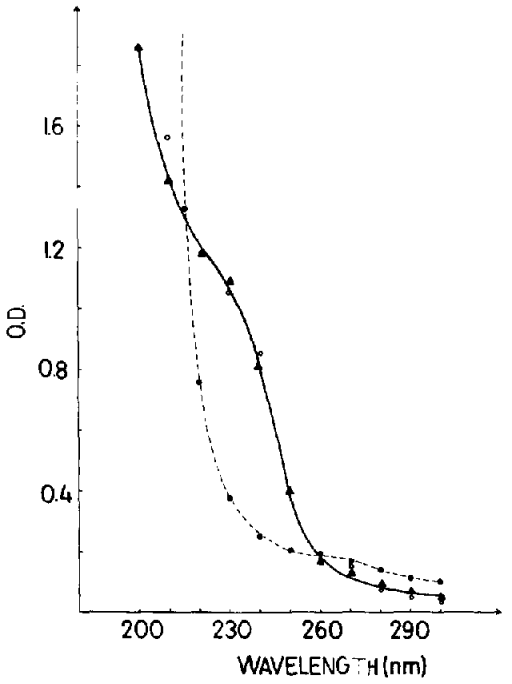

Fig.6. U.v. spectra for dermatan sulphate and octasaccharide before and after desulphation. The u.v. spectra were recorded in 0.005 M Tris-HCl buffer pH 7.5. Dermatan sulphate (–⊙–); octasaccharide before (–△–) and after desulphation (–○–).

Table 2
Sulphate release from various substrates in the presence of arylsulphatase B and subfraction VI (pH 6.0)

Substrate	Total amount of sulphate in the incubation mixture in μg	Percent of sulphate released after 14 h at 37°C in 0.5 M acetate buffer pH 5.8		
		Arylsulphatase B	Subfraction VI	Subfraction VI and Arylsulphatase B
Dermatan sulphate	50	0	4.4	5.0
Mucopolysaccharide fragment from Maroteaux-Lamy urine	24	0	2.1	37.0
Octasaccharide	48	1	1.3	76.0
Nitrocatechol sulphate	196	92	0	92.0

The reaction was carried out in the presence of 1.85 μg of arylsulphatase B and 4 μg protein of subfraction VI (p*I* 6.0.).

In addition, subfraction VI (p*I* = 6.0) did not contain β-glucuronidase, β-galactosidase, α- and β-*N*-acetylhexosaminidase or α-iduronidase activity and lost its biological activity upon boiling.

The requirement of two components for sulphate release from the natural substrate in vitro indicate the following: one of them may be responsible for the chemical conversion (arylsulphatase B), while the other component may modify the substrate or the enzymatic component in the reaction.

The substrate requirements of enzymes capable of sulphate release from mucopolysaccharides depended on the enzyme source. Substrate for the placental system described herein appears to be very similar to that from rat liver [20].

However, contrary to current data that indicate that desulphation takes place from the non-reducing end of the sugar moiety, the data presented here (high sulphate release, 75%, and high mol. wt. of the modified product) support the suggestion of Matalon et al. [6] that the internal sulphate group might be released as well.

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References

[1] Barton, R. W. and Neufeld, E. F. (1972) J. Pediatr. 80, 114.
[2] Stumpf, A. D., Austin, J. H., Crocker, A. G. and La France, M. (1973) Am. J. Dis. Child. 126, 747.
[3] Kihara, H., Fluharty, A. L. and Stevens, R. L., Comm. at the 25th Meeting of the American Society of Human Genetics, Atlanta, Georgia, Oct. 1973, 24–27.
[4] Fluharty, A. L., Stevens, R. L., Sander, D. L. and Kihara, H. (1974) Biochem. Biophys. Res. Commun. 59, 455.
[5] DiFerrante, N., Hyman, B. H., Klish, W., Donnelly, P. V., Nichols, B. L., Dutton, P. V. and Gniot-Szulzycka, J. (1974) John Hopkins Med. J. 135, 42.
[6] Matalon, P., Arbogart, B. and Dorfman, A. (1974) Biochem. Biophys. Res. Commun. 61, 6.
[7] O'Brien, J. F., Cantz, M. and Spranger, J. (1974) Biochem. Biophys. Res. Commun. 60, 1170.
[8] Fluharty, A. L., Stevens, R. L., Fung, D., Peak, S. and Kihara, H. (1975) Biochem. Biophys. Res. Commun. 64, 955.
[9] DiFerrante, N., Neri, C., Neri, M. and Hogsett, W. E. (1972) Conn. Tissue Res. 1, 93.
[10] Weber, K., Pringle, J. R. and Osborn, M. (1972) in: Methods in Enzymology XXXVI (Jakoby, W. B. ed.), pp. 3–27, Academic Press, New York, London.
[11] Robinson, D., Smith, J. N. and Williams, R. T. (1951) Biochem. J. 49, XXIV.
[12] Dodgson, K. S. (1961) Biochem. J. 78, 312.
[13] Wardi, A. H., Michos, G. A., Allen, W. S., Varma, R. and Varma, R. S. (1974) Biochem. Med. 11, 93.
[14] Colowick, S. P. and Kaplan, N. O. (1957) in: Methods in Enzymology Vol. 3, pp. 451–454, Academic Press, New York.
[15] DiFerrante, N., Nichols, B. L., Knudson, A. G., McCredie, B. K., Singh, J. and Donnelly, P. V. (1973) Birth Defects, Original Article Series 9, 31.
[16] Gniot-Szulzycka, J., (1972) Acta Biochem. Pol. 19, 181.
[17] Vesterberg, O. (1972) in: Methods in Enzymology XXII (Jakoby, W. B. ed.), pp. 389–430, Academic Press, New York.
[18] Pownall, H. J., Morrisett, J. D., Sparrow, J. T. and Gotto, A. M. (1974) Biochem. Biophys. Res. Comm. 60, 779.
[19] Suzuki, S., Saito, H., Yamagata, T., Anno, K., Seno, N., Kawai, Y. and Furuhashi, T. (1968) J. Biol. Chem. 243, 1543.
[20] Tudball, N. and Davidson, E. A. (1969) Biochim. Biophys. Acta 171, 113.