ARYLSULFATASE B DEFICIENCY IN MAROTEAUX-LAMY SYNDROME CULTURED FIBROBLASTS

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Summary: Fibroblasts derived from patients with Maroteaux-Lamy syndrome (mucopolysaccharidosis VI) contained about 10% of normal arylsulfatase B activity, other lysosomal enzymes being unaltered. The properties of residual arylsulfatase B in Maroteaux-Lamy fibroblasts were identical to the enzyme derived from control fibroblasts. The decreased mucopolysaccharide turnover in Maroteaux-Lamy fibroblasts was normalized by supplementation with an arylsulfatase B fraction from normal fibroblasts. This data is consistent with the suggestion that the primary biochemical defect in Maroteaux-Lamy syndrome is a deficiency of arylsulfatase B.

The Maroteaux-Lamy syndrome, mucopolysaccharidosis VI, is characterized by severe skeletal deformaties, gross corneal opacity, marked retardation of growth, and normal intellect [1]. Dermatan sulfate is the only mucopolysaccharide excreted in excess. This disorder is rare, but in the cases reported by Maroteaux et al. [2] and McKusick et al. [3] an autosomal recessive mode of inheritance is suggested. Barton and Neufeld [4], by the corrective factor technique in cultured cell systems, have shown that the factor deficient in Maroteaux-Lamy syndrome is different from the factors deficient in Hurler and Scheie, Hunter, and the two Sanfilippo syndromes. Stumpf et al. [5] have reported that tissue of patients with Maroteaux-Lamy syndrome have about 10% of the arylsulfatase B activity of control tissue. We present evidence that Maroteaux-Lamy fibroblasts are also deficient in arylsulfatase B activity. A preliminary communication of these findings has been presented [6].

MATERIALS AND METHODS

Fibroblast cultures - The Maroteaux-Lamy culture designated M/L-1 was derived from one of the affected sibs reported by McKusick et al. [3], and was obtained from the Repository for Mutant Human Cell Strains in Montreal. Cul-

tures designated M/L-2 and M/L-3 were provided by Dr. Michael M. Kaback and Dr. Elizabeth F. Neufeld. Cells were cultured in MEM (obtained as powdered medium No. F-11, Grand Island Biological Co.) supplemented with 24 mM sodium bicarbonate, 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethansulfonic acid) (Calbiochem), 0.5% aureomycin, and 10% fetal calf serum, adjusted to pH 7.4. For labeling mucopolysaccharides 30 μc/ml of ^{35}S-sulfate was added to the medium. After 3 days the radioactive media was removed, the cells rinsed, and unlabeled medium added. To test for metabolic correction the medium was supplemented with 3 units/ml of an arylsulfatase B fraction prepared from normal fibroblast extract by passage through DEAE-cellulose, adsorption to SP-Sephadex, and elution by a NaCl gradient. Radioactive cellular mucopolysaccharides were determined in dialyzed cell extracts similar to those used for enzyme assay.

Enzyme assays - Cells were harvested by trypsinization, suspended in 100 mM Tris-HCl, pH 7.5, and lysed by 6 cycles of freeze-thawing. Cell debris was sedimented (1 min, 14,000 x g) and the supernatant fluid dialyzed overnight against 1000 vol of 25 mM Tris-HCl, pH 7.5. Aliquots were used for protein determination [7], direct enzyme assays, and DEAE-cellulose chromatography. All enzyme activities including those from DEAE-cellulose fractions are referred to the protein present in dialyzed extracts. 4-Nitrocatechol sulfatase activities were determined by the A and B specific assays of Baum et al. [8] as modified in this laboratory [9]. β-Hexosaminidase activity was determined with β-p-nitrophenyl-N-acetylglucosaminide [10]; α-iduronidase with phenyl-α-iduronide by a modification of the procedure of Hall and Neufeld [11]; β-galactosidase, α-glucosidase and β-glucuronidase with 4-methylumbelliferyl derivatives by procedures optimized in this laboratory for fibroblast extracts. Fractions from the DEAE-cellulose chromatography were analyzed for arylsulfatase activity with 4-methylumbelliferyl sulfate. An aliquot (50 μliters) of each fraction and 100 μliters of substrate solution (10 mM 4-methylumbelliferyl sulfate; 100 mM sodium acetate, pH 5.5; 100 mM NaCl; and bovine serum albumin

0.2 mg/ml) were incubated at 37° for 60 min. One ml of 0.4 M glycine-NaOH, pH 10.5 was then added and the fluorescence determined at 365 nm excitation and 450 nm emission.

DEAE-Cellulose chromatography - DEAE-Cellulose (Microgranular DE-32, Whatman) equilibrated with 25 mM Tris-HCl, pH 7.5, was poured to give packed columns 0.8 x 6 cm. The columns were charged with dialyzed fibroblast extracts, washed through with the buffer and developed with 26 ml of a linear gradient, 0 - 0.5 M NaCl, in buffer.

RESULTS AND DISCUSSION

Examination of the sulfatase distribution in fibroblast extracts by DEAE-cellulose chromatography showed that cells from Maroteaux-Lamy cultures were deficient in arylsulfatase B (Fig. 1). The cationic arylsulfatase B (I) was not adsorbed by the ion exchanger, while anionic arylsulfatases were retained. On application of the salt gradient, a minor fraction with arylsulfatase activity (II) was first eluted followed by arylsulfatase A (III) [12]. The total activities in fractions I, II and III, as established by pooling appropriate tubes, are shown in Table I. Maroteaux-Lamy cells showed on the average a 90% deficiency of arylsulfatase B activity. The minor anionic arylsulfatase activity was also depressed while arylsulfatase A levels were within the normal range. A variety of lysosomal acid hydrolases were also assayed in the dialyzed extracts prior to ion exchange fractionation. With the exception of arylsulfatase B, all activities were present in normal amounts as shown in Table I. While a clear defect in the arylsulfatase B levels could be demonstrated for unfractionated extracts by the assay of Baum et al. [8] quantitation of the residual enzyme levels was unreliable when normal amounts of arylsulfatase A were present. Reliance of arylsulfatase B levels has to be placed on data with fractionated enzymes.

The properties of the residual arylsulfatase B from Maroteaux-Lamy fibroblasts could not be distinguished from those of normal enzyme. Utilizing 4-methylumbelliferyl sulfate as substrate, the pH activity profile was similar

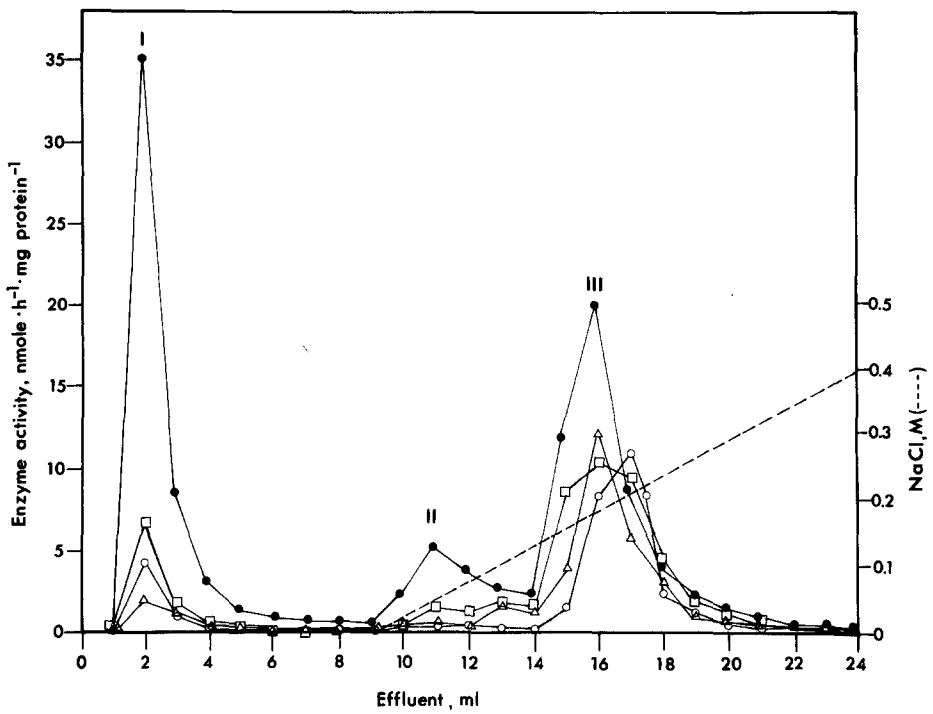

Fig. 1. The fractionation pattern of fibroblast arylsulfatase B on DEAE-cellulose. See methods for details (●—●) Control: (△—△) M/L-1; (○—○) M/L-2; and (■—■) M/L-3. The peaks are designated: I, arylsulfatase B; II, minor anionic arylsulfatase; and III, arylsulfatase A.

for enzyme from both sources with a broad peak at about pH 5.2. The K_m was around 5 mM for either enzyme. The rate of heat inactivation at 60° was also similar with 50% inactivation occurring in 30 min. Silver ions up to 0.2 mM did not inhibit either enzyme in contrast to arylsulfatase A which is markedly inhibited at this concentration [13].

Maroteaux-Lamy fibroblasts show an attenuated rate of decay of ^{35}S-labeled mucopolysaccharides [4]. When we supplemented the growth medium of such cells with a partially purified arylsulfatase B from control fibroblasts, enzyme activity of the cells was enhanced 4 fold and a normal decline in labeled-mucopolysaccharides was observed (Table II). A sample of 10-fold purified Maroteaux-Lamy factor from human urine [4] kindly provided by Dr. Elizabeth F. Neufeld was examined for arylsulfatases by DEAE-cellulose

TABLE I

ENZYME ACTIVITIES OF MAROTEAUX-LAMY FIBROBLASTS

Enzyme	Cell Strains			
	Control	M/L-1	M/L-2	M/L-3
DEAE-Cellulose fractions[a]	nmole/hr/mg extracted protein			
I - Arylsulfatase B	48	3.5	5.5	9.5
II - Minor anionic arylsulfatase	15	2.9	1.2	5.2
III - Arylsulfatase A	49	28	33	38
Direct assay				
Arylsulfatase B (Baum assay)	1,160	62[b]	2.0[b]	-
Arylsulfatase A (Baum assay)	1,720	880	1,100	1,380
β-Hexosaminidase	47,600	47,900	39,000	43,200
β-Galactosidase	550	710	810	670
α-Glucosidase	710	410	440	530
β-Glucuronidase	1,620	1,150	720	830
α-Iduronidase	44	31	31	-

[a]Column fractions were measured by hydrolysis of 4-methylumbelliferyl sulfate.
[b]Determinations from this assay are unreliable when there are very low quantities of arylsulfatase B in the presence of normal quantities of arylsulfatase A.

chromatography. Arylsulfatase B was present at a level commensurate with 10-fold enrichment from urine and was the only arylsulfatase detected.

Some apparent deficiencies of lysosomal hydrolases in tissues from patients with mucopolysaccharidoses have been attributed to the inhibitory effects of accumulated mucopolysaccharides [1]. While arylsulfatase B can be partially inhibited by dermatan sulfate, only a 40% depression of activity was achieved at 25 mg/ml. This is far in excess of the amount which could have been added with extracts of even heavily laden cells. Mixtures of Maroteaux-

TABLE II

NORMALIZATION OF MUCOPOLYSACCHARIDE TURNOVER BY AN ARYLSULFATASE B FRACTION

Cell Strain	^{35}S-MPS in cells returned to unlabeled medium	
	day 0	day 4
	CPM/mg extracted protein	
Control	17,600	2,300
M/L-1	18,700	9,770
M/L-1 plus arylsulfatase B		2,880

Lamy and normal cell fractions had the expected average enzyme activity. Moreover, cells from Hurler and Hunter syndrome patients, which also accumulate dermatan sulfate, do not show similar arylsulfatase B depressions. In fact, liver from a Hunter syndrome patient has been reported to have a somewhat enhanced level of this enzyme [14]. Mucopolysaccharide inhibition therefore could not account for the observed deficiency of arylsulfatase B activity. It thus appears that arylsulfatase B deficiency is probably a primary biochemical defect in Maroteaux-Lamy syndrome. It also appears that the minor anionic arylsulfatase in fraction II is generically related to arylsulfatase B. Finally, there is the question of whether the residual activity in Maroteaux-Lamy fibroblasts is due to a mutant enzyme or whether it represents an entirely different enzyme within a heterogeneous arylsulfatase B fraction. This can be resolved best when the physiological substrate specificity for arylsulfatases has been established.

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