REDUCED ARYLSULFATASE B ACTIVITY OF THE MUTANT ENZYME PROTEIN IN MAROTEAUX-LAMY SYNDROME

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SUMMARY

A mutant arylsulfatase B form is described in skin fibroblasts from a patient with Maroteaux-Lamy syndrome. This mutant enzyme is identical in its electrophoretic mobility and antigenic determinants to arylsulfatase B from normal fibroblasts but has a markedly reduced (15%) specific activity.

INTRODUCTION

Mucopolysaccharidosis type VI, Maroteaux-Lamy syndrome (MLS) is an autosomal recessive disorder which is characterized by hepatosplenomegaly, corneal opacities, skeletal malformations, and increased urinary excretion of dermatan sulfate [1,2]. In 1972, Barton and Neufeld [3] reported the presence of a protein fraction in normal human urine which could correct abnormal accumulation of $^{35}SO_4$ labeled mucopolysaccharide in fibroblasts from patients with MLS. Recently, liver, kidney, spleen, brain and cultivated fibroblasts from patients with MLS were shown to contain reduced activity of arylsulfatase B (ASB) [4].

The present study was undertaken to determine the nature of the residual ASB activity in patients with MLS. These studies demonstrate the presence of an antigenically cross-reacting material which has decreased enzymatic activity in skin fibroblasts from a patient with this disorder.

MATERIALS AND METHODS

Tissue Preparation: Fibroblasts were cultivated from skin biopsies from a normal control and MLS patient. The cultures were grown under conditions

Abbreviations: MLS, Maroteaux-Lamy syndrome; ASB, arylsulfatase B.

previously described [5] in Modified Eagle's Medium (GIBCO). Cells grown for three weeks in 100 mm Falcon Petri dishes reached a cell density of 1.6×10^7. The pH was maintained between 7.2 and 7.4. Mycoplasma monitoring was carried out routinely with mycoplasma agar medium (GIBCO). Fibroblast monolayers were washed three times with 0.05 M acetate buffer containing 0.05 M NaCl, pH 5.5, and then harvested with a rubber policeman. The cells were suspended in 0.03 M Tris-HCl buffer, pH 7.4, containing 0.2% Triton X 100 (1 ml buffer per 5 plates pellet) and disrupted by sonication for 60 seconds at 10,000 cycles (power setting 3) in a Branson Sonifier. The homogenate was stirred for 2 hours at room temperature and then dialyzed for 16 hours against 0.03 M Tris-HCl buffer, pH 7.4, (1,000 fold volume excess with two changes). The insoluble fraction was removed by centrifugation for 45 minutes at 105,000 x g.

The soluble fraction of normal human liver homogenate was prepared as previously described [6].

Fibroblast ASB Preparation: The soluble fibroblast fraction was added to DEAE-cellulose (cellex D, Bio-Rad) equilibrated with the same buffer. After stirring for 2 hours at 4° C, the DEAE-cellulose was removed by centrifugation. The protein fraction that was not adsorbed on the DEAE-cellulose in 0.03 M buffer contained only the ASB whereas arylsulfatase A and the minor sulfatase fraction with "ASB-like" activity [7] were adsorbed to the DEAE-cellulose and could be eluted at higher molarity (0.5 M sodium chloride in 0.05 M Tris-HCl buffer, pH 7.4).

Enzymatic Activity: ASB activity was determined using p-nitrocatechol sulfate (Sigma) as a substrate according to Baum et al. [8] in the same manner as previously described [6]. The reaction was performed for 10 minutes at 37° C and the liberated nitrocatechol was calculated taking 12,400 as the molar extinction coefficient [8]. The protein concentration of the samples was determined according to Lowry et al. [9] and the enzymatic activities were expressed as nmoles/min/mg protein.

Polyacrylamide Gel Electrophoresis: Polyacrylamide gel electrophoresis (7 cm discs) was carried out according to Davis [10] in 7.5% gels with β-alanine-acetic acid buffer, pH 4.0, for 3 hours at 4 mAmp/gel. Bands with arylsulfatase activity were located by incubating the gels in 10 mM p-nitrocatechol sulfate in 0.5 M sodium acetate-acetic acid buffer, pH 5.6, for 1 hour at 38° C. The gels were then transferred into 1 N NaOH for 2 to 3 minutes, and photographed before the fading of the red bands.

Immunological Procedures: The preparation of a monospecific antiserum against purified human liver ASB, the antibody titer and the IgG preparation are described elsewhere [6]. Double diffusion in agar gel and the demonstration of the residual enzymatic activity of the immun-precipitate were performed as previously described [6]. Single radial immunodiffusion was carried out according to Mancini et al. [11] as follows. To 30 ml of agarose (1% in phosphate buffered saline, pH 7.2, at 45° C), 150 μliters of the anti-ASB IgG fraction (4.17 mg protein) was added, mixed and poured into a disposable plastic Petri dish (9 cm in diameter). The plates were kept at 4° C for 6 to 8 hours afterwhich wells of identical size (2.9 mm in diameter) were punched out using an appropriate template. In each well, 20 μliters of enzyme preparation with known protein concentration and enzymatic activity were applied. Diffusion was completed in a humid chamber at room temperature for 48 hours. Proteins that did not precipitate in the immune complex were removed by exhaustive washing in phosphate buffered saline and the precipitin rings were stained with 1% amido black. The ring diameters were measured by measuring magnifyer (Bausch and Lomb) with a seven-fold magnification. The determination at each concentration was performed in duplicate.

RESULTS

The soluble protein fraction of normal fibroblasts and of MLS fibroblasts had protein concentrations of 7.4 mg/ml and 7.5 mg/ml respectively. Each of the preparations (200 μliters) was subjected to polyacrylamide gel electrophoresis

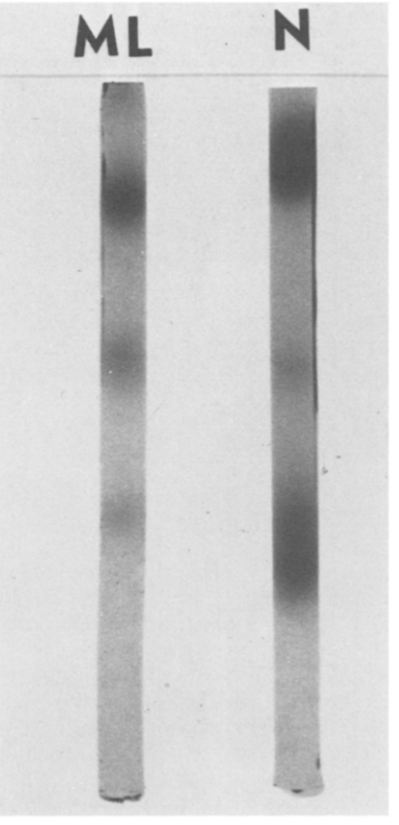

Figure 1: Polyacrylamide gel electrophoresis of normal [N] and Maroteaux-Lamy [ML] fibroblast lysates.

and the various bands with arylsulfatase activity were revealed. The results are shown in Figure 1. As seen in this figure, the major ASB band with fastest electrophoretic mobility in these conditions was shown to be largely diminished in the MLS preparation as compared to the normal fibroblasts.

The two fibroblast preparations and a crude liver homogenate were examined by double gel diffusion against anti-ASB antiserum (Figure 2, left side). A precipitin line of identity was demonstrated for the three preparations. When the residual enzymatic activity of the precipitin bands was examined (Figure 2, right side), only the normal liver and the normal fibroblast lines demonstrated enzymatic activity as depicted by this procedure.

In order to exclude the possibility that the antigenic cross-reacting

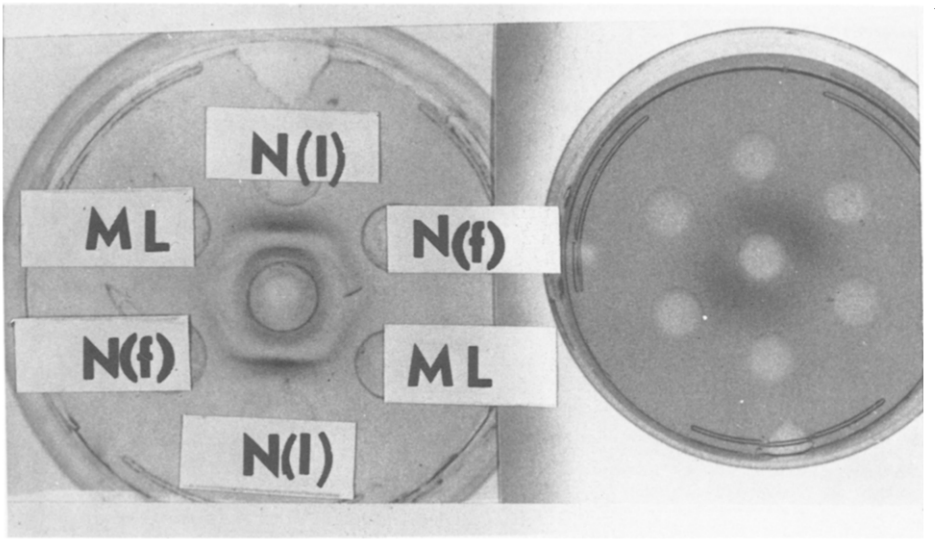

Figure 2: Double gel diffusion of normal liver [N(l)], normal fibroblast [N(f)], and Maroteaux-Lamy fibroblast [ML] lysates against anti-arylsulfatase B antiserum (central well). On the right side of the figure, the protein staining of the precipitin lines and on the left side, a duplicate plate in which the residual enzymatic activity of the lines are demonstrated.

materials in MLS is the "minor B component", the major ASB protein was separated from the fibroblast lysates by a DEAE-cellulose batch procedure as described in Materials and Methods. The ASB containing protein fraction was concentrated by vacuum ultrafiltrations. Only one enzymatically active band, corresponding to the major ASB, was shown by disc electrophoresis. When the normal and MLS ASB fractions were compared by double gel diffusion against anti-ASB antiserum, a precipitin line of identity was revealed for both preparations. The enzymatic activity for the normal fibroblast ASB preparation was 159 nmoles/min/mg protein whereas for the MLS ASB preparation it was 24 nmoles/min/mg protein.

Three dilutions of each of the two ASB preparations, normal and MLS, were prepared and the protein concentration and enzymatic activity of each of the different dilutions determined. The concentration of the antigenically cross-reacting material with anti-ASB antibodies was determined by single radial immunodiffusion. When the relative concentration of ASB in the

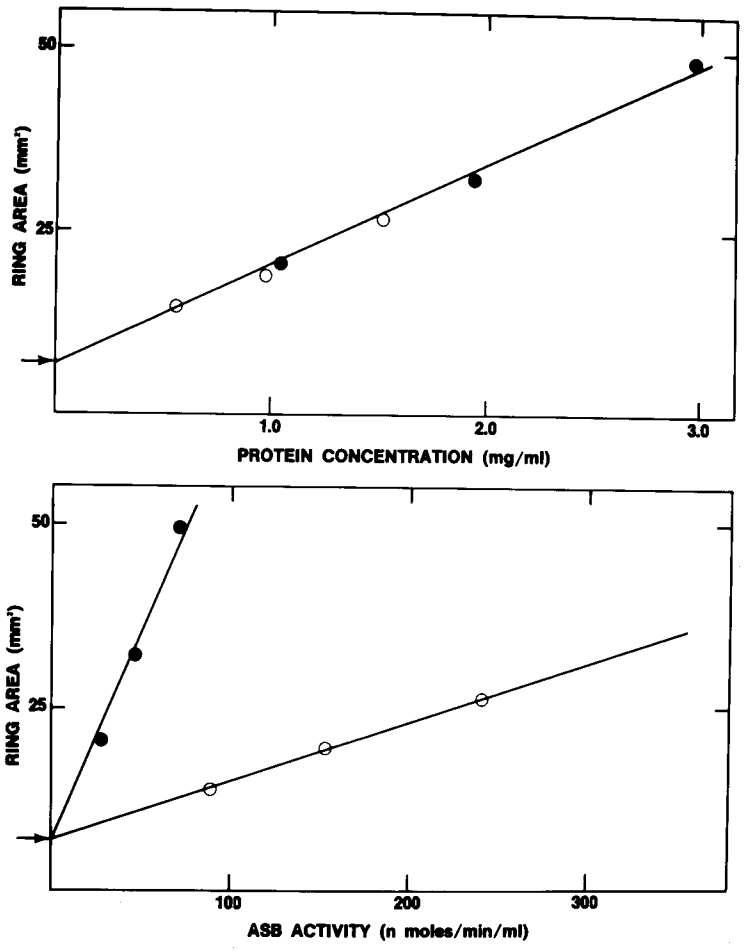

Figure 3: Single radial immunodiffusion of various dilutions of the protein fraction with arylsulfatase B from normal [o] and Maroteaux-Lamy [•] fibroblasts. Upper figure shows the correlation between the ring area to the protein concentration and the lower figure shows the correlation with the enzymatic activity. The arrow indicates the inner well area.

preparation was determined by ring area, it was proportional to the protein concentration (Figure 3, upper part). When the ring area was plotted against the enzymatic activity (Figure 3, lower part), it was shown that the relative enzymatic activity of MLS mutant protein was much lower.

DISCUSSION

This study demonstrated the presence of an immunologically cross-reacting

protein with ASB in MLS fibroblasts. This ASB mutant was identical in its electrophoretic mobility (Figure 1) to the normal fibroblast ASB. The antigenic determinants of the mutant enzyme were identical to those of normal fibroblast ASB and normal liver ASB as could be determined by double gel diffusion (Figure 2). Since the normal and mutant enzyme forms revealed an identical precipitin line, the antiserum prepared against normal ASB was used for the quantitative immunoassay of both ASB and its mutant. Utilizing this procedure, the mutant protein concentration of the MLS fibroblast ASB fraction was shown to be similar to that found for normal fibroblasts. In contrast, the relative enzymatic activity of the immunologically cross-reacting material in MLS was only 15% of normal (Figure 3). Fluharty *et al.* [12], using similar procedures for ASB preparation and utilizing a different synthetic substrate, 4-methylumbelliferyl sulfate, reported 10% residual enzymatic activity in the MLS ASB fraction.

O'Brien *et al.* [13] demonstrated a markedly reduced N-acetylgalactosamine-4-sulfatase activity towards dermatan sulfate in MLS fibroblasts. Recent studies by Matalon and Dorfman (personal communication) have indicated that the natural substrate for ASB is the sulfate at carbon-4 position of N-galactosamine in both dermatan sulfate and chondroitin sulfate. These observations suggest that the ASB mutant protein with its reduced activity for both synthetic and natural substrates is responsible for the biochemical aberrations of at least one form of MLS.

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