

Various Sulfatase Activities in Leukocytes and Cultured Skin Fibroblasts from Heterozygotes for the Multiple Sulfatase Deficiency (Mukosulfatidosis)

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Summary

In heterozygotes for multiple sulfatase deficiency (MSD), several sulfatase activities including arylsulfatases A, B1, B2, and C, and cholesterol sulfatase were 40-50% of normals in cultured skin fibroblasts and 70-80% of normals in leukocytes. In MSD patients, these enzyme activities were deficient or reduced. DEAE-Sepharose column chromatographic patterns of 4-methylumbelliferyl sulfatases A, B1, and B2 in leukocytes and cultured skin fibroblasts from MSD patients and heterozygotes were also consistent with the above data. These data indicate that several sulfatase activities in heterozygotes of MSD exhibited intermediate activities as observed in the heterozygote state of other autosomal recessive inherited diseases.

Abbreviations

MDS, multiple sulfatase deficiency
MLD, metachromatic leukodystrophy
4-MU, 4-methylumbelliferyl

Multiple sulfatase deficiency (MSD) is an autosomal recessive disorder, which is clinically characterized by gargoyl-like appearance, skeletal abnormality, hepatosplenomegaly, and ichthyosis (1, 2). Biochemically, the accumulation of sulfatide, acid mucopolysaccharides, and cholesterol sulfate accompanies deficiencies of arylsulfatases A, B, and C, mucopolysaccharide sulfatases (sulfoiduronate sulfatase, sulfamidase, galactosamine-4-sulfatase, galactosamine-6-sulfatase) and steroid sulfatases (cholesterol sulfatase, dehydroepiandrosterone sulfatase, estrone sulfatase, pregnenolone sulfatase) in patients' tissues and cultured skin fibroblasts (1, 2, 3, 7, 18).

This disorder is of considerable theoretical interest in genetics, because there are deficient activities of several different enzymes. In terms of the genetic controlling mechanisms of sulfatases, it is important to determine whether sulfatase activities in the heterozygote exhibit intermediate activities.

This report demonstrates that various sulfatase activities in heterozygotes were about one-half of controls in cultured skin fibroblasts and 70-80% of controls in leukocytes. In addition, the arylsulfatase characteristics in MSD patients were indistinguishable from those in normals.

MATERIALS AND METHODS

The clinical and biochemical findings of a Japanese case of MSD were reported previously (9).

Cell preparations. Leukocytes from MSD, metachromatic leukodystrophy (MLD), and their heterozygotes (father and mother) were prepared as described by Snyder and Brady (20). Fibroblasts were cultivated from skin biopsies from normal controls, MLD,

MSD, and a MSD obligate heterozygote (mother). Three different cell cultures of MSD proband were kindly obtained from Prof. H. Kihara (University of California, Pomona USA). Two MSD probands (GM 3245 and GM 2407) and two MSD heterozygote cell cultures (GM 2644 and GM 2667) were shipped from the Human Genetic Cell Repository, Camden, NJ. The cultures were grown under conditions described previously (7). The leukocyte or fibroblast cells were suspended in 0.05 M Tris-HCl buffer, pH 7.4 and dialyzed overnight against 0.02 M Tris-HCl buffer (pH 7.4). The cells were disrupted by sonication for 60 sec at 40 "K" cycles in a Branson sonifier. The homogenates were used for enzyme assays of arylsulfatase C, cholesterol sulfatase, and heparin N-sulfatase. For the determination of other sulfatases (arylsulfatases A and B), the homogenate was centrifuged at 100,000 × g for 30 min and the clear supernatant was used for enzyme assays. For the fractionation with DEAE-Sepharose column chromatography, the soluble fraction was used.

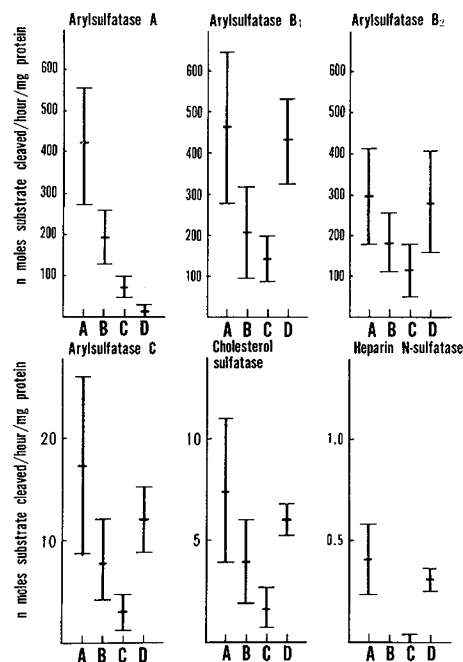


Fig. 1. Various sulfatase activities in cultured skin fibroblasts from patients with metachromatic leukodystrophy (MLD), multiple sulfatase deficiency (MSD), and their heterozygotes. (A) control subjects ($n = 10$); (B) heterozygotes of MSD ($n = 3$); (C) MSD patients ($n = 5$); and (D) MLD ($n = 3$). The activities were expressed as nmoles substrate cleaved/h/mg protein, mean \pm standard deviation. "n" indicates the number of different cell lines used.

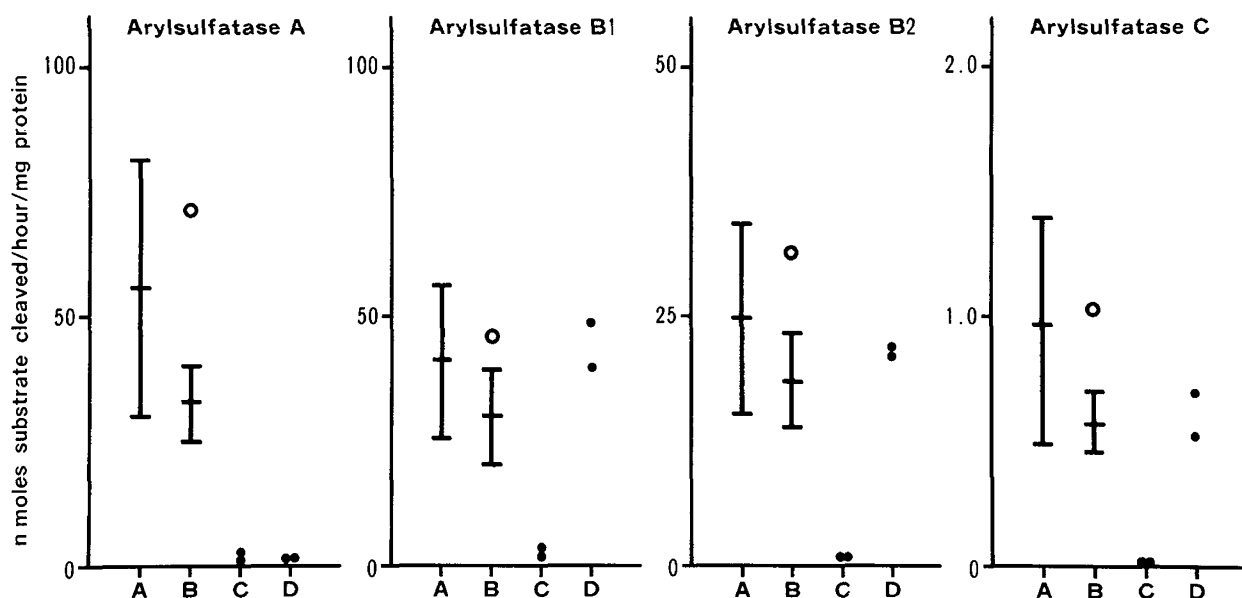


Fig. 2. Arylsulfatases A, B1, B2, and C activities in leukocyte from patients with metachromatic leukodystrophy (MLD), multiple sulfase deficiency (MSD), and their heterozygotes. (A), control subjects ($n = 8$); (B) heterozygotes of MSD ($n = 2$)*, open circles indicate a sister of MSD patient; (C), MSD patient; and (D), MLD patients. * The data of mean and standard deviation were obtained from at least two different collections of leukocyte specimens. The activities were expressed as nmoles substrate cleaved/h/mg protein, mean \pm standard deviation. "n" indicates the number of patients studied.

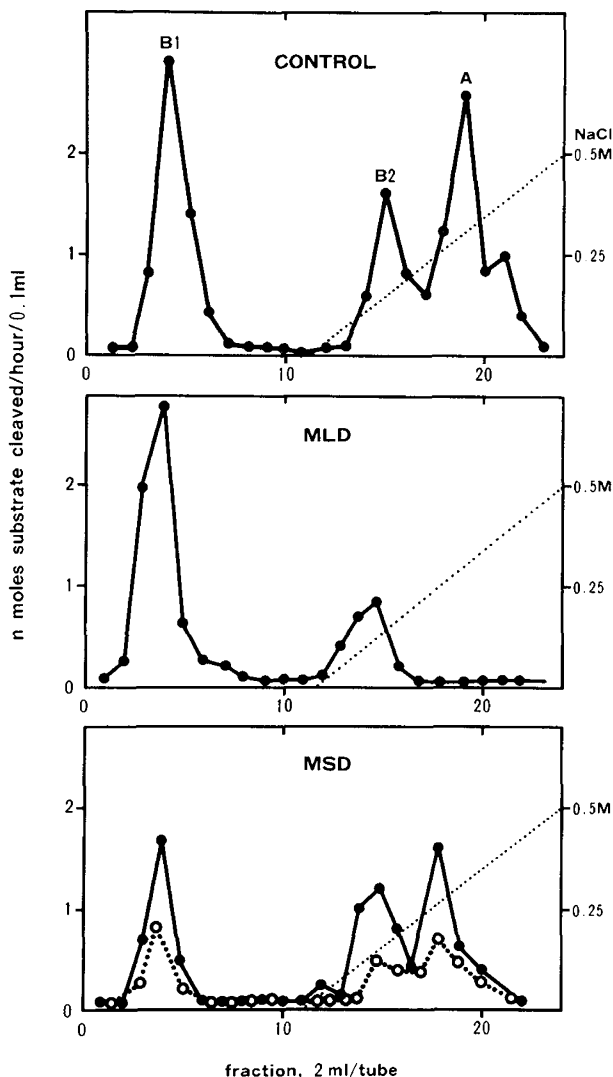


Fig. 3. DEAE-Sephacose column chromatographic patterns of 4-methylumbelliferyl (4-MU) sulfatase in cultured skin fibroblasts from control (top), metachromatic leukodystrophy MLD (middle) and multiple sulfase

Enzyme assay. Arylsulfatase A activity was determined using *p*-nitrocatechol sulfate as a substrate according to Baum *et al.* (4). Arylsulfatase B1 and B2 activities were determined using *p*-nitrocatechol sulfate by correction with DEAE-Sephacose column chromatographic patterns, because arylsulfatase B activity contained 30–40% of arylsulfatase A activity, as shown in Figure 1. 4-Methylumbelliferyl (4-MU) sulfatase was determined using 4-MU sulfate (Koch-Light) as follows: the incubation mixtures contained 100 μ l of 10 mM 4-MU sulfate dissolved in 0.2M sodium acetate buffer (pH 5.0) and 100 μ l of cell homogenates. The incubation was terminated by the addition of 0.2 M glycine-carbonate buffer (pH 10.8) after 30–60 min incubation. Arylsulfatase C was measured using 4-MU sulfate as a substrate according to Eto *et al.* (7). Cholesterol sulfatase was determined using [14 C]-cholesterol sulfate (specific activity, 10–25 Ci/mmol) (a gift from Dr. Iwamori, Department of Biochemistry, University of Tokyo), as described previously (8). Heparin N-sulfatase was measured by the method of Schmidt *et al.* (19), using radioactive (N-sulfonate [35 S]) heparin (Amersham, Chicago). Protein was estimated by the method of Lowry *et al.* (16).

DEAE-Sephacose column chromatography. The soluble fraction was subjected to DEAE-Sephacose column chromatography (11) for the fractionation of arylsulfatases from MLD, MSD, and their heterozygotes. The elution was performed by a continuous gradient of sodium chloride from 0–0.5 M NaCl. Each fraction after column chromatography was dialyzed against 5 mM sodium acetate buffer (pH 6.0) and studied for K_m , thermostability, optimal pH and effects of ions.

RESULTS

Various sulfatase activities in cultured skin fibroblasts (Fig. 1). Various sulfatase activities including arylsulfatases A, B1 and B2, and C, cholesterol sulfatase, and heparin N-sulfatase were shown in Figure 1. Arylsulfatase A activity was 16% less than that of controls in five different cells of MSD patients and 45% less in three cell lines of MSD heterozygotes. Arylsulfatases B1 and B2 activities were 30% and 36% of controls in MSD patients and 45% and 52% in MSD heterozygotes, respectively. Arylsulfatase C and cholesterol sulfatase were 20% less than that in patients and 43%

deficiency (MSD) (bottom) (MSD patient, \circ — \circ ; its heterozygote, \bullet — \bullet). The activities were normalized on the basis of 0.5 mg protein of fibroblast cells.

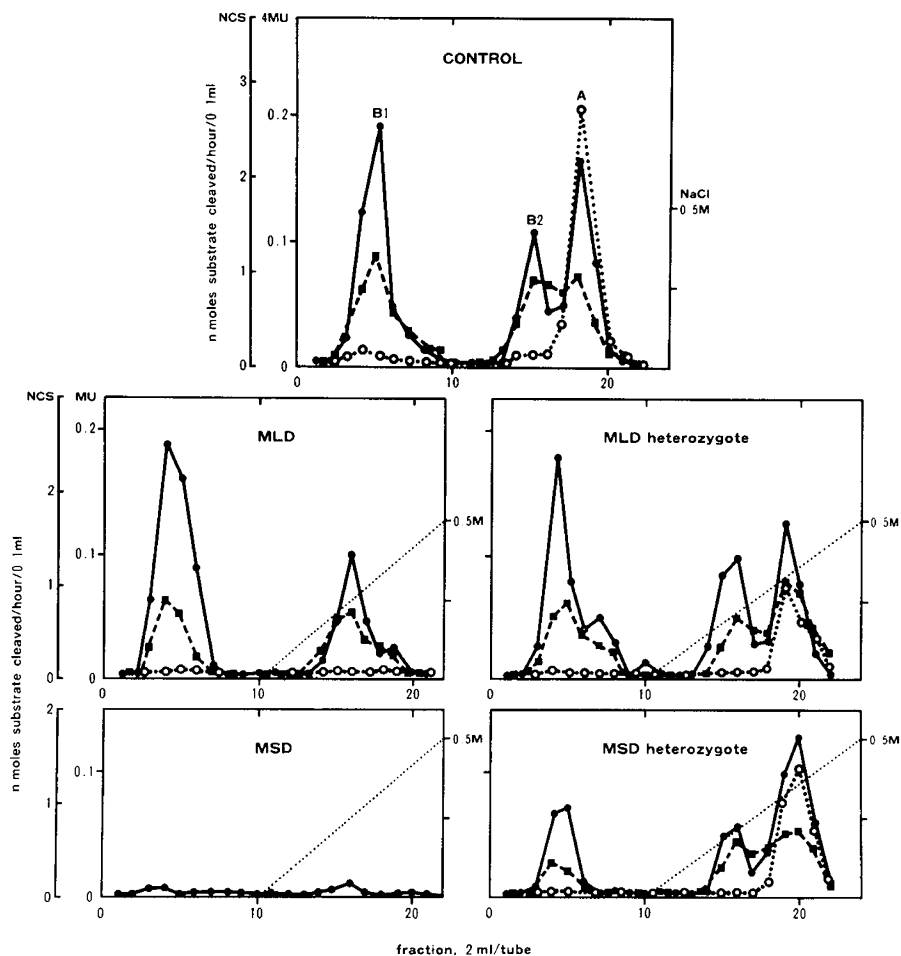


Fig. 4. DEAE-Sephrose column chromatographic patterns of 4-methylumbelliferyl (4-MU) and *p*-nitrocatechol (*p*-NC) sulfate sulfatase in leukocytes from controls, metachromatic leukodystrophy (MLD), MLD heterozygote, multiple sulfase deficiency (MSD) and MSD heterozygote. The activities were normalized on the basis of 5 mg protein. (●—●), 4-MU sulfatase (using 10 mM sulfate in 0.2 M acetate buffer, pH 5.3); (○—○), *p*-NC sulfate (reagent A by Baum's method; and (■—■), *p*-NC sulfatase (reagent B by Baum's method).

and 54% in heterozygotes for MSD, respectively. Heparin N-sulfatase was 0.5% in MSD patients.

Various sulfatase activities in leukocytes (Fig. 2). Arylsulfatase A activity in leukocytes from MSD patients and obligate heterozygotes were 5% and 60% less than that of controls, respectively. Arylsulfatases B1 and B2 activities were practically absent in MSD patients and 70% in its heterozygotes. Arylsulfatase C activity was deficient in MSD patients and 59% in heterozygotes. A sister of the Japanese case of MSD showed arylsulfatases A, B1, and B2, and C activities within normal ranges.

DEAE-sepharose column chromatography of 4-MU arylsulfatases from leukocytes and cultured skin fibroblasts. Figures 3 and 4 show the column chromatographic patterns of 4-MU and *p*-nitrocatechol arylsulfatases from cultured skin fibroblasts and leukocytes of patients with MLD, MSD, and their heterozygotes. In control fibroblasts, 4-MU arylsulfatase activities were fractionated into three major fractions, designated as A, B1, and B2. The fraction B2 is considered to be a minor arylsulfatase B reported by Fluharty *et al.* (11).

The MSD patient showed a deficiency of each component of the 4-MU arylsulfatases as well as of *p*-nitrocatechol arylsulfatase. In the heterozygote, each component of arylsulfatase activity was reduced to roughly one-half the control, as shown in Figure 3. The MSD patient and heterozygote did not show different elution patterns of arylsulfatases, as compared with those of controls. Using *p*-nitrocatechol sulfate as a substrate, these patterns were essentially similar to those of control. Arylsulfatases A, B1, and B2 activities in the MSD patient's leukocytes were practically absent.

Enzyme characteristics of 4-MU arylsulfatases A, B1, and B2.

Km values of arylsulfatases A, B1, and B2 in control leukocytes were 0.2, 0.1, and 0.5×10^4 M, respectively. In the MSD heterozygote these are 0.2, 0.3, and 0.3×10^4 M, respectively. Optimal pHs of arylsulfatases A, B1, and B2 in control leukocytes were 5.6, 5.2 and 5.2, respectively. The MSD heterozygote showed a similar optimal pH to those of control. Effects of ions by Ba^{++} , Mg^{++} , Ca^{++} , Ag^{++} , and Hg^{++} in MSD heterozygote were essentially similar to those of control (the data is not shown). Results obtained from cultured skin fibroblasts were similar to those obtained from leukocytes for both the MSD patient and its heterozygotes.

DISCUSSION

Several sulfatase activities including arylsulfatases A, B1, B2, C, cholesterol sulfatase and heparin N-sulfatase were deficient or reduced in activity in cultured skin fibroblasts and leukocytes from patients with MSD. Deficient activities of several sulfatases in cultured skin fibroblasts with MSD have been described previously (3, 7), whereas these enzyme activities in leukocytes from MSD patients have been described. Several sulfatase activities in MSD leukocytes were practically absent, as compared with those from MSD cultured skin fibroblasts in which significant amounts of residual activities were present. This difference might be due to culture conditions, because Fluharty *et al.* (12, 13) reported that alkaline pH in the culture medium induced arylsulfatase A in MSD fibroblasts and also fetal calf serum contained predominantly arylsulfatase B rather than arylsulfatase A (unpublished observation).

Several sulfatase activities in heterozygotes with MSD have not

been reported previously. As shown in Figures 1 and 2, several sulfatase activities in cultured skin fibroblasts were almost one-half of controls in three heterozygotes of MSD patients, whereas these enzyme activities in leukocytes from MSD heterozygotes overlapped with control individuals. These differences might be due to heterogeneous populations in leukocytes as compared with those of cultured skin fibroblasts. It is therefore advisable to detect heterozygotes in cultured skin fibroblasts.

The reduced activities of arylsulfatases A, B1, B2, and C in leukocytes and cultured skin fibroblasts were confirmed by the experiments with DEAE-Sepharose column chromatography. The presence of an abnormal peak with 4-MU sulfatases was not observed in either leukocytes or cultured skin fibroblasts preparations. Enzyme kinetics and characteristics of isolated 4-MU sulfatases in leukocytes and cultured skin fibroblasts from the heterozygote and the patient with MSD suggest that the reduced activities of the various sulfatases are due to quantitatively reduced normal enzymes, indistinguishable from the enzymes in normal controls. These findings also confirmed the results of Fiddler *et al.* (10) who showed that arylsulfatases A and B in this disorder are reduced in their levels of cross reacting materials against anti-arylsulfatases A and B antibodies.

Reduced activities of various sulfatases in MSD heterozygotes may support the hypothesis that the multiple sulfatase deficiencies in this disorder are probably due to a single mutation of a sulfatase gene controlling system. The previous hypotheses such as a mutation in a subunit, cofactor, or inhibitor common to these sulfatases can be ruled out by immunologic studies reported by Fiddler *et al.* (10). Of interest is that affected enzymes in this disorder are produced by both autosomal and X-linked chromosomes. Arylsulfatases A and B and heparin N-sulfatase are known to be localized in autosomal genes (arylsulfatase A in chromosome number 22 (6) and B in chromosome number 5) (6), whereas arylsulfatase C, cholesterol sulfatase, other steroid sulfatase and sulfatase are considered to be localized in X-chromosomes (steroid sulfatase in Xg locus) (17). Most recently, cell fusion studies in which MSD cells were fused with other sulfatase deficient disorder such as MLD, Maroteaux-Lamy syndrome, Sanfilippo A, steroid sulfatase deficient disorder and Hunter syndrome support the hypothesis that an enzyme deficiency in MSD is different from that causing specific sulfatase deficiencies (5, 14). Intermediate activities of various sulfatases in MSD heterozygotes suggest that a defect of post-translational process, like I-cell disease, is not likely because the heterozygotes of I-cell disease did not show the intermediate activities of various lysosomal enzymes (15). The occurrence of these different enzyme deficiencies in MSD patients suggests that there might be a defect in a gene controlling various sulfatase activities.

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