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PSEUDO ARYLSULFATASE A DEFICIENCY: EVIDENCE FOR A STRUCTURALLY ALTERED ENZYME

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SUMMARY: Analysis of arylsulfatase A from pseudo arylsulfatase A deficiency fibroblasts by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoradiochemical nitrocellulose blot radiography revealed two subunit bands which migrated faster than subunit bands of enzyme from normal fibroblasts. Immunoreactive material was present only at levels comparable to enzyme activity. These findings imply that arylsulfatase A in pseudodeficiency is structurally altered, but it is catalytically equivalent to normal arylsulfatase A. This altered enzyme must be the product of the pseudodeficiency gene since no immunoreactive product of the metachromatic leukodystrophy gene could be detected in metachromatic leukodystrophy cells by the procedure employed. It is not clear from the present data if the attenuated arylsulfatase A activity in pseudodeficiency results from a decreased rate of synthesis or an increased lability of the mutant enzyme.

In pseudo arylsulfatase A deficiency, there is an apparent profound deficiency of cerebroside sulfatase activity (cerebroside-3-sulfate 3-sulfohydrolase, EC 3.1.6.8) also known as arylsulfatase A (aryl-sulfate sulfohydrolase, EC 3.1.6.1) [1,2]. Although enzyme activities overlap the range in the arylsulfatase A deficiency neurodegenerative disorder MLD[1], PD appears to be a benign trait. Residual enzyme activity, albeit very low, is functionally adequate. For the most part, this trait has been recognized in one of the parents of a child affected with MLD. Most PD individuals are, therefore, obligate carriers of MLD [e.g., 3,4]. There have been several suggestions on the

[1] Abbreviations: MLD, metachromatic leukodystrophy; PD, pseudodeficiency; $NaDodSO_4$, sodium dodecyl sulfate; CRM, cross-reacting material.

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cause of PD, but the reason remains obscure. We present here subunit analysis of PD fibroblast arylsulfatase A by $NaDodSO_4$ polyacrylamide gel electrophoresis and immunoradiochemical nitrocellulose blot radiography. A characteristic alteration in enzyme subunit structure is shown in cells from 4 PD individuals. Possible explanations for the altered enzyme activity in PD are considered.

METHODS

Fibroblast Cultures and Preparation. Skin fibroblast cultures were obtained from four PD subjects who are also obligate carriers of MLD [5-7], patients with MLD, and laboratory personnel as controls. Fibroblasts were cultured to confluency in MEM-HEPES [6] and harvested by trypsinization. Cell pellets were washed twice with citrate-buffered saline and suspended in 3-4 volumes of buffer A (25 mM Tris-HCl, pH 7.5) containing 0.1% Triton X-100. Cells were lysed by six freeze-thaw cycles and centrifuged for 3 min at 8,000 x g in a Brinkmann Microfuge. The supernatant fluid was stored at -20°C.

Purification of Antiserum. A previously prepared antiserum raised in rabbits against pure human urine arylsulfatase A was used [8]. It was heated to 50°C for 20 min and applied to a DEAE-cellulose column. Twenty percent of the antibody reactive material was recovered in the void volume and 80% of the material was recovered by applying a linear gradient of NaCl. The latter was subjected to gel filtration on Sephadex G-200 and antibody reactive material was recovered where immunoglobulin G (7S) was expected. It was concentrated by ultrafiltration, sodium azide added to 0.02% and stored at -20°C.

DEAE-Cellulose Chromatography. Arylsulfatase A in fibroblast extracts was partially purified by a modification of the procedure described by Stevens [9]. Extracts (1-1.5 ml) were applied to 1 ml of DE-32 (Whatman) equilibrated with buffer A in a 2.5 ml disposable syringe. The resin was washed with 3 ml of buffer A and 3 ml of buffer A containing 0.1 M NaCl, and the enzyme was eluted with buffer A containing 0.5 M NaCl. Effluent from the latter was collected in 0.25 ml fractions and aliquots (control 5 µl; PD 10 µl) were assayed for arylsulfatase A activity by the procedure of Baum et al. [10] on a microscale. A unit of enzyme activity is defined as the amount which hydrolyzes 1 µmol of nitrocatechol sulfate/h.

Polyacrylamide Gel Electrophoresis and Electroblotting. Gel electrophoresis was carried out by the method of Laemmli [11] in a Bio-Rad model 220 vertical slab gel electrophoresis cell cooled with tap water. A 3% stacking gel and 10% separating gel were employed. Samples from DEAE-cellulose chromatography containing 0.5 units of arylsulfatase A were heated with $NaDodSO_4$ sample buffer in a boiling water bath for 3 min. Volumes were adjusted so 100-150 µl samples were applied to each lane. Electrophoresis was carried out at 45 mA until the bromphenol blue dye marker reached the bottom of the gel (approx 50 min) and continued for an additional 60 min.

After electrophoresis, proteins were transferred from polyacrylamide gels to nitrocellulose paper (Bio-Rad) by the proce-

dure of Towbin et al. [12]. Electroblotting was carried out with a Bio-Rad Trans-Blot Cell for 14-16 h at 100 mA at room temperature.

Antibody ^{125}I-Protein A Radiography. Air dried nitrocellulose sheets were carried through washing, antisera treatment, washing, ^{125}I-protein A (New England Nuclear) treatment, washing and radiography essentially as described by Ginns et al. [13]. Tris-saline (10 mM Tris-HCl, pH 7.5) was used instead of phosphate-buffered saline and rabbit anti-arylsulfatase A was the antibody.

CRM Titration. Titration for CRM to pure human urine arylsulfatase A was carried out by a double antibody protocol similar to one previously described [14].

RESULTS

Normal fibroblast arylsulfatase A yielded two bands on immunoradiochemical nitrocellulose blot radiography of material which had been subjected to NaDodSO$_4$ polyacrylamide gel electrophoresis (Fig. 1). Pure human liver arylsulfatase A [15] also yielded two subunit bands with identical migration rates (Fig. 1). We had shown previously that subunits of this enzyme were M_r= 59,000 and 63,000 [15]. A similar two-banded pattern with identical migration rates was obtained with arylsulfatase A derived from 5 normal fibroblast cell lines (Fig. 1).

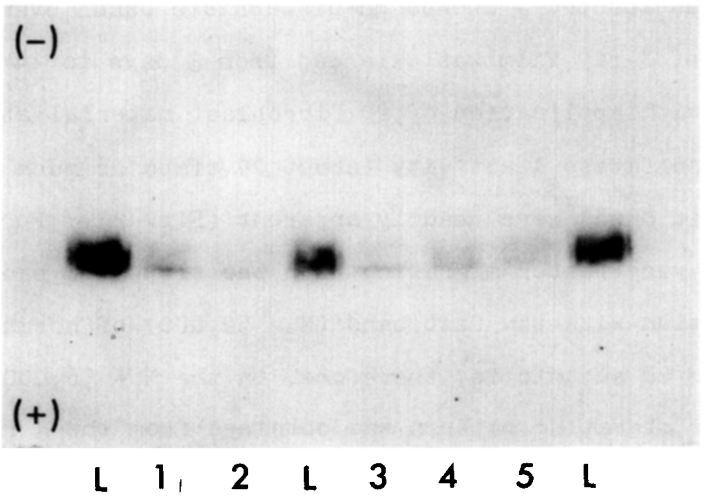

Fig. 1. Immunoradiochemical nitrocellulose blot radiograph of NaDodSO$_4$ polyacrylamide gel electrophoresis of normal fibroblast arylsulfatase A and pure human liver arylsulfatase A. Liver enzyme, L; enzyme from different normal fibroblast cell strains, 1-5.

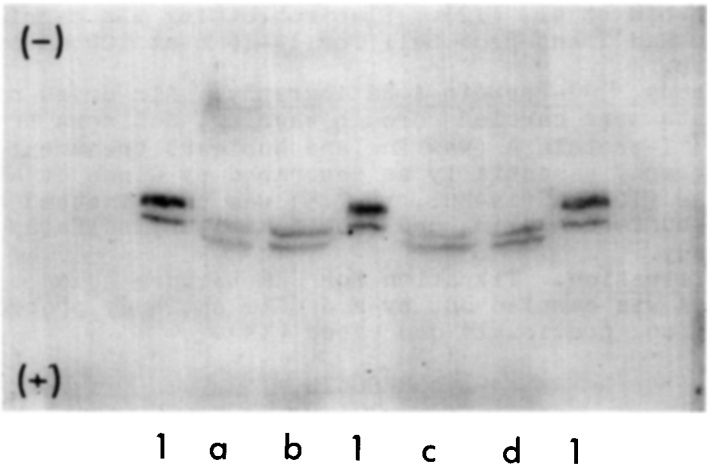

Fig. 2. Immunoradiochemical nitrocellulose blot radiograph of NaDodSO$_4$ polyacrylamide gel electrophoresis of arylsulfatase A from normal and pseudo arylsulfatase A deficiency fibroblasts. Enzyme from normal fibroblasts, 1; enzyme from different pseudodeficiency fibroblast cell strains, a-d.

Material from DEAE-cellulose chromatography of PD fibroblasts carried through the NaDodSO$_4$ polyacrylamide gel electrophoresis and immunoradiochemical nitrocellulose blot radiography procedure at the same protein level as normal fibroblast material unexpectedly showed no discernible bands even when exposure of X-ray film was extended from 3 days to 14 days. However, with application of PD fibroblast material at equivalent arylsulfatase A activity (about 20 times as much protein) two subunit bands were readily apparent (Fig. 2). Furthermore, the bands were faster migrating with the slower PD band in juxtaposition with the fast band (M_r= 59,000) of normal enzyme. The faster PD subunit is, therefore, in the M_r= 56,000 range. The faster migrating pattern was obtained from the 4 PD fibroblast cell lines tested (Fig. 2). Late infantile, juvenile and adult MLD fibroblast preparations gave no detectable bands in this subunit region when tested at the same protein level as PD fibroblasts (not shown). (MLD fibroblasts are devoid of

detectable arylsulfatase A activity, so DEAE-cellulose chromatography fractions were selected to parallel those from PD fibroblast extracts.)

CRM to arylsulfatase A was present in PD fibroblasts at levels considerably below the level present in normal fibroblasts. However, the amount present was nearly equivalent to enzyme activity. One unit of anti-arylsulfatase A (defined as the amount precipitating 1 unit of urine enzyme) precipitated 1.25, 1.29 and 1.13 units of enzyme in 3 PD cell lines compared to 1.38 units of control fibroblast enzyme[2].

DISCUSSION

There have been different suggestions on the cause of reduced arylsulfatase A activity in PD. Dubois et al. [16] postulated a defect in a regulatory gene leading to underproduction of the normal arylsulfatase A gene product. Langenbeck et al. [17] proposed a mutation at the arylsulfatase A locus giving rise to an altered form of the enzyme with attenuated catalytic activity. By this hypothesis, an individual with the PD trait who is a parent of a child with MLD would be a mixed heterozygote of the *pd* and *mld* alleles. Zlotogora et al. [18] concluded that enzyme activity data from two PD families were compatible for *pd* - *mld* allelism. Chang et al. [19] provided corroborating data for *pd* - *mld* allelism by showing lack of complementation in heterokaryons of PD and MLD fibroblasts. They also found only 2 arylsulfatase A activity bands from PD fibroblasts on isoelectric focusing while normal fibroblasts gave 6 activity bands [19].

The present findings demonstrate that arylsulfatase A in PD fibroblasts is structurally altered providing direct evidence

[2] The higher than unity enzyme precipitated in fibroblasts has been obtained reproducibly. We have attributed this to partial inactivation of urine arylsulfatase A.

in support of the *pd - mld* allelism hypothesis. However, the concomitant decrease in CRM to arylsulfatase A and enzyme activity indicates that the mutant enzyme is normal or nearly normal catalytically. Therefore, it must be presupposed that the mutation has either affected the rate of synthesis or resulted in an increased turnover of the enzyme molecule. Decreased size of the mutant enzyme could be due to loss of polypeptide or carbohydrate segments. Deglycosylation would be expected to affect the stability of the enzyme, and the observed size change is very similar to the size change caused by treatment of normal arylsulfatase A with endo-β-N-acetylglucosaminidase H [20]. Such an alteration, however, should affect the recognition marker system and lead to mucolipidoses-like conditions [21] rather than the normal phenotype. Elucidation of the putative missing segments in PD arylsulfatase A should provide clues on the nature of mutation in PD and provide some explanation for the normal function in the presence of a very low level of enzyme.

While this manuscript was in preparation, we learned that Bach and Neufeld [22] obtained similar results. It should be noted that one of our PD cultures (lane c, Fig. 2) was obtained from Dr. Bach and is the same one employed in their investigation.

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