Am J Hum Genet 35:228–233, 1983

Two Allelic Forms of Human Arylsulfatase A with Different Numbers of Asparagine-Linked Oligosaccharides

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SUMMARY

The biosynthesis of arylsulfatase A in human skin fibroblasts was studied by labeling cells and isolating arylsulfatase A using immune precipitation and polyacrylamide gel electrophoresis under denaturing and reducing conditions. Arylsulfatase A was synthesized as precursor polypeptides of 62 kDa or 59.5 kDa. Cell lines synthesizing either or both polypeptides were found. The results of a family study were consistent with the assumption that the two arylsulfatase A polypeptides are of allelic nature. In various heterozygous cell lines, the two polypeptides were formed at equal or different rates. The relative rate of biosynthesis was constant for an individual cell line, suggesting that both allelic products were under separate genetic control. In a group of 21 unrelated individuals, the gene frequency of alleles for the 62- and 59.5-kDa precursor forms was 3:1.

The two allelic forms of the arylsulfatase A polypeptides were converted into a 57-kDa form by endo-β-*N*-acetylglucosaminidase H, an enzyme specifically removing asparagine-linked oligosaccharides of the high-mannose (and hybrid) type. The apparent difference in the number of asparagine-linked oligosaccharides suggests that the two allelic genes differ in a region coding the sequence Asn-X-Thr(Ser), which is required for attachment of asparagine-linked oligosaccharides.

INTRODUCTION

Arylsulfatase A is a lysosomal enzyme whose physiological function is desulfation of cerebroside sulfatide [1]. Other naturally occurring substrates are semino lipid [2, 3], psychosine sulfate [4], and lactosyl sulfatide [5], all containing galactosyl

Received April 28, 1982; revised June 2, 1982.

This work was supported by grant SFB-104 from the Deutsche Forschungsgemeinschaft. A. W. was the recipient of an Alexander von Humboldt fellowship.

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3-sulfate residues and ascorbate 2-sulfate [6]. The interest in arylsulfatase A was greatly stimulated by the finding that catalytic activity of arylsulfatase A is diminished or absent in a group of lysosomal storage disorders, including metachromatic leukodystrophy and its variants, multiple sulfatase deficiency, I-cell disease, and pseudo-Hurler polydystrophy (for review, see [7, 8]). During studies of the biosynthesis and postribosomal modification of arylsulfatase A, we observed that two arylsulfatase A polypeptides differing in their molecular weight may be formed in human skin fibroblasts [9]. Here we provide evidence that the two arylsulfatase A polypeptides are allelic gene products. The two polypeptides differ in number of asparagine-linked oligosaccharides.

MATERIALS AND METHODS

L-[4,5-^{3}H]leucine (specific activity 50 Ci/mmol), [2-^{3}H]mannose (specific activity of 16 Ci/mmol), and ^{14}C-methylated protein standards were from New England Nuclear, Boston, Mass. The monospecific antiserum raised in goat against arylsulfatase A from human placenta was that previously described [10].

Cell Culture

Diploid human skin fibroblasts were grown at 37°C/5% CO_2 in Eagle's minimal essential medium supplemented with 10% fetal calf serum (Boehringer-Mannheim, Mannheim, West Germany), nonessential amino acids, and antibiotics as described [11]. The mutant cell lines were sent to our laboratory for diagnostic purposes.

Isolation of [^{3}H]Arylsulfatase A

The conditions for labeling of fibroblasts with either [^{3}H]leucine or [^{3}H]mannose were those described in [12] with the modifications given in [10]. The medium was supplemented with 10 mM NH_4Cl, and the labeling period was 24 hrs. Arylsulfatase A was immune precipitated from the medium [10] and analyzed by discontinuous polyacrylamide gel electrophoresis under denaturing and reductive conditions [13] followed by fluorography [14]. The radioactivity incorporated into polypeptides visualized by fluorography was determined as described [15]. The treatment with endo-β-*N*-acetylglucosaminidase H of media prior to immune precipitation of arylsulfatase A was performed as in [16].

RESULTS AND DISCUSSION

In [10], we reported the synthesis of an arylsulfatase A subunit as a precursor of 62 kDa. Less than 15% of the precursor was secreted in the medium, and within 7 days the remainder was intracellularly converted into a 60.5-kDa product, which represents one of the mature forms of arylsulfatase A. In the mature enzyme isolated from human placenta, another polypeptide with an apparent mol. wt. of 57 kDa was observed [10]. During the study on synthesis of arylsulfatase A in normal and mutant fibroblasts, a second precursor polypeptide of arylsulfatase A with an apparent mol. wt. of 59.5 kDa was observed in some cell lines [9]. In figure 1 (top), cell lines producing either the 62-kDa precursor (cell lines L. and 81RD5) or the 59.5-kDa precursor (cell lines S. K. and C.) are shown. The remaining cell lines shown in figure 1 (top) that synthesize both precursors fall into two groups. In one group, represented by cell lines V. and B., the two precursors were synthesized at approximately equal amounts, whereas in another group that includes cell

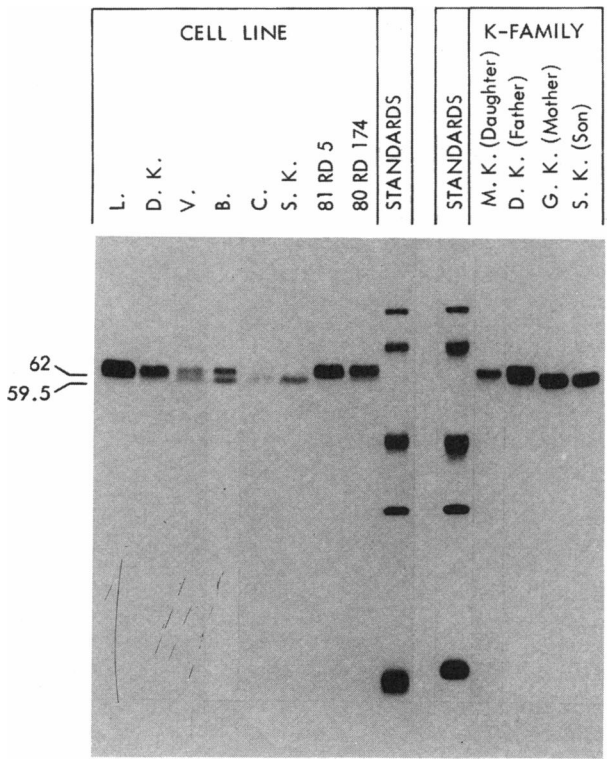

FIG. 1.—Arylsulfatase A precursor from secretions of human skin fibroblasts. *Top*, Fibroblasts from eight unrelated donors were incubated in the presence of [^{3}H]leucine and 10 mM ammonium chloride. Fluorogram shows the arylsulfatase A precursors isolated from the secretions. *Bottom*, Arylsulfatase A precursors from the fibroblasts obtained from the parents and sister of a male individual (S. K.) homozygous for the 59.5-kDa allele of arylsulfatase A. The migration of the 62- and 59.5-kDa precursors of arylsulfatase A is indicated. The following [^{14}C]-methylated protein standards were used: phosphorylase b, 92,500; bovine serum albumin, 69,000; ovalbumin, 46,000; carbonic anhydrase, 30,000; and cytochrome c, 12,300.

lines D. K. and 80 RD 174, the 62- and 59.5-kDa precursors are synthesized at a rate of approximately 5:1.

Several of these cell lines were repeatedly assayed over a period of 15 months. Within this period, neither the type(s) of precursor synthesized nor the ratio in which the 62- and 59.5-kDa precursors were produced were subject to variation.

To establish the allelic nature of the two arylsulfatase A precursor polypeptides, we studied the fibroblasts of relatives of a male individual producing only the 59.5-kDa precursor (cell line S. K.).

The fibroblasts of his mother also produced only the 59.5-kDa precursor, whereas those of his father and his sister synthesized the 62- and 59.5-kDa precursors at a ratio of 5:1 (fig. 1*B*). These results are consistent with the assumption that the mother and the son are homozygous for the arylsulfatase A allele coding for the 59.5-kDa precursor and the father and the sister are heterozygous for the two arylsulfatase A alleles.

In total, we have studied arylsulfatase A synthesis in 25 cell lines. Among these, 21 cell lines were derived from unrelated donors and four were from members of one family. The cell lines were submitted to our laboratory for diagnosis of lysosomal storage disorders. In 17 of these cell lines, lysosomal storage disorders were diagnosed. Among the 25 cell lines, 12 were homozygous for the 62-kDa allele and three for the 59.5-kDa allele. At least one control cell line was within each group. The gene frequency was calculated from the 21 cell lines derived from unrelated donors. Within this group, 12 cell lines were homozygous for the 62-kDa allele, one homozygous for the 59.5-kDa allele, and eight heterozygous, corresponding to a relative percentage of 57:5:38. These values approximate the theoretical distribution (56:6:38) expected from the Hardy-Weinberg law for gene frequencies of .75 and .25 for the 62- and 59.5-kDa alleles, respectively.

The heterozygotes fell into two groups. In one, the ratio of the products of the 62- and 59.5-kDa alleles was about 1:1, and in the other it was 5:1 (cell lines D. K., 80 RD 174, and M. K. in fig. 1). This observation remains unexplained. It is possible that the genes coding the 62.5-kDa and/or the 59.5-kDa products exist in more than one allelic form, differing in their rate of expression or stability of the products. Alternatively, the genes may be controlled by regulatory element(s) that itself occur(s) in allelic forms.

Human skin fibroblast arylsulfatase A was previously characterized as a glycoprotein with two oligosaccharides of the high-mannose type in about 90% of the arylsulfatase A polypeptide chains. The high-mannose oligosaccharides linked to asparagine residues in the polypeptide are cleavable by endo-β-*N*-acetylglucosaminidase H. The removal of the two high-mannose oligosaccharides leads to a reduction of the apparent molecular weight by 5 kDa. Differences in apparent molecular weight of arylsulfatase A polypeptides may therefore be due to a different length of the polypeptide and/or to a different number (or structure) of oligosaccharides. To distinguish between the two possibilities, arylsulfatase A derived from homozygous cell lines was treated with endo-β-*N*-acetylglucosaminidase H (fig. 2). The 62-kDa precursor was converted into a 57-kDa product as described in [10]. The product obtained from the 59.5-kDa precursor could not be distinguished from that of the 62-kDa precursor in polyacrylamide gel electrophoresis. In control experiments with arylsulfatase A labeled in its carbohydrate with [2-^{3}H]mannose, it was shown that more than 80% of the radioactivity can be released from the enzyme by treating it with endo-β-*N*-acetylglucosaminidase H (fig. 2). From these results, we conclude that the 62-kDa precursor polypeptide contains two asparagine-linked oligosaccharide side chains cleavable by endo-β-*N*-acetylglucosaminidase H and that the lower apparent molecular weight of the 59.5-kDa precursor is due to the absence of one of these side chains. Attachment of high-mannose oligosaccharides to asparagine residues requires the sequence Asn-X-Thr(Ser) in the accepting polypeptide [17]. The lack of one asparagine-linked oligosaccharide in the 59.5-kDa precursor suggests the absence of one of the two carbohydrate accepting amino acid sequences. At the level of DNA, the two alleles may therefore differ in a sequence that codes for an Asn-X-Thr(Ser) acceptor site in the 62-kDa allele.

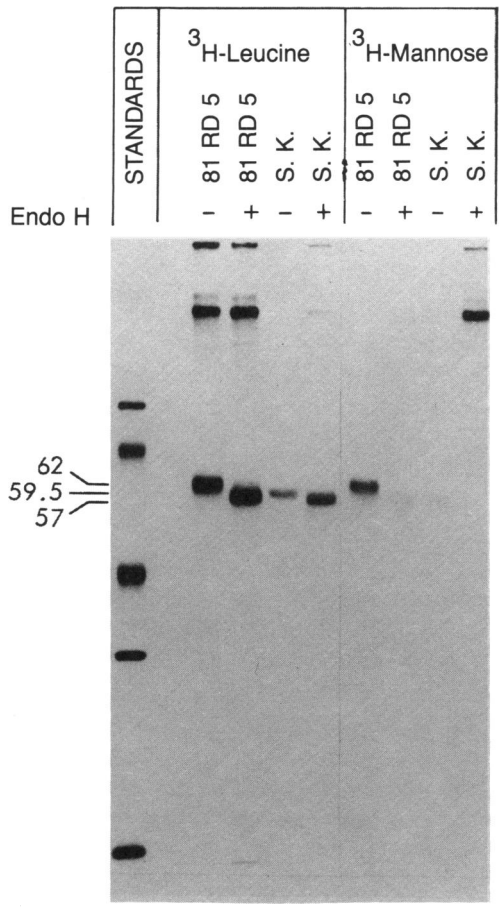

FIG. 2.—Effect of endo-β-*N*-acetylglucosaminidase H on arylsulfatase A. The enzyme was immune precipitated from secretions of cells labeled in presence of 10 mM NH_4Cl with either [^{3}H]leucine or [^{3}H]mannose. Aliquots untreated (−) or treated (+) with the endoglycosidase were analyzed by gel electrophoresis and fluorography. The migration of arylsulfatase A before (62 and 59.5 kDa) and after (57 kDa) treatment is indicated.

At present, it is impossible to correlate the catalytic activity with the allelic forms of arylsulfatase A and the rate of their expression, since the greater part of cell lines are derived from patients with lysosomal storage disorders. Correlation of the alleles with catalytic activity or other parameters depends on screening of a larger population based on a more simple and preferably nonradioactive assay for arylsulfatase A alleles.

ACKNOWLEDGMENTS

We thank Dr. H. Harris, Philadelphia, for critical reading of the manuscript.

REFERENCES

1. MEHL E, JATZKEWITZ H: Cerebroside 3-sulfate as a physiological substrate of arylsulfatase A. *Biochim Biophys Acta* 151:619–627, 1968

2. YAMATO K, HANDA S, YAMAKAWA T: Purification of arylsulfatase A from boar testes and its activities towards seminolipid and sulfatide. *J Biochem (Tokyo)* 75:1241–1247, 1974
3. FLUHARTY AL, STEVENS RL, MILLER RT, KIHARA H: Sulfoglycerogalactolipid from rat testis: a substrate for pure human arylsulfatase A. *Biochem Biophys Res Commun* 61:348–354, 1974
4. ETO Y, WIESMANN U, HERSCHKOWITZ NN: Sulfogalactosylsphingosine sulfatase. Characteristics of the enzyme and its deficiency in metachromatic polydystrophy in human cultured skin fibroblasts. *J Biol Chem* 249:4955–4960, 1974
5. HARZER K, BENZ HU: Deficiency of lactosyl sulfatide sulfatase in metachromatic leukodystrophy (sulfatidosis). *Hoppe-Seylers Z Physiol Chem* 355:744–748, 1974
6. ROY AB: L-Ascorbic acid 2-sulfate, a substrate for mammalian arylsulfatases. *Biochim Biophys Acta* 377:356–363, 1975
7. DULANEY JT, MOSER HW: Sulfatide lipidosis: metachromatic leukodystrophy, in *The Metabolic Basis of Inherited Disease*, 4th ed, edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, New York, McGraw-Hill, 1978, pp 770–809
8. MCKUSICK VA, NEUFELD EF, KELLY TE: The mucopolysaccharide storage diseases, in ibid., pp 1282–1307
9. HASILIK A, WAHEED A, CANTZ M, VON FIGURA K: Impaired phosphorylation of lysosomal enzymes in fibroblasts of patients with mucolipidosis III. *Eur J Biochem* 122: 119–123, 1982
10. WAHEED A, HASILIK A, VON FIGURA K: Synthesis and processing of arylsulfatase A in human skin fibroblasts. *Hoppe-Seylers Z Physiol Chem* 363:425–430, 1982
11. CANTZ M, KRESSE H, BARTON RW, NEUFELD EF: Corrective factors for inborn errors of mucopolysaccharide metabolism. *Methods Enzymol* 28:884–897, 1972
12. HASILIK A, NEUFELD EF: Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursors of higher molecular weight. *J Biol Chem* 255:4937–4945, 1980
13. LAEMMLI UK: Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970
14. BONNER WM, LASKEY RA: A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Eur J Biochem* 46:83–88, 1974
15. WAHEED A, HASILIK A, VON FIGURA K: Enhanced breakdown of arylsulfatase A in multiple sulfatase deficiency. *Eur J Biochem* 123:317–321, 1982
16. HASILIK A, VON FIGURA K: Oligosaccharides in lysosomal enzymes: distribution of high-mannose and complex oligosaccharides in cathepsin D and β-hexosaminidase. *Eur J Biochem* 121:125–129, 1981
17. MARSHALL RD: Glycoproteins. *Annu Rev Biochem* 41:673–702, 1972