The sulfatases constitute a conserved family of enzymes that specifically hydrolyze sulfate esters in a wide variety of substrates such as glycosaminoglycans, steroid sulfates, or sulfolipids. By modifying the sulfation state of their substrates, sulfatases play a key role in the control of physiological processes, including cellular degradation, cell signaling, and hormone regulation. The loss of sulfatase activity has been linked with various severe pathophysiological conditions such as lysosomal storage disorders, developmental abnormalities, or cancer. A novel member of this family, arylsulfatase G (ASG), was initially described as an enzyme lacking arylsulfatase activity and localized to the endoplasmic reticulum. Contrary to these results, we demonstrate here that ASG does indeed have arylsulfatase activity toward different pseudosubstrates like 4-nitrocatechol sulfate and 4-methylumbelliferyl sulfate. The activity of ASG depends on the Cys-84 residue that is predicted to act as a strong, competitive ASG inhibitor. ASG is active as an unprocessed 63-kDa monomer and shows an acidic pH optimum as typically seen for lysosomal sulfatases. In transfected cells, ASG accumulates within lysosomes as indicated by indirect immunofluorescence microscopy. Furthermore, ASG is a glycoprotein that binds specifically to mannos 6-phosphate receptors, corroborating its lysosomal localization. ARSG mRNA expression was found to be tissue-specific with highest expression in liver, kidney, and pancreas, suggesting a metabolic role of ASG that might be associated with a so far non-classified lysosomal storage disorder.

Sulfatases represent a family of enzymes essential for the degradation and remodeling of sulfate esters. In mammals, sulfatases are involved in the turnover of various sulfated substrates such as glycosaminoglycans (heparin, heparan sulfate, dermatan sulfate, keratan sulfate), steroid hormones (e.g. dehydroepiandrosteron 3-sulfate), and sulfolipids (e.g. cerebrosides-3-sulfate) (1, 2). Furthermore, they have important regulatory functions in modulating heparan sulfate-dependent cell signaling pathways (3–9) as well as in the activation of sulfated hormones during biosynthesis (10, 11). In vitro, sulfatases display stringent specificities toward their individual substrates and have low functional redundancy. Their active sites contain a unique Cα-formylglycine (FGly) that is post-translationally generated in the endoplasmic reticulum by oxidation of a conserved cysteine residue (12–14). A genetic defect of the formylglycine-generating enzyme (FGE) leads to multiple sulfatase deficiency, a rare but fatal inherited disease in which all sulfatases are catalytically inactive due to a lack of FGly (1, 13–15).

Twelve of the 17 sulfatases encoded in the human genome have been characterized biochemically (16). Based on their subcellular localization they can be divided into lysosomal and non-lysosomal enzymes. The latter are found either at the cell surface (Sulf1, Sulf2), in the endoplasmic reticulum (aryl sulfatases C, D, and F), or in the Golgi apparatus (aryl sulfatase E) and act at neutral pH. In contrast, all lysosomal sulfatases (aryl sulfatases A and B, iduronate-2-sulfatase, heparan-NSulfatase, glucosamine-6-sulfatase, and galactosamine-6-sulfatase) share an acidic pH optimum (17). The genetic deficiency of each of these six lysosomal sulfatases causes specific and severe lysosomal storage disorders, namely metachromatic leukodystrophy and mucopolysaccharidoses type VI, II, IIIA, IIIB, and IV, respectively, which highlights the essential and non-redundant function of these enzymes (2). In affected patients, the degradation of a specific sulfated compound is blocked, leading to its accumulation in the lysosomes and in the extracellular fluids. Lysosomal storage impairs autophagic delivery of bulk cytosolic contents to lysosomes, finally resulting in accumulation of toxic proteins, cellular damage, and apoptosis (18). The total number of lysosomal hydrolases, according to proteomic analyses, is estimated to be in the range of 50–60 (19). In principle, a genetic mutation of any of these proteins can cause a lysosomal storage disorder. For many sulfated substrates like sulfo-proteins (tyrosine-, threonine- or serine-O-sulfate (20), the selectin ligand 6-sulfo sialyl LewisX, as well as the heparan sulfate constituent N-acetylgalactosamine-3-sulfate and glucuronate-2-sulfate), the corresponding sulfatases and possible associated storage disorders have not been identified yet. Thus, the discovery and characterization of novel lysosomal enzymes will likely be important in the identification and molecular understanding of so far non-classified inherited genetic diseases.

The mammalian arylsulfatase G (ASG) was identified in 2002 through bioinformatic searches of expressed sequence tag databases (21). The ARSG gene is located on chromosome 17q24.2, **This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.**

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2 and a supplemental reference.

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2 The abbreviations used are: FGly, Cα-formylglycine; 4-MUS, 4-methylumbelliferyl sulfate; ARSG, arylsulfatase G; ER, endoplasmic reticulum; M6PR, mannose 6-phosphate receptor; pNCS, p-nitrocatechol sulfate; pNPS, p-nitrophenyl sulfate; MES, 4-morpholineethanesulfonic acid.
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consists of 11 exons, and encodes a 525-amino acid protein that shares a high degree of similarity with all sulfatases, in particular with arylsulfatase A (50% sequence similarity and 37% identity). The enzyme was tentatively classified as an arylsulfatase, although no activity toward the commonly used arylsulfate pseudosubstrates p-nitrocatechol sulfate (pNCS) and 4-methylumbelliferyl sulfate (4-MUS) could be detected. In overexpressing COS-7 cells, ASG was found to be localized in the endoplasmic reticulum. In this study, however, we demonstrate for the first time that ASG is an active arylsulfatase enzyme of the lysosome.

EXPERIMENTAL PROCEDURES

Construction of Expression Plasmids—The full-length cDNA sequence encoding human ASG, designated KIAA1001 (accession number NM_014960.1, 22), was obtained from the Kazusa Institute (Kisarazu, Chiba, Japan). Two sequence isoforms of ASG are known. Whereas genomic and expressed sequence tag sequences, both for human and murine ASG, contain a GCA (Ala) codon in position 501, this codon is replaced by CCA (Pro) in the KIAA1001 clone (21). The ASG A501P cDNA was amplified by PCR, thereby adding a 3′-RGS-His _{6}, -encoding sequence followed by a stop codon and a HindIII site (reverse primer 5′-CCCAAGCTTAGTGATGGTGATGGTGATGCG-3′/H11032). All constructs were full-length cDNA fragments, whereas genomic DNA was linearized with HindIII and NcoI, which cuts in the endogenous Kozak sequence of ASG, and cloned into pMPSV-EH-ASA (23), thus exchanging the entire coding region of arylsulfatase A in-frame to that of ASG. The insert was then subcloned as a 5′-EcoRI-3′-HindIII/blunt fragment into the EcoRI/Smal-opened multiple cloning site of pC-neo (Prömegene). The QuikChange mutagenesis protocol (Stratagene) was used to generate the wild-type ASG-encoding sequence with a GCA codon in position 501 (accession number NM_014960.2). For this purpose, complementary primers were used (forward 5′-CGACAACATCTCCAGCCAGA-TTACACTCAGG-3′, reverse 5′-CCTGAGTGTAATCTGCTGGAGATGTTGTCG-3′). The ASG C84A mutant was prepared accordingly (forward 5′-GCTGCTCCACC-GCCTCAACCTCCCCG-3′, reverse 5′-CGGGAGGTTAGGC-GCGGTGAGGGCAGC-3′). All constructs were full-length sequenced in the coding region to preclude any PCR-derived errors. Unless otherwise stated, all experiments were performed with wild-type ASG.

Cell Culture and Transfections—HT1080 human fibrosarcoma cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (PAA Laboratories, Pasching, Austria) and 1% penicillin/streptomycin (Invitrogen). All cell culture dishes (Nunc) or Cellmaster PS roller bottles (Greiner Bio-One) were supplemented with 10% fetal calf serum (PAN Biotech GmbH, Aidenbach, Germany) and 1% penicillin/streptomycin (Invitrogen). AsG-bearing cells were grown in Dulbecco’s modified Eagle’s medium—HT1080 cells stably overexpressing ASG-His were grown to confluency. The cells were harvested by rubber policeman and extracted by sonication in lysis buffer (10 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.4). For the Western blotting. A mouse monoclonal antibody against the RGS-His _{6} epitope was used as a primary antibody (Qiagen). Signals were detected using a horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes) and ECL detection reagent (Pierce). Signals were quantified using the AIDA 4.06 software package (Raytest, Straubenhardt, Germany).

Enzymatic Assays—Activities of ASG toward pNCS or p-nitrophenyl sulfate (pNPS) were assayed using 10 mM pNCS or pNPS in either 0.5 M sodium acetate, (pH 4.5–6.0), MES (pH 6.5), or Tris-HCl (pH 7.0–8.0). The enzyme was tentatively classified as an arylsulfatase, and screened by Western blotting for ASG expression. Purification from Cell Culture Supernatants—HT1080 cells stably overexpressing ASG-His were grown to near confluence in growth medium containing 10% fetal calf serum on 15-cm cell culture dishes (Nunc) or Cellmaster PS roller bottles (Greiner Bio-One). During ASG production, the amount of serum was reduced to 1%. The conditioned medium was collected every 48–72 h, cleared by spinning, and subjected to ammonium sulfate precipitation (50% w/v). The precipitate was dialyzed overnight at 4 °C against binding buffer (20 mM Tris-HCl, 500 mM NaCl, 40 mM imidazole, pH 7.4). Fraction peaks were eluted with buffer B (20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 6.0), and applied onto a RESOURCE S 1-ml cation exchange column (GE Healthcare). Elution was carried out using a gradient with buffer B (20 mM MES, 1 M NaCl, pH 6.0). The ASG-His protein eluted at 140 mM NaCl. Peak fractions were pooled and concentrated by speed vac, if necessary. Proteins were analyzed by SDS-PAGE on 15% polyacrylamide gels and stained with Roti-Blue colloidal Coomassie (Carl Roth, Karlsruhe, Germany). Protein concentrations were determined using Coomassie Plus Bradford reagent (Pierce). Average yields were ~40 μg of purified ASG/liter of medium.

Peptide N-Glycosidase F and Endoglucosaminidase H Treatment—HT1080 cells stably overexpressing ASG-His were grown to confluency. The cells were harvested by rubber policeman and extracted by sonication in lysis buffer (10 mM HEPES, 0.5 M NaCl, pH 7.4). The lysate was cleared by centrifugation (72,000 × g, 20 min, 4 °C). Cell lysates and purified ASG samples were denatured and subjected to treatment with peptide N-glycosidase F and endoglucosaminidase H (both from Roche Applied Science) as described (24). In case of peptide N-glycosidase F deglycosylation, samples were denatured with 0.5% SDS and 0.2% β-mercaptoethanol at pH 7.2 for 5 min at 95 °C. Samples were mixed with peptide N-glycosidase F in incubation buffer containing 12% Triton X-100 at pH 7.2. For endoglucosaminidase H treatment, samples were denatured at pH 5.0 with 0.01% SDS and 0.7% β-mercaptoethanol for 5 min at 95 °C and subjected to deglycosylation. In both cases, samples were incubated at 37° C for 2 or 24 h and analyzed by Western blotting.

Western Blot—For Western blot analysis, a mouse monoclonal antibody directed against the RGS-His _{6} epitope was used as a primary antibody (Qiagen). Signals were detected using a horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes) and ECL detection reagent (Pierce). Signals were quantified using the AIDA 4.06 software package (Raytest, Straubenhardt, Germany).
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pH 5.6 using 0.5~30 mM pNCS in 0.5 mM sodium acetate. Absorbances were measured at 515 nm (ε<sub>515</sub> = 12400 M<sup>-1</sup> cm<sup>-1</sup>) in the case of pNCS or at 405 nm (ε<sub>405</sub> = 18000 M<sup>-1</sup> cm<sup>-1</sup>) for pNPS. Inhibition kinetics included Na<sub>2</sub>HPO<sub>4</sub>, NaHSO<sub>4</sub>, or warfarin (Sigma-Aldrich) at the indicated concentrations. Activity measurements with 4-MUS were performed accordingly, using 10 mM 4-MUS in the respective buffers. Reactions were stopped by addition of 150 μl of 1 M Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>, pH 10.7. The fluorescence of 4-methylumbelliferone, compared with a calibration curve, was measured with an excitation wavelength of 360 nm and an emission wavelength of 465 nm. All absorbance and fluorescence measurements were performed using an infinite M200 microplate reader (TECAN, Crailsheim, Germany). The temperature optimum was determined using a gradient thermocycler.

**Immunofluorescence**—Stably transfected HT1080 cells expressing ASG-His were grown on poly-L-lysine-covered coverslips for 24 h and labeled with 50 nM LysoTracker Red DND-99 (Molecular Probes) for 2 h in serum-free Dulbecco’s modified Eagle’s medium. The cells were briefly washed with phosphate-buffered saline, fixed with 4% paraformaldehyde for 20 min at room temperature, and permeabilized with 0.1% Triton X-100 for 10 min. After blocking with 2% fetal calf serum, the cells were incubated with a mouse monoclonal anti-RGS-His<sub>6</sub> antibody (Qiagen) for 1 h. The primary antibody was detected with an Alexa-488-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes). Immunofluorescence images were obtained on a Leica DM5000 B microscope equipped with an HCX PL APO ×100 oil immersion objective.

Mannose 6-Phosphate Receptor Binding Assay—10 μg of purified ASG were incubated overnight at 4 °C with an Affigel-10-based affinity matrix (2-ml column volume; Bio-Rad) to which a 1:1 mixture of MPR46/MPR300 purified from goat had been immobilized as described (25). The column was washed four times with 2 ml of MPR binding buffer (50 mM imidazole, pH 6.5, 150 mM NaCl, 5 mM Na-β-glycerophosphate, 2 mM EDTA, 10 mM MgCl<sub>2</sub>, 0.2% NaN<sub>3</sub>) and then three times with 2 ml of MPR binding buffer containing 5 mM glucose 6-phosphate to remove unspecifically bound proteins. Mannose 6-phosphate-containing proteins were eluted with 10 ×1 ml of 5 mM glucose 6-phosphate in MPR binding buffer (26). Wash and eluate fractions were analyzed by Western blotting.

**Gel Filtration Analysis**—Purified ASG was subjected to gel filtration on a Superdex 75 3.2/30 PC column (GE Healthcare), equilibrated with either 50 mM NaAc, 150 mM NaCl, pH 5.6, or 50 mM Tris-HCl, 150 mM NaCl, pH 7.4. Proteins were eluted at a flow rate of 40 μl/min using an ÄKTA Ettan LC system (GE Healthcare). Fractions were examined for ASG activity by pNCS assays.

**Reverse Transcription PCR Expression Analysis**—Reverse transcription PCR experiments were performed using a panel of normalized cDNAs prepared from eight normal human tissues (MTC panel human I; Clontech). An internal 880-bp fragment from human ARSG was amplified by PCR (forward primer 5′-TTCATCCAGCGTGCAAGACACCAGC-3′ and reverse primer 5′-CTTACAAATGGCTTCGCCGTGTC-3′). PCR was carried out for 35 cycles with 62 °C annealing temperature. Normalization was confirmed by primers specific for glyceraldehyde-3-phosphate dehydrogenase.

**RESULTS**

**Purification and Arylsulfatase Activity of ASG**—To investigate the biochemistry and cell biology of ASG, we generated HT1080 cell lines stably expressing human ASG with a C-terminal RGS-His<sub>6</sub> tag. The secreted protein was purified from the conditioned medium using a combination of Ni<sup>2+</sup> affinity chromatography (lane 3), and after RESOURCE 5 cation exchange chromatography (lane 4) were separated by SDS-PAGE. Proteins were stained with Coomassie (A) or detected by Western blotting using an anti-RGS-His<sub>6</sub> antibody (B). Molecular mass standards (lane M) are shown on the left. The intense band at 66 kDa in lanes 1 and 2 corresponds to bovine serum albumin from fetal calf serum. To determine the pH optimum of enzymatic activity, ASG was incubated with either 10 mM pNCS or 10 mM 4-MUS at pH 4.5–8.0 for 1 h at 37 °C using 13 or 26 ng of ASG/assay, respectively (C). The temperature optimum of ASG activity was analyzed using 10 mM pNCS at pH 5.6 and incubation for 1 h at 36–74 °C (D). Substrate turnover was linear during incubation (not shown). Activities under optimum conditions were set to 100%. Error bars represent S.D. from three independent experiments.
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Characterization of ASG Mutants/Isoforms—The active site Cα-formylglycine residue is essential for catalysis in all human sulfatases that have been characterized so far (2, 13). The so-called sulfatase signature 84-CSPSR-88 of ASG predicts Cys-84 to be post-translationally modified to FGly. To confirm the importance of FGly in ASG, we mutagenized Cys-84 to Ala and compared the activity of the mutant to the wild-type enzyme by performing pNCS activity assays with cell lysates from stably transfected cells. We also analyzed the activity of the ASG A501P isoform encoded by the sequence of the human expressed sequence tag clone KIAA1001 (see “Experimental Procedures” and Refs. 21, 22). The ASG C84A mutant showed no activity above background levels resulting from endogenous sulfatases (Fig. 2). Compared with wild-type ASG, the A501P isoform displayed only ~35% activity. Wild-type ASG was used for all further studies.

Arylsulfatase Substrates, Kinetics, and Inhibitors of ASG—To further investigate the enzymology of ASG, we analyzed the influence of substrate concentration on the activity toward pNCS (Fig. 3A). ASG follows Michaelis-Menten kinetics and shows hyperbolic substrate saturation. At substrate concentrations above 15 mM substrate inhibition is observed. The double-reciprocal Lineweaver-Burk transformation (Fig. 3B) yields the following kinetic constants: $K_m = 4.2$ mM, $V_{max} = 63.5$ μmol/(min·mg) = 63.5 units/mg. Compared with other arylsulfatases, these values fall within the normal range. Typical activities toward pNCS are 40–100 units/mg (27). Because of its limited solubility at acidic pH, no substrate saturation could be obtained with 4-MUS as a substrate (data not shown). With an estimated $V_{max}$ of only 0.2–0.4 units/mg and a $K_m$ of 5–15 mM, 4-MUS represents a rather poor substrate for ASG. Compared with pNCS, the cleavage of the closely related pNPS, merely lacking one hydroxyl group, is even slower (~0.05 units/mg at pH 5.6), indicating a stringent substrate specificity that might also apply for physiological substrates.

Many sulfatases are inhibited by their product, sulfate, or by its analog, phosphate (28). Interestingly, inhibition of ASG by phosphate is much stronger than by sulfate (Fig. 3C). Whereas sulfate has an IC$_{50}$ value of ~1 mM, inhibition by phosphate shows an IC$_{50}$ of ~50 μM at 10 mM pNCS (Fig. 3C, inset). To determine the type of inhibition, substrate saturation curves were recorded at different phosphate concentrations, which are presented in Fig. 3D as Lineweaver-Burk plots. The regression lines show a common intercept on the ordinate axis as expected for a competitive inhibitor. From the slopes of the regression lines plotted against inhibitor concentrations, a $K_i$ value of 17 μM was extrapolated. In contrast to arylsulfatase E (29), ASG is not inhibited by warfarin even at concentrations close to the solubility limit (data not shown).

ASG Is Active in the Monomeric State—To analyze whether or not ASG forms dimeric or oligomeric complexes, purified ASG was subjected to gel filtration on a Superdex 75 column at either pH 5.6 or 7.4. Fractions were tested for sulfatase activity (Fig. 4). At both pH values, neither protein UV peaks nor sulfatase activities provided any indication for dimerization or oligomerization of ASG as described for its closest relative, arylsulfatase A (30). ASG eluted as a 63-kDa protein, which corresponds to the monomeric molecular mass also observed in SDS-PAGE. Thus, unlike, for example, arylsulfatase B, ASG does not undergo lysosomal processing (31).

Characterization of the ASG Glycosylation State—The ASG sequence contains four potential N-glycosylation sites (asparagine residues 117, 215, 356, and 497). Treatment with peptide N-glycosidase F reduces the apparent sizes of both the intracellular and the secreted protein from a broad, glycosylated band at 63 kDa (with micro-heterogeneity in its N-glycans) to a sharp band of ~53 kDa (Fig. 5A). This difference in size suggests that all four N-glycosylation sites are utilized, assuming that the average mass is ~1.9 kDa for a high mannose and ~2.9 kDa for a complex type oligosaccharide (24). Furthermore, the intracellular ASG is largely sensitive toward treatment with endoglucoinamidase H, whereas the secreted ASG is resistant (Fig. 5A, lane 8). Thus, high mannose type oligosaccharides added to ASG in the ER are processed to complex type N-glycan structures during maturation in the Golgi. The intracellular ASG represents a mixture containing mainly high mannose N-glycans, one of which, however, was endoglucoinamidase H-resistant in the majority of ASG molecules (Fig. 5A, lane 4). Endoglucoinamidase H treatment did not result in a complete deglycosylation even at extended incubation times and increased endoglucoinamidase H concentrations.

ASG Binds to Mannose 6-Phosphate Receptors—Most lysosomal proteins are transported to the lysosomes via the mannose 6-phosphate receptor (M6PR) pathway (32, 33). In a comprehensive proteome analysis of lysosomal proteins, an affinity column with immobilized M6PRs has successfully been used to identify novel lysosomal candidate proteins (26). Here, we used this affinity column to assay whether or not ASG binds to...
M6PRs in vitro (Fig. 5B). The M6PR matrix was incubated with purified ASG and washed with glucose 6-phosphate to remove unspecifically bound protein. As expected for a lysosomal protein, ASG eluted specifically with mannose 6-phosphate (Fig. 5B, lanes E1–4) but not with glucose 6-phosphate (lanes W5–7), thus providing further evidence for a lysosomal localization. About 28% of ASG loaded onto the affinity matrix was recovered in the elution fractions. The observed binding efficiency corresponds to those of reference lysosomal proteins and is probably due to partial modification with mannos 6-phosphate residues in the overexpressing cells (26).

Intracellular Localization of ASG—To corroborate the lysosomal localization of ASG as suggested by its acidic pH optimum and binding to M6PRs, we analyzed the localization of overexpressed ASG in HT1080 cells by immunofluorescence. For lysosomal staining, LysoTracker Red was used, an aldehyde-fixable, fluorescent amine that accumulates within acidic cell compartments, predominantly lysosomes, trans-Golgi, and perinuclear late endosomes (34, 35). Cells were incubated with LysoTracker Red for 2 h prior to fixation, permeabilization, and incubation with anti-RGS-His6 antibodies to detect ASG. The ASG staining (Fig. 6) revealed vesicular spots throughout the cytoplasm, similar to the distribution observed for the lysosomal arylsulfatase A (36). Although some of the LysoTracker signal was lost during the fixation and permeabilization steps, most of the ASG-positive vesicular structures co-localized with the LysoTracker staining, thus substantiating ASG as a lysosomal sulfatase. The detection of lysosomal structures was obscured in many smaller cells due to the intense perinuclear ER/Golgi staining. ER localization of ASG is most likely due to folding/maturation problems as commonly observed for many overexpressed proteins entering the secretory route. This might explain why ASG has been characterized previously as an ER-localized protein in transiently transfected COS-7 cells using Myc-tagged ASG (21). Co-immunofluorescence analysis using lysosomal membrane glycoprotein 1 (LAMP1) as an alternative marker of lysosomes/late endosomes confirmed the predominantly lysosomal localization of ASG also in low expressing HT1080 cells (see supplemental Fig. S2).

Tissue-specific Expression of ASG—Tissue expression analysis can add important information about the biological role of sulfatases in vivo. Previous reverse transcription PCR experiments with five different mouse tissues revealed a rather ubi-
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DISCUSSION

Of the 17 known human sulfatases (16), six have been reported to be localized in lysosomes. As typically seen for lysosomal enzymes, all of these sulfatases have acidic pH optima ranging from 3.8 to 5.7 (17). Moreover, all six sulfatases have been linked with six individual lysosomal storage disorders (2). Here we show that the human arylsulfatase G also belongs to this important group of lysosomal enzymes.

ASG is a stable 63-kDa glycoprotein that hydrolyzes different pseudosubstrates at acidic pH but has only ~10% activity at pH 7.5. Mutation of the active site Cys residue that is predicted to be post-translationally converted to FGly leads to a complete loss of enzymatic activity, emphasizing the importance of FGly for catalysis. The ASG A501P isoform, corresponding to the KIAA1001 expressed sequence tag clone (22), displays significantly reduced activity. The observed differences in comparison with wild-type ASG could be caused by compromised protein folding due to the presence of a proline residue. It should be noted that the Ala codon is conserved in the genomes of mouse, rat, cow, and even chicken and zebrafish.

In transfected HT1080 cells, ASG co-localizes with the lysosomal markers LysoTracker Red and LAMP1. Furthermore, ASG binds to immobilized mannose 6-phosphate receptors, which are required for transport of most lysosomal proteins into the lysosomes. Combined with the acidic pH optimum of enzymatic activity, these findings demonstrate that ASG is an active, lysosomal arylsulfatase. Although the immunofluorescence data do not fully exclude the possibility that ASG has a dual lysosomal/ER localization, this seems unlikely with respect to enzyme function.

Arylsulfatase A, the sulfatase with highest homology to ASG, is a lysosomal sulfatase that acts as an octamer at acidic pH (30). Deficiency of arylsulfatase A causes metachromatic leukodystrophy, a lysosomal storage disorder associated with progressive demyelination of the central and peripheral nervous system. During biogenesis, arylsulfatase A forms dimeric complexes in the ER that are transported via the mannose 6-phosphate receptor pathway to the lysosomes. Because of the pH shift, the arylsulfatase A dimers oligomerize to octamers, thereby stabilizing the enzyme against lysosomal proteases.
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Inability to octamerize, as observed for the common P426L allele of arylsulfatase A, contributes to metachromatic leukodystrophy pathology (30). In contrast to arylsulfatase A, gel filtration analysis of ASG showed no indication for a possible dimerization or oligomerization. The active state of ASG is the unprocessed 63-kDa monomer.

Kinetic analysis revealed a strong, competitive inhibition of ASG by phosphate (K_i = 17 μM). This inhibition might represent an additional regulatory mechanism to control ASG activity. For instance, secreted ASG that has escaped from lysosomes would be entirely inactivated in the extracellular environment due to the neutral pH as well as the high phosphate concentrations (e.g. 0.6–1.3 mM in blood serum). To the best of our knowledge, absolute intralysosomal phosphate concentrations have not been directly determined; however, the uptake of phosphate into human fibroblast lysosomes has been characterized as a saturable transport system with K_M = 5 μM (38). This suggests that ASG activity could be modulated and restricted to specific biological conditions by changes in phosphate concentrations. Inhibition of ASG by sulfate is probably of less relevance, because physiological sulfate concentrations (0.3–0.5 mM in blood serum) (39) do not result in a significant reduction of ASG activity.

The physiological substrates of ASG remain to be characterized. For many sulfated biomolecules, the corresponding sulfatases have not been identified so far. The lysosomal localization and differential expression with highest levels in liver and kidney suggest the loss of ASG activity to be associated with a previously unidentified lysosomal storage disorder. A comprehensive analysis of physiological substrates will be required in order to elucidate the metabolic role of ASG. The generation of ASG knock-out mice is in progress in our laboratory and will hopefully help in identifying the physiological substrates and in understanding the biological significance of this sulfatase, which might ultimately lead to the identification of ASG-deficient patients. Such patients could then be treated by either enzyme replacement, substrate reduction, enzyme enhancement, or gene therapy as currently being tested and practiced for other lysosomal enzyme deficiencies. The finding that ASG contains mannos-6-phosphate particularly qualifies this sulfatase for enzyme replacement therapy.

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