Abstract

Arylsulfatase A (ARSA) and B (ARSB) have been regarded as lysosomal enzymes because of their hydrolytic activity on synthetic aromatic substrates and the lysosomal localization of their enzymatic activity. Using sea urchin embryos, we previously demonstrated that the bulk of ARS is located on the cell surface of the epithelium, colocalizing with sulfated polysaccharides, and that it does not exhibit enzymatic activity. To examine whether ARSA and ARSB exist on the cell surface in mammalian tissues, we raised antibodies against ARSA and ARSB and examined immunohistochemically their localization in the liver using light and electron microscopy. Here we show that mammalian ARSA and ARSB exist on the cell surface of sinusoidal endothelial cells, hepatocytes, and sinusoidal macrophages (Kupffer cells), as well as in the lysosome. They are also colocalized with heparan sulfate proteoglycan. These results suggest that ARSA and ARSB also may function in the cell surface of mammals. This is the first report to show cell-surface localization of ARS in mammalian somatic cells. The extracellular localization of ARS will provide new insight for human ARS deficiency disorders, such as metachromatic leukodystrophy and mucopolysaccharidosis VI.

Key words

Arylsulfatase A, B · Metachromatic leukodystrophy · Mucopolysaccharidosis type VI · Lysosomal storage disease · Kupffer cell · Sinusoidal endothelial cell · Liver

Introduction

Arylsulfatase (ARS) is a lysosomal enzymes that hydrolyzes arylsulfates. Different types of ARS, from A through K, have been cloned from humans. The genetic deficiency of ARSs cause severe lysosomal storage disorders. A deficiency of ARSA causes metachromatic leukodystrophy (MLD). A deficiency of ARSB causes mucopolysaccharidosis type VI syndrome (MPS VI). A deficiency of ARSC causes ichthyosis, which is a metabolic disease with an X-linked recessive inheritance.

Using enzyme histochemical methods, it has been shown that enzymatic activity of ARSs is localized in the endoplasmic reticulum and lysosomes in various tissues. Immuno-histochemical and biochemical studies show that ARSs A, B, and G are localized in the lysosome, ARSs C, D, and F are localized in the endoplasmic reticulum, and ARS E is localized in the Golgi apparatus.

It has been shown that sea urchin ARS mainly exists on the apical surface of the embryonic cells, and the possible function of ARS as a component of extracellular matrix has been suggested. We speculated that mammalian ARS, or at least a part of the subfamily, could also exist on the cell surface. Among the ARS genes, the ARSA and ARSB have been well studied. Thus, we focused the investigation on ARSA and ARSB. In this study we produced polyclonal antibodies against ARSA and ARSB, and using these antibodies we examined the localizations of these two enzymes in the liver using light and electron microscopy. Here we show that mammalian ARSA and ARSB also mainly exist on the cell surface. We suggest that ARSA and ARSB might have novel functions in the cell surface or near the cell surface of the extracellular space.
Materials and methods

Animals

Adult Wistar rats (male) and ICR mice (male) were used. Their use was approved by the Animal Care Committee of Kyorin University School of Medicine.

Reagents

Cy3-labeled affinity purified goat antirabbit IgG and Cy2-conjugated donkey antirat IgG were from Jackson Immunoresearch (West Grove, PA, USA). DAPI (4′,6′-diamidino-2-phenylindole dihydrochloride) was obtained from Boehringer Mannheim (Mannheim, Germany).

Antibodies

To examine the localization of ARS in mammalian cells, we raised polyclonal antibodies to mouse ARSA and rat ARSB. When we started this study, sufficient knowledge of the amino acid sequence of mouse ARSB appropriate for antibody production was not available.

Polyclonal antiserum ARSA and antirat ARSB antibodies were raised against synthetic peptides QYDAAMTFGEGPA (GenBank accession no.: BC011284, amino acids 464–483) and VASPLLKQKGVKSRELMHITPSQIAKGEDPA (GenBank accession no.: BC011284, amino acids 327–346), respectively, by immunizing rabbits. (NCBI Reference Sequences accession no.: NM_033443, amino acids 121–135) and QYDAAMTFGESQ (GenBank accession no.: BC011284, amino acids 464–483) and VASPLLKQKGVKSRELMHITPSQIAKGEDPA (GenBank accession no.: BC011284, amino acids 327–346), respectively, by immunizing rabbits. Their use was approved by the Animal Care Committee of Kyorin University School of Medicine.

Immunoblot analysis

The specificity of anti-ARS antibodies was determined by Western blotting analysis. Mouse Swiss3T3 cells were dissolved in sample buffer [final concentration: 100 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.04% bromophenol blue, 2% β-mercaptoethanol] and boiled for 5 min. Whole liver tissue of a male rat of the Wistar strain, weighing 200–250 g, was homogenized in a homogenization buffer containing 15 mM Tris-HCl (pH 7.5), 150 mM NaCl, 500 μM ethylenediaminetetraacetic acid (EDTA), 500 μM diethyloretiol (DTT), 2 mM phenylmethanesulfonfonyl fluoride (PMSF), and protease inhibitor cocktail (1:1000 dilution; Sigma-Aldrich) in accordance with the manufacturer’s instructions. The lysate was centrifuged at 35000 g for 30 min. The clear supernatant was dissolved in the sample buffer and boiled for 5 min. Proteins were analyzed on 12% (Swiss3T3) or 10% (rat liver) acrylamide gels by SDS-polyacrylamide gel electrophoresis (PAGE) and transblotted onto a polyvinylidene fluoride (PVDF) membrane (Immobilon Transfer Membranes; Millipore, Bedford, MA, USA). The membrane was reacted with antiserum ARSA (1:10000) or antirat ARSB antibodies (1:2000), followed by horseradish peroxidase-conjugated goat antirabbit secondary antibodies (KPL, Rockford, IL, USA), followed by detection with Super Signal West Dura Extended Duration Substrate (PIERCE, Gaithersburg, MA, USA) as an enzymatic substrate. The chemiluminescent signal was detected by X-ray film.

Fluorescent immunohistochemistry

The tissues were fixed with 4% paraformaldehyde–phosphate-buffered saline containing 0.9 mM CaCl$_2$ and 0.9 mM MgCl$_2$ (PBS(+)) at 4°C for 1 h. After washing with PBS(+), nonfrozen sections (40 μm thick) were cut with a microslicer DTK-1500 (D.S.K., Kyoto, Japan). The sections were washed with PBS(+), incubated with 0.05% Triton-X100–PBS(+) for 30 min, blocked with 5% normal donkey serum–PBS(+) for 1 h, and then the sections were incubated with the anti-ARSA (1:500) or anti-ARSB (1:1000) antibodies and rat monoclonal heparan sulfate proteoglycan antibody (1:100) (Upstate Biotechnology, Lake Placid, NY, USA) at 4°C for 24 h. The antigen was detected by incubating with Cy3-conjugated donkey antirabbit IgG antibody (1:100) and Cy2-conjugated donkey antirat IgG (1:100) (Jackson Immunoresearch) at 4°C for 2 h. Nuclei were stained with TO-PRO-3 (Molecular Probe, Eugene, OR, USA). After washing with PBS(+), the specimens were mounted in 90% glycerol-0.1 M Tris-HCl buffer (pH 8.5) containing 0.5 mM p-phenylene diamine, and observed under a laser confocal scanning microscope (MRC-1024; BioRad, Hercules, CA, USA).

When PBS without Ca$^{2+}$ and Mg$^{2+}$ (PBS(−)) was used for all processes of fixation and immunoreaction instead of PBS(+), positive staining was scarcely observed.

Immunoelectron microscopy

The method of fixation, sectioning, and blocking was the same as the immunohistochemistry except that endogenous peroxidase activity was blocked by incubating sections with 0.3% H$_2$O$_2$–PBS (+) at room temperature for 30 min. The horseradish peroxidase (HRP)-labeling was carried out according to the method described previously.$^{19}$ After incubation of the sections with anti-ARSA or anti-ARSB antibodies, the specimens were reacted with HRP-conjugated donkey antirabbit IgG antibody (Dako, Kyoto, Japan) at 4°C for 24 h. After washing with PBS(+), the antigen was visualized with 3,3′-diaminobenzidine (DAB) (0.5 mg/ml) containing 0.05% H$_2$O$_2$. The specimens were postfixed with 1% OsO$_4$ in 0.1 M phosphate buffer (pH 7.3), dehydrated through a graded series of ethanol solutions, exposed to propylene oxide, and embedded in Epon 812. Ultrathin sections were prepared, stained with lead citrate, and observed under a transmission electron microscope (JEM-1010) (JEOL, Tokyo, Japan).