SULFATION OF CHENODEOXYCHOLIC ACID BY RAT BRAIN CYTOPLASMIC FRACTION
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We have found three bile acids, cholic acid, chenodeoxycholic acid (CDCA), and deoxycholic acid, in the rat brain tissue. Although CDCA is the most abundant brain bile acid, it may, therefore, usually bind to some proteins in the brain tissue to avoid the toxic expression based on its lipophilicity. Dehydroepiandrosterone, one of neurosteroids, undergoes sulfation at C-3 position by the action of SULT2A1, and the produced sulfate is excreted from brain via the organic anion transporting polypeptide. CDCA is converted into its sulfate conjugate by SULT2A1 in the liver. Therefore, the brain CDCA may also undergo sulfation for its detoxification and improvement in its clearance. In this study, we investigated the sulfation of 3α-hydroxyl group in CDCA by rat brain tissue using a highly sensitive and selective liquid chromatography/electrospray ionization mass spectrometry. CDCA as a substrate was mixed with the rat brain cytoplasmic fraction, and then incubated at 37°C after adding 3'-phosphoadenosine 5'-phosphosulfate. The incubation mixture was mixed with 18O-labeled deoxycholic acid 3-sulfate as an internal standard, and the products were extracted by a solid-phase extraction method. In the incubation mixture, we found CDCA 3-sulfate, which was produced by the enzymes in the rat brain cytoplasmic fraction, although the activity for CDCA sulfation was less than that for dehydroepiandrosterone sulfation. The results clearly suggested that the sulfation activity toward 3α-hydroxyl group of CDCA existed in the rat brain. This enzymatic conversion of CDCA may be important for regulation of the brain CDCA content.

SPECIFIC ANALYSIS OF SMALL MOLECULE-BINDING PROTEINS
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Although the affinity extraction method using a gel directly immobilized small molecules is commonly used for identification of the target proteins of physiological active small molecules and drugs, it often leads to the insufficient information because of its non-specific bindings. To overcome this problem, we have developed the specific affinity extraction method for small molecule binding proteins (N. Mano et al., Anal. Chem., 2006: 78, 4668-4675). We use a unique cleavable affinity gel prepared in our laboratory, which has disulfide linker between a small molecule and its support. By the use of the mouse ascites including the bile acid antibody and serum albumin as bile acid-binding proteins, we have demonstrated that the mild cleavage reaction of disulfide linker using dithiothreitol cleaves significantly the disulfide bonds in the linker without any reductive reactions of intra-molecular disulfide bonds in protein molecules captured on the immobilized small molecules. In this study, we applied this method to identify the chenodeoxycholic acid (CDCA)-binding proteins in the rat liver tissue. We mixed the rat liver cytoplasmic or nuclear fractions with the CDCA-immobilized cleavable affinity gel, and then mildly washed with phosphate buffer (pH 7.4). Dithiothreitol was added into the mixture to cleavage of the disulfide linker, and the CDCA-binding proteins were recovered in the supernatant. The extracted proteins were separated by SDS-PAGE and analyzed by MALDI-TOF MS after in-gel digestion to identify their CDCA-binding proteins. The results suggested that CDCA binds to several proteins in the rat hepatocytes.