

脂質アシルCoA：ウシ脳のミトコンドリア  
のスフィンゴシンアシル転移酵素：可  
溶化と膜脂質リポソームでの再構成

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**Fatty Acyl-Co A: Sphingosine Acyltransferase in Bovine  
Brain Mitochondria: Its Solubilization and Reconstitution  
onto the Membrane Lipid Liposomes**

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**ABSTRACT** Fatty acyl-Co A: sphingosine acyltransferase (ceramide synthase, EC 2.3.1.24) is mainly localized in the microsomal and mitochondrial membranes. Attempts to isolate the enzyme have failed, largely because there has been little or no detection of the enzyme activity in detergent extracts. In this study, we solubilized the membrane-bound enzyme from bovine brain mitochondria with a Tris-HCl buffer containing 2 % Triton X-100 and, after removal of the detergent, reconstituted it with the membrane lipid liposomes. The specific activity of the reconstituted enzyme was approx. 8 times higher than that of the solubilized enzyme. We next examined the lipid dependence of the enzyme, using various phospholipid liposomes. The ability of phospholipids to enhance the activity of solubilized ceramide synthase was specific and structure-related. The most potent stimulator was phosphatidylserine liposomes, suggesting an important role of the net negative charges. This paper also describes a highly reproducible high-performance liquid chromatographic (HPLC) procedure for the determination of ceramide synthase activity. Combination of the HPLC method with the reconstituted enzyme system appears to be suitable for elucidating the characteristics of this enzyme.

抄録 スフィンゴシンアシル転移酵素は主にミクロソームやミトコンドリアの膜に存在する。著者らはウシの脳のミトコンドリアから膜結合性の酵素を可溶化し、膜脂質リポソームで再構成した。再構成した酵素の比活性は可溶化した酵素の約8倍であった。可溶化したセラミド合成酵素の活性を高めるリン脂質の能力は、特異的で構造相関があった。最も効果的な促進因子は実効陰荷電が重要な役割をもつと示唆されるホスファチジルセリンリポソームであった。また再構成した酵素系とHPLCによるセラミド合成酵素活性測定法の組み合わせは、この酵素の性質を解明するために有用な方法である。

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