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# The international debate on Expression and characterization of recombinant human acyl-CoA synthetase (ACSL4)

**Shunataro Hara**
Showa University, Japan

Acyl coenzyme A synthetase long-chain family members (ACSLs) are a family of enzymes that convert long-chain free fatty acids into their acyl-CoAs. Among ACSL isozymes, ACSL4 has been hypothesized to modulate the metabolic fates of polyunsaturated fatty acids including arachidonic acid. In the present study, to investigate the enzymatic and protein characteristics of ACSL4, the cDNA for human ACSL4 was cloned from human epithelial colorectal adenocarcinoma Caco-2 cells and then recombinant ACSL4 enzyme containing a C-terminal His-tag was expressed in Spodoptera frugiperda 9 (Sf9) cells using the baculovirus expression system. ACSL4 enzyme activity was detected in 10,000xg supernatants of ACSL4-expressing Sf9 cell lysates and then partially purified by nickel affinity column chromatography. We further investigated the substrate specificity of recombinant human ACSL4 by LC/MS and found that ACSL4 enzyme preferred various kinds of polyunsaturated fatty acids including docosahexaenoic acid, docosapentaenoic acid, eicosopentaenoic acid, and dihomo-γ-linolenic acid, as well as arachidonic acid as a substrate. On the other hand, oleic acid, linoleic acid and linolenic acid were poor substrates, although these fatty acids contain unsaturated bonds. These results confirmed the importance of ACSL4 in maintenance of membrane phospholipid bearing polyunsaturated fatty acid.

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