Evidence of Isozymes for Δ6 Fatty Acid Desaturase in Rat Hepatocytes

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The expression of Δ6 fatty acid desaturase, previously identified, was suppressed almost completely by hyper expression of the corresponding antisense gene in a transformant of the rat hepatic cell line BRL-3A. Conversion rates of [1-14C] linoleic acid, α-linolenic acid, and tetracosapentaeanoic acid into the respective Δ6 fatty acids were equivalent to those in control cells. This finding suggested that all of these reactions were catalyzed by at least two Δ6 desaturase isozymes in rat hepatocytes.

Key words: antisense; Δ6 desaturase; isozyme; polyunsaturated fatty acids; rat hepatocyte

In mammals, polyunsaturated fatty acids such as arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3) are synthesized from dietary linoleic acid (LA; C18:2n-6) and α-linolenic acid (ALA; C18:3n-3), respectively, according to multi step pathways with fatty acid desaturation and elongation reactions (Fig. 1).1 The Δ6 desaturase is involved in the first, limiting step of the pathways and is thus a key enzyme to maintain the homeostasis of cellular unsaturated fatty acids. The Δ6 desaturation of oleic acid (C18:1n-9) also is needed for de novo synthesis of mead acid (C20:3n-9), which synthesis is induced by essential fatty acid deficiency. We previously identified a Δ6 desaturase in rat liver.2 Expression analysis in a yeast suggested that this enzyme catalyzes the Δ6 desaturation of both LA and ALA but not oleic acid. This finding rules out the involvement of the isolated enzyme in the n-9 pathway. On the other hand, it was recently reported3 that the Δ6 desaturase also acts on tetracosapentaeanoic acid (C24:5n-3) to generate tetracosahexaenoic acid (C24:6n-3) for biosynthesis of docosahexaenoic acid, the pathway of which has been demonstrated biochemically by Sprecher et al.4 Therefore, this kind of Δ6 desaturase participates in at least three reactions for the conversion of LA, ALA, and C24:5n-3 into their respective Δ6 fatty acids.

Nonetheless, in an experiment with hepatic microsomes in vitro, N-ethyl maleide inhibited the Δ6 desaturase activity in LA but not in ALA.5 In addition, a difference in the inhibitory effect of trans-9,12-linoleic acid was observed in Δ6 desaturation reactions toward LA and C24:5n-3 in vitro.6 These results suggested that there are two or more Δ6 desaturases for the n-6 and n-3 pathways. We need to find whether cloned Δ6 desaturase mainly governs one, two, or all of these possible reactions before studying the molecular mechanisms underlying the regulation of Δ6 desaturation. In this paper, a hepatic cell line, in which an antisense gene for the Δ6 desaturase was hyper expressed to suppress the corresponding intrinsic activity, was established to see the degree of its contribution to each of the reactions.

Full-length cDNA for rat liver Δ6 desaturase was ligated into a mammalian expression vector, pCEP4 (Invitrogen, Carlsbad, CA), carrying a hygromycin resistance gene, so that the 3'-end of the insert was just downstream (at the KpnI site) of a cytomegalovirus promoter. The resulting plasmid, pCEP4-Δ6d, or a control plasmid, pCEP4, prepared with an EndoFree Plasmid Maxi Kit (Qiagen, Bothell, WA)
was then used to transfect a rat hepatic cell line, BRL-3A (Human Science Research Resources Bank, Osaka, Japan), with Transfectam reagent (Promega, Madison, WI) as described by the manufacturer. The cells were maintained in a medium (Dulbecco's Modified Eagle Medium-Nutrient Mixture F-12 containing 10% fetal bovine serum; Invitrogen) at 37°C for 24 h in an incubator with 5% CO₂. Stable transfectants were selected by the addition of hygromycin B (Invitrogen) to the culture medium to the final concentration of 200 µg/ml, and several cell lines were cloned by limiting dilution.

When a 70%-confluent culture was obtained in a 100-mm dish, the culture was treated with LA, ALA, or docosapentaenoic acid (DPA; C22:5n-3; 80.5 nmol/dish) bound to bovine serum albumin (free of essential fatty acids; 15.6 nmol/dish; Sigma, St. Louis, MO) prepared by incubation of such a mixture at 37°C for 2 h. DPA was used here because the radiolabeled C24:5n-3 used for the experiment mentioned below was not available. After further cultivation until cells were confluent (24 h), the cells were removed from the plates with 0.05% trypsin-EDTA and suspended in 10 mM phosphate-buffered saline, pH 7.4. Cell lysates were then used in western blotting with anti-Δ6 desaturase antibody (Fig. 2). In the control experiments, a positive signal was detected on a component with a molecular mass of 47 kDa, which was smaller than that calculated from the deduced amino acid sequence (52.4 kDa). It is likely that the Δ6 desaturase in the polyacrylamide gel was charged more negatively than other proteins because of an excess of dodecyl sulfate bound to the large hydrophobic regions in this protein. In fact, the antibody used reacted with a 43 to 47-kDa component in rat liver microsomes and in a yeast expressing the Δ6 desaturase gene (unpublished). On the other hand, in samples of antisense-expressing cells cultivated with the same kind of fatty acid, the intensity of the signal was less than 3% of that in the respective controls (Fig. 2). This finding showed that the expression of the antisense gene suppressed the accumulation of the corresponding protein in the cells.

To examine the effects of suppression of the target protein on Δ6 desaturase activity, we cultivated the BRL-3A transformants under the same conditions except that a part (18 nmol) of the fatty acid to be added was replaced by the same amount of the respective radiolabeled fatty acid ([1-14C]LA, ALA, or DPA; 1.85 kBq/nmol; American Radiolabeled Chemicals, Inc., St. Louis, MO). Cellular fatty acids were then extracted, methyl-esterified, and resolved by thin-layer chromatography. When LA was added to the culture, cellular incorporation of LA and accumulation over time of γ-linolenic acid (C18:3n-6), dihomo-γ-linolenic acid (C20:3n-6), and arachidonic acid were observed in both antisense-expressing and control cells with little if any difference (Fig. 3A). When ALA was added, the amounts of eicosapentaenoic acid (C20:5n-3) and DPA increased together with the increasing amount of incorporated ALA, which was again independent of the expression of the antisense gene (Fig. 3B). Similarly, the addition of DPA caused the generation of C24:6n-3 and docosahexaenoic acid at equivalent levels in both transformants (Fig. 3C). These results, taken together with those in the western analysis, suggested the existence of an isozyme(s) for the Δ6 desaturase in BRL-3A cells.

One of the candidates for such an isozyme is a rat homologue of FADS3, the gene of which has been...
Fig. 3. Δ6 Desaturation Activity in the BRL-3A Transformants.

The antisense-expressing transformant (+; pCEP4-Δ6d) or control one (−; pCEP4) was cultivated in the presence of 14C-labeled LA (A), ALA (B), or DPA (C) bound to bovine serum albumin free of essential fatty acid. Total lipid was extracted from the cells (5 × 10⁶) with chloroform-methanol (2:1, v/v) and treated with 10% methanolic hydrochloride (Tokyo Kasei, Tokyo, Japan) at 60°C for 3 h. The fatty acid methyl esters were resolved on a silica gel 60 HPTLC plate with a concentration zone (Merck, Darmstadt, Germany), which was immersed with 10% silver nitrate and dried before use. The plate was developed with toluene-acetone (95:5, v/v; panel A) or toluene-acetonitrile (90:10, v/v; panels B and C) and then analyzed on a BAS1000 imaging analyzer (Fuji Film, Kanagawa, Japan). The components were identified by scraping of the bands and eluting them with hexane followed by analysis by gas-liquid chromatography as described previously.8) The experiment was repeated three times with similar results. S, 14C-labeled fatty acid used as a substrate for each experiment.

found in the vicinity of the Δ6 and Δ5 desaturase genes in the human genome.7) Its deduced amino acid sequence contains three histidine clusters, two large hydrophobic regions, and a cytochrome b₅-like domain, all of which are characteristic of a certain group of fatty acid desaturases.2) However, an expression experiment is necessary to identify its substrate specificity since this cannot be predicted from sequence information only. The other candidate is a linoleoyl-CoA desaturase purified by Okayasu et al.,9) the observed molecular mass of which was 66 kDa, which differs from that of our enzyme (43–47 kDa). Isolation of the corresponding gene will be required although amino acid sequence of the purified enzyme has not been reported.

This study showed that, in a rat hepatic cell line, at least two Δ6 desaturases participate in all three Δ6 desaturation steps, unlike in previous reports5,6) that suggested that different kinds of the enzyme may govern the different reactions in human hepatocytes.

References


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