Cloning and Identification of a Novel C18-Δ9 Polyunsaturated Fatty Acid Specific Elongase Gene from DHA-producing Isochrysis galbana H29

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Abstract Isochrysis galbana, a marine prymnesiophyte microalga, is able to produce a high level of long chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA, C22:6n-3). In this article, a novel gene (IgASE2) that encoded a C18-Δ9 polyunsaturase fatty acids specific (C18-Δ9-PUFAs-specific) elongase was isolated and characterized from DHA-rich microalga, I. galbana H29. A full-length cDNA of 1653 bp was cloned by rapid-amplification of cDNA ends (RACE) PCR techniques. The IgASE2 contained a 786 bp ORF encoding a protein of 261 amino acids that shared 87% identity with the reported Δ9-elongase IgASE1, a 44 bp 5' untranslated region and an 823 bp 3' untranslated region. The function of IgASE2 was demonstrated by its heterologous expression in Saccharomyces cerevisiae. In S. cerevisiae, IgASE2 elongated linoleic acid (LA, C18:2n-6), α-linolenic (ALA, C18:3n-3) to eicosadienoic acid (EDA, C20:2n-6) and eicosatrienoic acid (ETra, C20:3n-3). The conversion ratios of LA to EDA and ALA to ETra were 60.47 and 58.36%, respectively. However, IgASE2 could not catalyze the elongation reactions of oleic acid (OA, C18:1n-9) and other fatty acids. These results confirmed that IgASE2 had C18-Δ9-PUFAs-specific elongase activity.

Keywords: Δ9-elongase, polyunsaturated fatty acids, Isochrysis galbana, arachidonic acid, eicosapentaenoic acid

1. Introduction

The very long chain polyunsaturated fatty acids (LCPUFAs), arachidonic acid (ARA; C20:4n-6), eicosapentaenoic acid (EPA; C20:5n-3), and docosahexaenoic acid (DHA; C22:6n-3) are important components of neuronal cells in brain and retina tissues and are considered to have profound effects on cell function and development. ARA and EPA are precursors for the biosynthesis of 22-carbon polyunsaturated fatty acids and eicosanoids, including the 2-group prostaglandins, 4-group leukotrienes, thromboxanes, and lipoxins that serve as biological effectors involved in inflammatory, immune responses and cell signaling [1,2]. Several studies have reported that polyunsaturated fatty acids (PUFAs) have the potential to reduce heart disease as well as to improve vision sensitivity and reading ability [3,4]. Additionally, Wathes et al. [5] recently reported that PUFAs were involved in male and female reproductive functions.

LCPUFAs are synthesized through an alternative series of chain elongation and desaturation reactions by the same enzymatic mechanisms. Two pathways involving the conversion of essential fatty acids LA and ALA into LCPUFAs have been found according to different desaturation and elongation reactions (Fig. 1). The common pathway is that in which both LA and ALA are first desaturated by a Δ6 desaturase, and then two carbons are added to the hydro-
carbon chains by a chain-elongating system; subsequently, ARA and EPA are yielded by a further desaturation [6,7]. This pathway is referred to as the Δ6 (ω3-Δ6, ω6-Δ6) pathway [8], because the first step, Δ6 desaturation, is a rate-limiting step. The alternative pathway for biosynthesis of 20-carbon polyunsaturated fatty acids in some organisms is that in which both LA and ALA are first elongated into EDA and ETrA, respectively, and then desaturated, producing dihomo-γ-linolenic acid (DGLA) and eicosatetraenoic acid (ETA) for subsequent conversion to ARA and DHA, respectively [9]. This pathway is referred to as the Δ8 (ω3-Δ8, ω6-Δ8) pathway [8]. In contrast to the common pathway, the first step in the alternative pathway (Δ8 pathway), Δ9 elongation, is a rate-limiting step [8,10]. It is apparent that the identification of different Δ9-elongases is very important to the Δ8 pathway.

Desaturases and elongases involved in PUFA production have been intensively studied in recent years, and a number of desaturase and elongase genes have been isolated. The isolated elongase genes appeared to be related to the ELO gene family of S. cerevisiae [11]. In contrast, only several Δ9 elongase genes were identified. To date, only four C18-Δ9-PUFAs-specific elongases (IgASE1 from the microalga Isochrysis galbana [8], Emihu-d9E from Emiliania huxleyi, Pavpi-d9E from Pavlova pinguis and Pavsa-d9E from Pavlova salina [12]), have been identified and they were the same type of enzymes specific to the synthesis of the C20:2 (n-6) and C20:3 (n-3) PUFAs. Recently, three multifunctional enzymes with Δ9 elongase activities (TaELO [13], TaNE [14] and MALCE1 [15]) were reported. TaELO from Thraustochytrium aureum has Δ9, Δ6, and Δ5-elongation activities as well as the elongation activity of monounsaturated fatty acid. TaNE from T. aureum ATCC 34304 has Δ9, Δ6, and Δ5 elongation activities and Δ5 desaturation activities, and MALCE1 displays wide substrate specificity including Δ9 elongation activity.

Isochrysis galbana, a marine microalga, is rich in DHA and EPA. Here, we reported a novel Δ9-fatty acid elongase, IgASE2, catalyzing specifically the Δ9 elongation reaction. Its gene was isolated from the DHA-rich microalga I. galbana H29, and is heterologously expressed in the yeast INVSc 1. The substrate conversion ratio of IgASE2 is significantly higher than that of IgASE1 [8].

2. Materials and Methods

2.1. Cultivation of I. galbana
I. galbana H29 was obtained from the Tianjin Key Laboratory of Marine Resources and Chemistry, College of Marine Science and Engineering, Tianjin University of Science & Technology. The alga was grown in f/2 medium at 20°C in white light for 12 h/day.

2.2. Total RNA isolation and cloning of full-length cDNA
The I. galbana cultures were harvested by centrifugation at 3,000 × g for 5 min, and total RNA was isolated from the