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SHORT COMMUNICATION

Cloning and characterization of a plastidic glycerol 3-phosphate dehydrogenase cDNA from *Dunaliella salina*

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Summary

A cDNA encoding a nicotinamide adenine dinucleotide (NAD^+)-dependent glycerol 3-phosphate dehydrogenase (GPDH) has been cloned by rapid amplification of cDNA ends from *Dunaliella salina*. The cDNA is 3032 base pairs long with an open reading frame encoding a polypeptide of 701 amino acids. The polypeptide shows high homology with published NAD^+-dependent GPDHs and has at its N-terminal a chloroplast targeting sequence. RNA gel blot analysis was performed to study GPDH gene expression under different conditions, and changes of the glycerol content were monitored. The results indicate that the cDNA may encode an osmoregulated isoform primarily involved in glycerol synthesis. The 701-amino-acid polypeptide is about 300 amino acids longer than previously reported plant NAD^+-dependent GPDHs. This 300-amino-acid fragment has a phosphoserine phosphatase domain. We suggest that the phosphoserine phosphatase domain functions as glycerol 3-phosphatase and that, consequently, NAD^+-dependent GPDH from *D. salina* can catalyze the step from dihydroxyacetone phosphate to glycerol directly. This is unique and a possible explanation for the fast glycerol synthesis found in *D. salina*.

Introduction

The enzyme that catalyzes the reversible conversion of dihydroxyacetone phosphate (DHAP) to glycerol 3-phosphate, DHAP reductase, also known as glycerol 3-phosphate dehydrogenase (GPDH)

Abbreviations: bp, base pair; cDNA, DNA complementary to RNA; DHAP, dihydroxyacetone phosphate; EST, expression sequence tag; GPDH, glycerol 3-phosphate dehydrogenase; NAD, nicotinamide-adenine dinucleotide; ORF, open reading frame; RACE, rapid amplification of cDNA ends

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(EC 1.1.1.8), is found in many eubacteria and eukaryotes. Nicotinamide-adenine dinucleotide (NAD^+)-dependent GPDH is an important enzyme in glycerol metabolism. In algae *Dunaliella*, the reversible conversions between glycerol and DHAP are metabolized by two distinct reactions. For glycerol synthesis, DHAP is converted to glycerol 3-phosphate catalyzed by NAD^+-dependent GPDH, and then glycerol 3-phosphate is converted to glycerol catalyzed by glycerol 3-phosphatase. For glycerol dissimilation, glycerol is converted to dihydroxyacetone catalyzed by glycerol dehydrogenase, and then dihydroxyacetone is converted to DHAP catalyzed by dihydroxyacetone kinase (Ben-Amotz and Avron, 1981; Haus and Wegmann, 1984; Sussman and Avron, 1981).

The roles of NAD^+-dependent GPDH isoenzymes in yeast, *Saccharomyces cerevisiae*, have been detailed (Albertyn et al., 1994; Ansell et al., 1997). It is known that heterologous expression of GPDH genes in yeast can increase glycerol production (Watanabe et al., 2004). Therefore, the NAD^+-dependent GPDH genes were considered as the key genes of glycerol synthesis. In higher plants and algae, GPDH is referred to as DHAP reductase, because at physiological pH and substrate, the enzyme is essentially inactive as a dehydrogenase (Gee et al., 1988a, b).

Dunaliella salina, a photosynthetic organism, devoid of a rigid cell wall, has the remarkable characteristic of surviving large osmotic stresses, by adjusting intracellular levels of glycerol to concentrations balancing the external osmotic pressure. Therefore, the cells maintain a constant volume independent of the external salinity (Avron, 1986; Sadka et al., 1991). Based on the previous studies, we hypothesize that the mechanism of glycerol synthesis in *D. salina* is similar to *S. cerevisiae*, in that different GPDH isoforms plays different roles (Ansell et al., 1997). Although three isoforms of DHAP reductases in *Dunaliella tertiolecta* have been separated by a diethylaminoethyl cellulose column and characterized individually (Gee et al., 1989, 1993; Ghoshal et al., 2002), there are no correspondent genes or expression information about these isoforms. In order to understand the glycerol synthesis mechanism in *Dunaliella*, it is essential to clone and characterize these GPDH genes.

Materials and methods

Algae and growth condition

D. salina strain 435 was obtained from Institute of Hydrobiology, Chinese Academy of Sciences. The algae grew in a controlled-environment chamber with 16 h lighting at 25 °C and 8 h darkness at 15 °C. The composition of the growth medium was 1.5 M NaCl, 5.0 mM $NaNO_3$, 5.0 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM $NaH_2PO_4 \cdot 2H_2O$, 1.0 mM KCl, 10.0 mM $NaHCO_3$, 0.3 mM $CaCl_2 \cdot 2H_2O$ and a mixture of micronutrients (Pick et al., 1986). Cells in exponential phase were used for treatment. Oxidative stress treatment was performed by adding 5 μL H_2O_2 to 50 mL cell culture (0.2 mM H_2O_2 in medium). Osmotic stress treatment was performed by adding 5.85 g NaCl to 50 mL cell culture (3.5 M NaCl in medium).

Isolation of GPDH expression sequence tag (EST) from *D. salina*

The GPDH EST was isolated by random sequencing of clones from a cDNA library prepared from *D. salina* cells grown in 1.5 M NaCl. The cDNA library was constructed as part of *D. salina* EST sequencing program that was devoted to comprehensive characterization of gene expression when external NaCl concentration changed. The PCR primers used for EST amplification were: primer E1 (5-CGTATCCTGGGCATCGACTG-3) and primer E2 (5-GAAGAAGGTGTCATCGCGCA-3).

Rapid amplification of cDNA ends (RACE)

3′ RACE was performed using the 3′ RACE kit (3′-Full RACE Core Set) (TaKaRa). 5′ RACE was performed using the 5′ RACE kit (SMART RACE cDNA amplification kit) (Clontech). Total RNA was extracted using trizol reagent (Invitrogen) and was used for cDNA synthesis.

For 3′ RACE, first-strand synthesis was carried out in the presence of oligo-dT primers (TaKaRa). PCR amplification of the 3′ end of *D. salina* GPDH cDNA was carried out with the following primers: gene-specific forward primer F1 (5-AGTTCATCTCCCCCT-CAGTGCGCGA-3), gene-specific forward nested primer F2 (5-AGGCCTGGGCCCAGAAGAGGATCG-3) and 3sites Adaptor primer (TaKaRa). The PCR product with F1 primer and 3sites Adaptor primer was diluted at the ratio of 1:100 and re-amplified with F2 primer and 3sites Adaptor primer.

For 5′ RACE, first-strand synthesis was carried out in the presence of 5′CDS primer (Clontech) and the SMART II primer (Clontech). PCR amplification of the 5′ end of *D. salina* GPDH cDNA was carried out with the following primers: gene-specific reverse primer R1 (5-CGCCCGGCACATCCGCAAGCAG-3), gene-specific reverse nested primer R2 (5-TGCC-CAGGATACGGGACACCAT-3) and universal primer UPM (Clontech). The PCR product with R1 primer