Note

Cloning and Sequencing of a Synechococcus Gene Encoding a Protein Very Similar to Mammalian Aldehyde Dehydrogenases

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Received July 12, 1994

We report cloning and sequencing of a gene encoding a putative aldehyde dehydrogenase in Synechococcus sp. PCC7942. It was found that this phototrophic microorganism has a protein very similar to mammalian class-3 aldehyde dehydrogenases. A mutant strain lacking this gene was hypersensitive in growth to an aromatic aldehyde.

Organisms generally respond to osmotic stress by increasing the intracellular concentrations of a limited number of solutes, which have been termed compatible solutes.1) One of the prominent compatible solutes in bacteria is glycinebetaine. Although enteric bacteria are unable to synthesize glycinebetaine from glucose or other carbon sources, E. coli K-12 can convert choline to glycinebetaine under conditions of osmotic stress, and has a NAD+–linked glycinebetaine aldehyde dehydrogenase (BADH, EC 1.2.1.8), specified by the betB gene.2) Many diverse species of plants also accumulate glycinebetaine in response to osmotic stress resulting from salinity or drought.3) In contrast to bacteria, plants are able to carry out de novo synthesis of glycinebetaine, which proceeds in chloroplasts by a two-step oxidation of choline via the intermediate glycinebetaine aldehyde. A gene encoding the second enzyme (BADH) in this pathway has been cloned from spinach.4) In this study we tried to isolate a gene encoding BADH from a cyanobacterium (Synechococcus sp. PCC7942), which is a phototrophic microorganism that harbors a photosynthetic apparatus similar in structure and function to that in chloroplasts in plants. Here we characterized a Synechococcus gene encoding a putative aldehyde dehydrogenase. To our surprise, however, the gene we isolated was found to resemble much more closely a set of mammalian aldehyde dehydrogenases (ALDH) than BADH. E. coli and spinach BADHs are a significantly similar in their amino acid sequences.2,3) Comparison of the amino acid sequences enable us to design a pair of degenerated oligonucleotide mixtures that could be used for polymerase chain reaction (PCR) amplification. The two oligonucleotides we thus designed contain all possible coding sequences corresponding to the amino acid sequences. (L/M)ELGGK (5'–primer) and EE(L/V)FGPV (3'–primer), respectively. Using these primers, PCR amplification was done for the Synechococcus total chromosomal DNA with denaturing (94°C for 1 min), annealing (54°C for 1 min), and polymerization with Taq polymerase (72°C for 1 min). A DNA fragment of about 400 bp in length was reproducibly amplified under the conditions used (data not shown). This DNA fragment was cloned onto the plasmid pUC19. We then attempted to clone the entire gene by screening a Synechococcus DNA library, constructed in λ DASH, with the DNA fragment as a probe. This yielded a positive λ phage, which carried an about 8-kb DNA

Fig. 1. Cloning and Sequencing of the aldA Gene from Synechococcus sp. PCC7942.

The bold-faced arrow shows the coding sequence of the aldA gene encoding a putative ALDH. The kanamycin-resistance gene (Km+) was inserted into the BamHI site, as indicated, to isolate an insertional inactivation mutant of aldA. The resultant aldA− mutant cells, as well as the wild-type cells, were streaked on a conventional solid medium, and then were grown phototrophically (lower panel). Note that paper disks containing 100% benzaldehyde was placed at the center of the streaked line.

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**Fig. 2. The Amino Acid Sequence of the Putative ALDH of Synechococcus.**

The amino acid sequence of the putative ALDH of Synechococcus (upper) was aligned with that of the rat class-3 ALDH (lower). Identical amino acids were highlighted by asterisks. Analyses were done with the GENETYX program from Software Development Co.
insert. A set of subcloned DNA was generated on pUC19, and then an about 1.9-kb EcoRI–BglII DNA region, which hybridized with the probe, was sequenced (Fig. 1) (the nucleotide sequence data will appear in the GenBank/EMBL/DDJB Nucleotide Sequence Data Libraries under the accession number D15079). The region analyzed was found to contain an open reading frame (ORF) of 1377 pb, which could encode a protein consisting of 459 amino acids (aa) (Figs. 1 and 2).

The amino acid sequence deduced for the ORF was compared with those of BADHs from E. coli and spinach. The computer-aided inspection indeed indicated that the Synechococcus gene-product resembles both the E. coli and spinach BADHs, with 25% amino acid identity (124aa/489aa) for E. coli and 26% identity (131aa/497aa) for spinach. However, a family of eukaryotic ALDHs showed the highest degree of similarity to the novel Synechococcus sequence, namely a rat tumor-associated ALDH isoform (class 3, EC 1.2.1.5) had 47% identity (212aa/453aa),

as shown in Fig. 2. A human class-3 ALDH homologue also had a strongly similarity. ALDHs have been isolated mainly from the livers of a variety of higher vertebrates species, i.e., two major isoforms (EC 1.2.1.3) coexist in mammalian livers.

Although the Synechococcus sequence showed a significant similarity also to these class-1 (cytosolic) and class-2 (mitochondrial) ALDHs (data not shown), it is apparent that it is more closely related in its primary structure to the class-3 ALDH. In this context, only a few putative ALDH sequences are available for prokaryotes so far, namely ALDHs from Pseudomonas oleovorans, Acetobacter polyoxogenes, and E. coli. These prokaryotic ALDHs show a significantly lower degree of amino acid sequence identity with the Synechococcus protein described here than the eukaryotic class-3 ALDHs.

As far as we know, ALDH activity in Synechococcus species had not been reported. Furthermore, it is not known whether or not the Synechococcus gene product described here has any ALDH activity. However, it is of interest to address this issue. In this context, mammalian ALDHs display a wide substrate specificity and notably participate in clearance of alcohol-derived acetaldehydes. However, the inducible class-3 ALDHs were proposed to preferentially oxidize aromatic aldehyde substrates. To gain an insight into the nature of the putative Synechococcus ALDH,

an inactivation mutant of the corresponding gene was isolated by inserting the kanamycin-resistance (neo) gene (Fig. 1). This was confirmed by Southern hybridization analysis with an appropriate probe, suggesting that this gene appears not to be essential. When these mutant cells were grown in a conventional medium, we were not able to find any noticeable phenotype for the knock-out mutant. However, when the sensitivity for cell growth to external benzyaldehyde was examined on a solid medium, the mutant showed significantly higher sensitivity for growth to this particular aromatic aldehyde than the wild-type (see the panel in Fig. 1).

Thus, it is tempting to speculate that the Synechococcus gene may be involved in degradation of certain aromatic aldehydes. In any case, the Synechococcus gene (named tentatively here aldA) most likely encodes a unique ALDH which has strong homology to mammalian ALDHs. A search for a BADH gene in Synechococcus, if present, remains to be done.

Acknowledgments. We wish to thank Dr. N. Murata for the kind gift of the Synechococcus DNA library in phage λ DASH. This study was supported by a Grant-in-Aid for Scientific Research on a Priority Area (No. 04273013) from the Ministry of Education, Science, and Culture of Japan.

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