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Genes Encoding Two Isocitrate Dehydrogenase Isozymes of a Psychrophilic Bacterium, *Vibrio* sp. Strain ABE-1

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The genes coding for two structurally different isocitrate dehydrogenase isozymes (IDH-I and IDH-II) of a psychrophilic bacterium, *Vibrio* sp. strain ABE-1, were cloned and sequenced. Open reading frames of the genes (*icdI* and *icdII*) are 1,248 and 2,229 bp in length, respectively. The amino acid sequences predicted from the open reading frames of *icdI* and *icdII* corresponded to the N-terminal amino acid sequences of the purified IDH-I and IDH-II, respectively. No homology was found between the deduced amino acid sequences of the isozymes; however, the IDH-I, a dimeric enzyme, had a high amino acid sequence identity (74.3%) to the *Escherichia coli* IDH. The deduced amino acid sequence of the IDH-II, a monomeric enzyme, was not related to any known sequence. However, the IDH-II had an amino acid sequence homologous to that of a cyanogen bromide-cleaved peptide containing a putative active-site methionyl residue of the monomeric IDH of *Azotobacter vinelandii*. The two genes (*icdI* and *icdII*) were found to be tandemly located in the same orientation. Northern (RNA) blot analyses showed that the two genes are transcribed independently. Primer extension experiments located single transcriptional start sites 39 and 96 bp upstream of the start codons of *icdI* and *icdII*, respectively. The amount of *icdI* transcript but not *icdII* increased when *Vibrio* sp. strain ABE-1 cells were cultured in acetate minimal medium.

The NADP-dependent isocitrate dehydrogenase (IDH) which catalyzes the oxidative decarboxylation of D-isocitrate to 2-oxoglutarate has been purified from a number of bacteria. On the basis of the subunit structure, the bacterial IDHs are usually either homodimers with subunit molecular weights of ca. 45,000 or monomeric enzymes of polypeptide molecular weights of 80,000 to 100,000. In contrast to eucaryotic cells which contain structurally different IDH isozymes (8), many bacteria seem to possess only one, either the dimeric or the monomeric IDH type. For instance, IDH enzymes from *Escherichia coli* (4), *Thermus thermophilus* (11), *Bacillus stearothermophilus* (16), and *Rhodopseudomonas sphaeroides* (6) have been shown to be dimers, and those from *Azotobacter vinelandii* (7), *Rhodomicrobium vannielii* (19), and *Vibrio parahaemolyticus* (12) are monomers.

Vibrio sp. strain ABE-1, a psychrophilic bacterium, appears to be unique for the coexistence of IDH isozymes with different subunit structures (18, 25). One, IDH-I, is a homodimer of thermostability comparable to that of the enzymes from mesophiles, and the other, IDH-II, is a monomer with extreme lability above 20°C. In addition, immunochemical properties and N-terminal amino acid sequences of the isozymes have been shown to be different from each other (18) but showed similarities to those of the respective types of other bacterial IDHs (12).

The genes encoding dimeric IDHs of *E. coli* (35) and *T. thermophilus* (21) have been cloned and sequenced, respectively. There is significant homology between these predicted amino acid sequences, and the amino acid residues which are suggested to interact with isocitrate (17) are also conserved in the two enzymes. Recently, homology between the deduced amino acid sequences of *Saccharomyces cerevisiae* (9) and pig heart (32) mitochondrial IDHs were reported to be very high, but the eucaryotic enzymes exhibit low amino acid sequence similarity to the *E. coli* IDH. However, Soundar and Colman (32) reported that the amino acid residues which are implicated in metal-isocitrate binding in the *E. coli* enzyme (17) are located in the same general region of the eucaryotic enzymes. These findings have implications for the molecular evolution of IDH. But the relation between dimeric and monomeric IDHs is still unclear, since no sequence of a monomeric IDH has been determined.

Since *Vibrio* sp. strain ABE-1 possesses structurally different IDH isozymes (18, 25), investigation of the genes encoding the isozymes is necessary to elucidate the physiological and evolutionary basis of the coexistence of the isozymes at the molecular level. In this paper, we report the cloning and sequencing of the genes coding for the IDH isozymes. It was found that the two genes are closely adjacent, and the predicted amino acid sequence of the IDH-I is very similar to that of the *E. coli* enzyme but quite different from that of IDH-II. Some regulatory aspects of the expression of the cloned genes are also presented.

MATERIALS AND METHODS

Materials. Restriction endonucleases *Ban*I and *Sac*II and T4 polynucleotide kinase were obtained from Toyobo (Osaka, Japan). Other restriction endonucleases and enzymes for DNA manipulation were obtained from Takara Shuzo (Kyoto, Japan) or Nippon Gene (Toyama, Japan). All other reagents were of analytical grade.

Bacteria, phages, plasmids, and media. The obligately psychrophilic bacterium *Vibrio* sp. strain ABE-1 (34) was grown at 15°C as described previously (24). The composition of a succinate or acetate medium was the same as described previously (18). *E. coli* XL1-Blue (31), used to propagate plasmids for purification of plasmid DNA, was cultured in 2× TY medium (28) containing 1.6% Bacto Tryptone (Difco), 1%

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yeast extract (Difco), and 0.5% NaCl at 37°C. *E. coli* DEK2004 (*trp icd recA*, a gift from P. E. Thorsness) was used as a host for transformation by *Vibrio* sp. strain ABE-1 *icd* genes. The medium for the growth of this strain was LB or was morpholinepropanesulfonic acid (MOPS) based (23), supplemented with 0.5 mM glutamate and 0.5% glucose or 2% acetate. Ampicillin and tetracycline were added to culture media at a concentration of 50 to 250 and 15 μg/ml, respectively. Phage λDASH (Stratagene) was used as a vector for construction of a genomic library, and plasmid pBluescript (Stratagene) was used for subcloning *Vibrio* sp. strain ABE-1 *icd* genes. pTK512, a plasmid containing the *E. coli icd* gene and conferring overproduction of the enzyme protein (35), was a kind gift from P. E. Thorsness.

DNA isolation and construction of a enomic library. Chromosomal DNA was isolated and purified from *Vibrio* sp. strain ABE-1 cells by the method of Bendbrook et al. (1) with some modifications. Washed cells were suspended in 100 volumes of 0.5 M sucrose with continuous shaking for 1 h. The cells were collected by centrifugation at 3,000 × *g*, resuspended in 8 volumes of 20 mM Tris-HCl (pH 7.5) containing 1 mM EDTA and 0.1% lysozyme, and incubated at 37°C for 3 h. After the incubation, 20% lauroyl sarcosyl sulfate and proteinase K were added to the suspension to give a final concentration of 1% and 500 μg/ml, respectively, and the cells were lysed by successive incubation at 60°C for 10 min and 37°C overnight. The chromosomal DNA purified by ultracentrifugation through a CsCl density gradient was dialyzed exhaustively against TE (10 mM Tris-HCl [pH 7.5] containing 1 mM EDTA) and stored at 4°C until use. DNAs from plasmid and phage were isolated by the usual methods (28). The *Vibrio* sp. strain ABE-1 genomic library was constructed as follows: aliquots of the chromosomal DNA were partially digested with restriction enzyme *Sau*3AI, and the digest was fractionated by agarose gel (0.8%) electrophoresis. The 10- to 22-kbp fragments were ligated to λDASH vector (Stratagene) cut with *Bam*HI.

Synthesis of oligonucleotides. Oligonucleotides used as hybridization probes were synthesized on a Milligen model 7500 or Applied Biosystems model 381A DNA synthesizer.

Southern and plaque hybridization. Taking the difference between the N-terminal amino acid sequences of IDH-I and IDH-II into consideration, we synthesized 26- and 35-mer nucleotide probes for the *icdI* and *icdII* genes encoding the IDH-I and IDH-II, respectively: 5′-GA(A,G)GGIGA(C,T)GG IAT(A,C,T)GGIGTIGA(A,G)GT-3′ corresponded to the N-terminal amino acid sequence from amino acids 32 to 40 of the IDH-I protein (18), and 3′-TT(C,T)TA(A,G,T)TA(A,G,T)AT (A,G)TGITA(A,G,T)TGICT(A,G)CT(C,T)CGIGGICG-5′ corresponded to the sequence from amino acid 6 to 17 of the IDH-II protein (18), where I denotes inosine. The oligonucleotides were labeled with [γ-^{32}P]ATP by use of T4 polynucleotide kinase. Chromosomal DNA isolated from *Vibrio* sp. strain ABE-1 was digested with several restriction enzymes: *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I. The restriction fragments were separated on an agarose gel (1.0%) and blotted on a nylon membrane. The membrane was soaked at 44°C for 5 h in prehybridization solution containing 6× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 5% Irish cream liqueur (Baileys, Dublin, Ireland), 0.5% sodium dodecyl sulfate (SDS), and 10 μg of sonicated calf thymus DNA per ml. Hybridization was carried out at 44°C overnight in the prehybridization solution supplemented with the labeled probes. The membrane was successively washed in 2× SSC at room temperature for 30 min and then in 2× SSC containing 0.1% SDS at 44°C for 2 h with a change of washing buffer.

Plaque hybridization (28) was carried out by using the same probes and conditions described above.

DNA sequencing. All nucleotide sequences were obtained by using the dideoxy method (29), alkaline-denatured double-stranded templates (14), and the Sequenase kit (United States Biochemicals) according to the manufacturer's instructions. Overlapping deletions were generated by the method of Henikoff (15). Sequences were analyzed and compared by using the computer programs of Genetyx (Software Development Co., Tokyo, Japan).

Enzyme assay. The procedures for preparation of a sonic extract of bacterial cells and determination of IDH activity were described previously (18). The temperature for assay of IDH-I and *E. coli* IDH activities was 40°C and that for IDH-II activity was 20°C. One unit of the enzyme activity is defined as the amount of the enzyme catalyzing the reduction of 1 μmol of $NADP^+$ per min. Protein concentration was determined by the method of Bradford (3).

Western blot analysis. The procedures for purification of IDH isozymes and preparation of antibody raised against IDH-I or -II were described previously (18). The purified IDH isozymes and the proteins in a sonic extract of bacterial cells were separated by SDS-polyacrylamide (7.5%) gel electrophoresis, transferred to nitrocellulose, and subjected to Western blot (immunoblot) analysis with the ECL Western blotting detection system (Amersham) and anti-IDH-I or anti-IDH-II antibody from rabbit.

Northern (RNA) blot analysis. Total RNAs from *Vibrio* sp. strain ABE-1 cells grown in peptone-meat extract, succinate, and acetate media were isolated by the method of Case et al. (5). The RNAs (10 μg) were separated on an agarose gel (1.5%) containing 0.66 M formaldehyde and transferred to a nylon membrane and then hybridized with the labeled *Hin*dIII-*Eco*RI fragment of *icdII* or the *Sac*I-*Sac*I fragment of *icdI* (see Fig. 1). The membrane was successively washed in SSC containing 0.1% SDS as follows: 2× SSC at room temperature for 20 min, 1× SSC at 65°C for 10 min, and 0.1× SSC at 65°C for 20 min.

Primer extension analysis. We synthesized the following 31- and 32-mer oligonucleotides chemically as the primers for *icdI* and *icdII*, respectively: 5′-GGGATTGTTAGGTACTGATAA CTTACCGTCG-3′, complementary to the internal region from bases 2823 to 2793 of *icdI*, and 5′-GCTTGAATAATGG GTAATAAAGAATACGTCGC-3′, complementary to that (from bases 326 to 295) of *icdII*. The primers were 5′ end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Total RNA (600 μg) isolated from *Vibrio* sp. strain ABE-1 cells was hybridized with either of the labeled primers. The primer extension reaction (28) was done by using Rous-associated virus 2 reverse transcriptase (Takara Shuzo Co.), and products were analyzed by electrophoresis on a 6% polyacrylamide sequencing gel.

Nucleotide sequence accession number. The nucleotide sequence data reported here have been deposited in the DDBJ, GenBank, and EMBL data bases under accession number D1047.

RESULTS

Isolation of recombinant DNA. Genomic Southern hybridization analyses with synthetic oligonucleotide probes for either the *icdI* or the *icdII* gene revealed that each of the *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I digests contained a single fragment that hybridized strongly with the probe, except that no fragment which hybridized with the probe for *icdII* was detected in the *Hin*dIII digest (data not shown). To isolate the

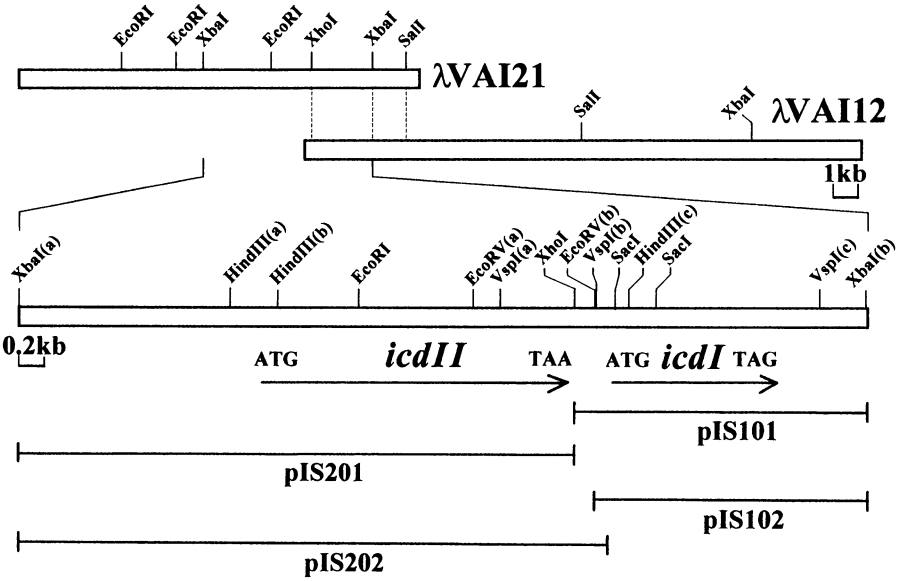

FIG. 1. Mapping of the genomic clones containing the *icdI* and *icdII* genes. Arrows represent the open reading frames of the *icdI* and *icdII* genes. Some restriction sites are distinguished by letters indicated in parentheses. The restriction fragments used in construction of plasmids (pIS101, pIS102, pIS201, and pIS202) are shown in lines below the restriction maps.

genes encoding the IDH isozymes, approximately 20,000 recombinants of the genomic library constructed with phage carrying 10- to 22-Kbp *Sau*3AI fragments of the *Vibrio* sp. strain ABE-1 chromosomal DNA were screened with each of the oligonucleotide probes described above. Numerous positive signals were obtained in both cases. From them, the strongest positive plaque corresponding to each *icd* gene was selected for further characterization. From analyses of restriction enzyme maps of the recombinant DNAs, it was found that the two clones (designated λVAI12 and λVAI21 for the clones containing *icdI* and *icdII* genes, respectively) were partially overlapping and the *icdI* gene was immediately downstream of the *icdII* gene (Fig. 1). An *Xba*I(a)-*Xho*I fragment of λVAI21 was subcloned into pBluescript SK(+) (Stratagene) to yield the plasmid pIS201. Similarly, the *Xho*I-*Xba*I(b) fragment of λVAI12 was subcloned into pBluescript KS(+) to yield the plasmid pIS101. These plasmids were used for all subsequent sequencing experiments.

Nucleotide and deduced amino acid sequences. Both strands of the inserts in plasmids pIS101 and pIS201 were sequenced. The complete nucleotide sequences of the *icd* genes including the flanking regions and the deduced amino acid sequences are shown in Fig. 2. Open reading frames (ORFs) of the two *icd* genes are separated from each other by a spacer of 276 bases. *icdII* contains a coding region of 2,229 bp, and there is a putative ribosome binding site, GGAA (30), 6 to 9 bases upstream of the ATG codon. The coding region of *icdI* is about half as long as that of *icdII* and contains 1,248 bases. A putative ribosome binding site, GGAG, is also present 9 to 12 bases upstream of the ATG codon. The deduced amino acid sequences of the proteins agree with the previously determined N-terminal amino acid sequences of the corresponding purified proteins (boxed residues in Fig. 2), except for three residues. The formylmethionine residue was removed from both proteins, and an aspartic acid residue instead of a glycine residue at position 26 of the IDH-I protein was found in *icdI*. Downstream of each ORF, there is a sequence [at nucleotide positions 2,551 to 2,578 and 4,020 to 4,047 from the *Hin*dIII(a) site] of a terminator-like stem-loop structure (27). The former is composed of a 12-bp stem with a 4-nucleotide loop (free energy of association, −24.7 kcal/mol), and the latter is composed of a 12-bp stem with a 6-nucleotide loop (free energy of association, −19.6 kcal/mol).

The molecular weights calculated from the deduced amino acid sequences of IDH-I and -II were 45,013 and 80,493, respectively, in agreement with those determined by SDS-polyacrylamide gel electrophoresis (18). The amino acid compositions calculated from the sequences were in good agreement with those obtained by chemical analysis of the acid hydrolysates (18). The homology score (36) between the amino acid sequences of IDH-I and -II was found to be less than 29, not a significant value. The analysis (33) of IDH-II failed to detect any repeated amino acid sequences within the protein. These results indicate that the two IDH isozymes of *Vibrio* sp. strain ABE-1 did not originate from a common ancestral gene, and internal gene duplication was not involved in the evolution of *icdII*. The G+C contents of the ORFs of *icdI* and *icdII* were 38.4 and 39.7%, respectively, slightly higher than that (33%) of chromosomal DNA (34). Codon utilization of both *icd* genes exhibited a preference of A or T in the third base, and the percentages of the codons using A or T in the third base were 74.7 and 74.8% in *icdI* and *icdII*, respectively.

Transcriptional start point. The initiation site of each *icd* gene of *Vibrio* sp. strain ABE-1 was determined by primer extension analyses. Essentially the same profiles were obtained by using different RNAs isolated from the cells grown on peptone-meat extract, succinate, or acetate. Typical results are shown in Fig. 3. The initiation sites for the *icdI* and *icdII* genes were found to be G-2704 and T-141 (Fig. 2), respectively. Therefore, the putative promoter motifs (27) at −10 and −35 for *icdI* and *icdII* were TACTTT (nucleotides 2,692 to 2,697) and TTTAGT (nucleotides 2,668 to 2,673) and TTTATA (nucleotides 106 to 111) and AACTAT (nucleotides 130 to 135), respectively. These results indicate that each of the *icd* genes of *Vibrio* sp. strain ABE-1 is separately expressed.

Expression of the *icd* genes. Expression of *icdI* or *icdII* in *E.*

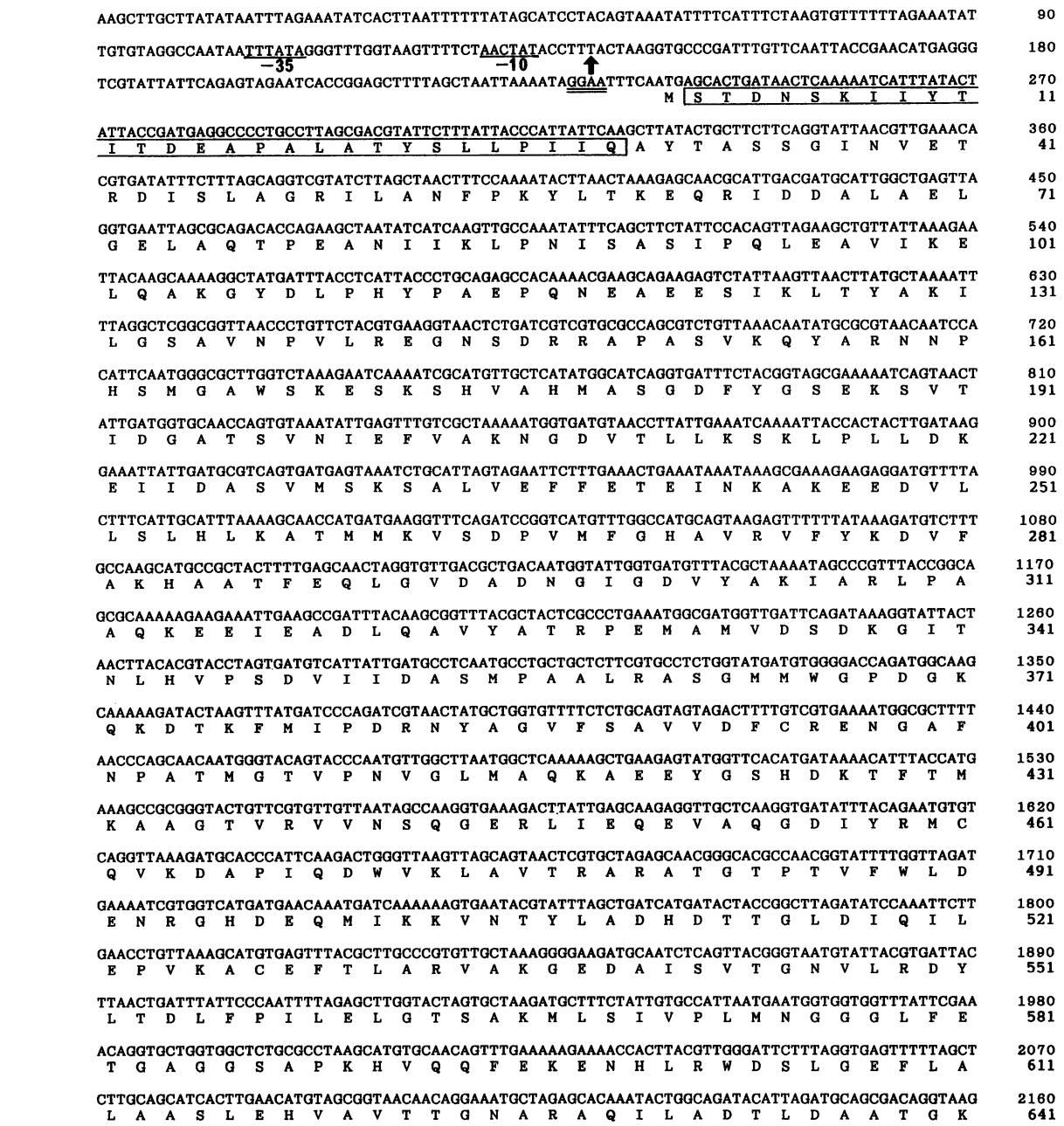

FIG. 2. Nucleotide and deduced amino acid sequences of the *icdI* and *icdII* genes. The sequence from *Hin*dIII(a) to the *Vsp*I(c) site shown in Fig. 1 are presented. Arrowheads indicate the transcriptional start points. The underlined sequences are the possible promoter sequences. The doubly underlined sequences are the putative ribosome-binding sites. N-terminal amino acid sequences determined by chemical analysis of the proteins (12) are boxed. The probable stem-loop structures located in the downstream region of the stop codons (indicated by asterisks) of the translation are shown by two opposing arrows.

coli DEK2004, a mutant defective in *icd*, was determined by measuring IDH activity or by Western blot analysis of a sonic extract prepared from *E. coli* cells transformed by a plasmid harboring either *icdI* or *icdII*. The expression plasmid for *icdI*, pIS102, was constructed by digesting pIS101 with *Eco*RV, which cleaves at the restriction site present between a putative terminator-like sequence and promoter region of *icdI* (Fig. 1 and 2), and ligating it to *Eco*RV- and *Xba*I-digested pBluescript KS(+). The expression plasmid for *icdII*, pIS202, which contains the above terminator-like sequence located downstream of *icdII* ORF, was constructed by using the *Xba*I-*Sac*I fragment of λVAI21 and pBluescript SK(+). For expression of *icdI*, the transformed cells were cultured at 37°C, while the culture temperature for the expression of *icdII* was 15°C because of the extreme thermolability of IDH-II protein (24). Expression level (1.49 U/mg of protein) of the *icdI* gene in *E. coli* cells, which were cultured in a peptone broth or glucose minimal medium at 37°C, was about 10 times higher than the activity in a sonic extract of *Vibrio* sp. strain ABE-1 grown at 15°C. Thermolabile IDH activity, which was completely inac-

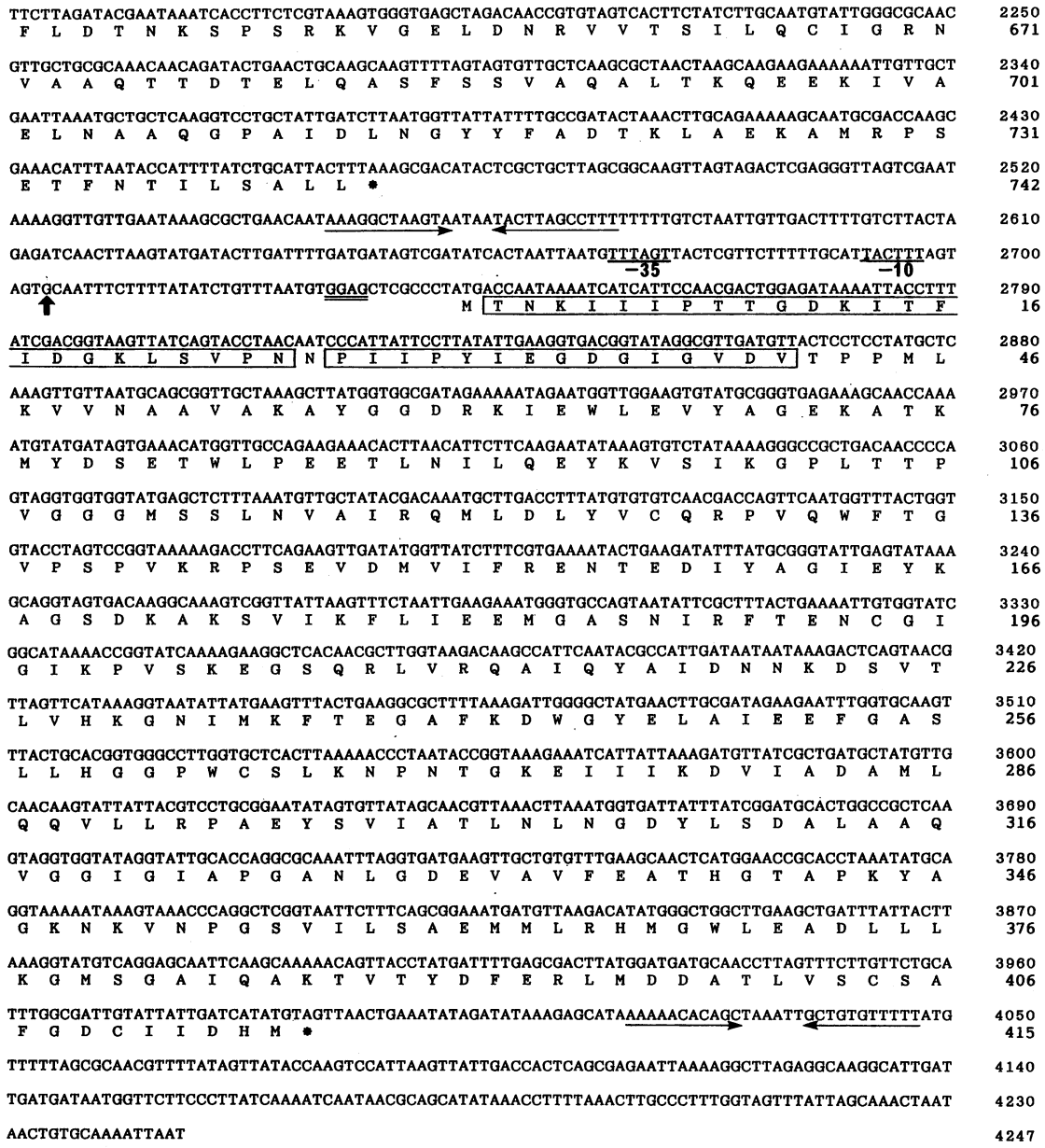

FIG. 2—*Continued.*

tivated by heating the extract at 40°C for 10 min, was detected in a sonic extract prepared from *icdII*-transformed cells grown at 15°C, and the level (0.11 U/mg of protein) of the activity was approximately three times lower than the value found in a sonic extract of *Vibrio* sp. strain ABE-1. The addition of isopropyl-β-D-thiogalactoside to the culture medium did not affect the expression levels of the *icd* genes (data not shown). Western blot analysis employing immunoglobulin G raised against IDH-I or IDH-II demonstrated the presence of a protein band corresponding to the purified IDH-I or IDH-II in a sonic extract prepared from the transformed cells (Fig. 4).

Northern analyses of *icdI* and *icdII* transcripts. In order to examine whether the closely adjacent *icd* genes of *Vibrio* sp. strain ABE-1 are transcribed independently, Northern blots prepared with total RNA isolated from *Vibrio* sp. strain ABE-1 cells grown on various carbon sources were hybridized with either the 343-bp *Sac*I-*Sac*I fragment [internal to *icdI*, at positions 2735 to 3077 from the *Hin*dIII(a) site] or the 620-bp *Hin*dIII(b)-EcoRI fragment [internal to *icd*II, at positions 322 to 941 from *Hin*dIII(a) site]. Two single bands corresponding to approximately 1.4 and 2.4 kb hybridized to the probes prepared from *icdI* and *icdII*, respectively (Fig. 5). No transcript larger than 2.4 kb was detected. In addition, the estimated sizes of the transcripts were in good agreement with those of the respective ORFs plus their flanking regions. These results indicate again the separate expression of *Vibrio* sp. strain ABE-1 *icd* genes, and the promoter- and terminator-like structures found in 5′ and 3′ regions of each ORF apparently act as the true promoters and terminators.

The amount of *icdII* mRNA in *Vibrio* sp. strain ABE-1 cells

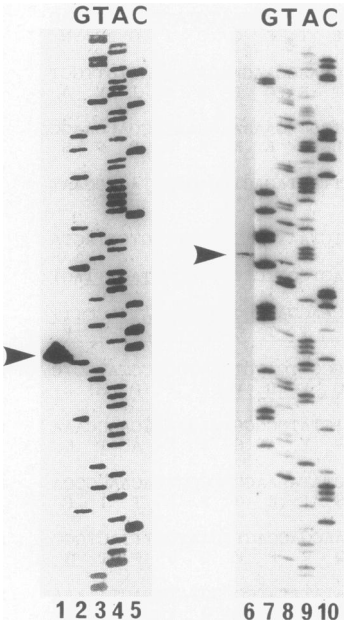

FIG. 3. Primer extension analysis. Primer extension products employing primers complementary to *icdI* and *icdII* are shown in lanes 1 and 6, respectively. RNA (600 μg) used in these experiments was obtained from *Vibrio* sp. strain ABE-1 cells grown in peptone-meat extract medium. The sequencing ladders of the DNAs obtained by using the same primers are shown in lanes 2 to 5 and 7 to 10. Arrows indicate the transcriptional initiation sites.

did not change significantly by changing the carbon source in the medium, whereas that of *icdI* increased when acetate was used as the sole source of carbon and energy (Fig. 5A, lane 3). These results were in agreement with the previous observation that the specific activity of IDH-I was increased in the cells grown in acetate medium (18).

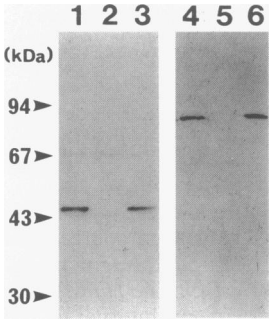

FIG. 4. Western blot analysis of polypeptides present in the sonic extract prepared from *E. coli* cells transformed by a plasmid bearing either *icdI* or *icdII*. Lane 1, purified IDH-I (0.2 μg of protein); lane 2, pBluescript KS(+)-transformed cell control (10 μg of protein); lane 3, pIS102-transformed cells (10 μg of protein); lane 4, purified IDH-II (0.2 μg of protein); lane 5, pBluescript SK(+)-transformed cell control (10 μg of protein); lane 6, pIS202-transformed cells (10 μg of protein). Anti-IDH-I and anti-IDH-II antibodies were used for lanes 1 to 3 and 4 to 6, respectively. The cells transformed by pIS102 or pBluescript KS(+) were cultured at 37°C, while the cells harboring pIS202 or pBluescript SK(+) were cultured at 15°C. Molecular mass standards are shown on the left.

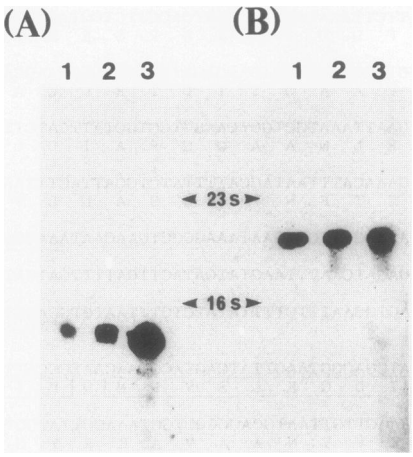

FIG. 5. Northern hybridization of the *icdI* and *icdII* genes. Total RNAs (10 μg per lane) extracted from *Vibrio* sp. strain ABE-1 cells grown on peptone-meat extract (lane 1), succinate (lane 2), or acetate (lane 3) were electrophoresed in a 1.5% agarose gel containing 0.66 M formaldehyde. After blotting the RNAs onto a nylon membrane, hybridization was performed by using the radiolabeled *Sac*I-*Sac*I DNA fragment of *icdI* (A) or the *Hin*dIII(b)-*Eco*RI DNA fragment of *icdII* (B). Positions of 23S and 16S rRNAs are shown between the panels.

DISCUSSION

We have cloned the two genes from a psychrophilic bacterium, *Vibrio* sp. strain ABE-1, *icdI* and *icdII*, encoding dimeric (IDH-I) and monomeric (IDH-II) IDH isozymes, respectively. Proof of the identity of each gene was obtained by comparing the predicted amino acid sequence and the N-terminal amino acid sequence of the respective IDH isozyme. In addition, the identities of the *icd* genes were confirmed from expression of the genes in *E. coli*. Interestingly, the two genes were found to be closely adjacent with a spacer of 276 bases in the order *icdII-icdI* (Fig. 1 and 2). In this spacer region, 85 bases downstream of the stop codon of *icdII*, there appears to be a rho-independent, terminator-like structure followed by a promoter, a Shine-Dalgarno region, and the start codon of *icdI*. Another promoter and ribosome-binding region are also seen upstream of the initiation codon of *icdII*, and another terminator-like sequence is downstream of the stop codon of *icdI*. These structural characteristics of the genes suggest that the two genes are expressed separately. Strong evidence that this is true was obtained by primer extension (Fig. 3) and Northern blot analyses of RNA isolated from *Vibrio* sp. strain ABE-1 cells (Fig. 5).

The bacterial *icd* gene has been cloned from three other sources, *E. coli* (35), *T. thermophilus* (21), and *Rhizobium meliloti* (20). The genes of *E. coli* and *T. thermophilus* have been shown to code for dimeric IDH. The nucleotide sequence of the *R. meliloti* gene is not yet available. Alignment of the deduced amino acid sequences of the bacterial dimeric IDHs is shown in Fig. 6. The amino acid sequence of IDH-I showed high identity (74.3%) with that of the *E. coli* enzyme (35). The level of amino acid sequence identity between the *T. thermophilus* enzyme and IDH-I was 37.3%. Ser-113 in the *E. coli* enzyme (2, 35) has been identified at the site for phosphorylation by isocitrate dehydrogenase kinase/phosphatase when the enzyme was inactivated in the cells grown on acetate as a sole carbon source. This residue was conserved in IDH-I at the same position; however, the specific activity of IDH-I in *Vibrio* sp. strain ABE-1 increased when this bacterium was grown on

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V-IDH-I  MTNKIIIPTTGDK-ITFIDG-KLSVPNNPIIPYIEGDGIGVDVTPPMLKVVNAAVAKAYGGD  60
E-IDH    MESKVVVPAQGKK-ITLQNG-KLNVPENPIIPYIEGDGIGVDVTPAMLKVVDAAVEKAYKGE  60
T-IDH    ---PLITTETGKKMHVLEDGRKL-------ITVIPGDGIGPECVEATLKVLEAA--KAP---  47

V-IDH-I  RKIEWLEVYAGEKATKWYDSETWLPEETLNILQEYKVSIKGPLTTPVGGGWSSLNVAIRQWL 122
E-IDH    RKISWMEIYTGEKSTQVYGQDVWLPAETLDLIREYRVAIKGPLTTPVGGGIRSLNVALRQEL 122
T-IDH    LAYEVRE-AGAS--VFRRGIASGVPQETIESIRKTRVVLKGPLETPVGYGEKSANVTLRKLF 106

V-IDH-I  DLYVCQRPVQWFTGVPSPVKRPSEVDMVIFRENTEDIYAGIEYKAG-SDKAK-SVI--KFLI 180
E-IDH    DLYICLRPVRYYQGTPSPVKHPELTDMVIFRENSEDIYAGIEWKAD-SADAE-KVI--KFLR 180
T-IDH    ETYANVRPVREFPNVPTPYAG-RGIDLVVVRENVEDLYAGIEHMQTPSVAQTLKLISWKGSE 167

V-IDH-I  EEMGASNIRFTENCGIGIKPVSKEGSQRLVRQAIQYAIDNNKDSVTLVHKGNIMKFTEGAFK 242
E-IDH    EEMGVKKIRFPEHCGIGIKPCSEEGTKRLVRAAIEYAIANDRDSVTLVHKGAIMKFTEGAFK 242
T-IDH    --------------------------KIVRFAFELARAEGRKKVHCATKSNIMKLAEGP-K 201

V-IDH-I  DWGYELAIEEFGASLLHGGPWCSLKNPNTGKEIIIKDVIADAMLQQVLLRPAEYSVIATLNL 304
E-IDH    DWGYQLAREEFGGELIDGGPWLKVKNPNTGKEIVIKDVIADAFLQEILLRPAEYDVIACMNL 304
T-IDH    RAFEQVAQ--------------------EYPDIEAVHIIVDNAAHQLVKRPEQFEVIVTTNM 243

V-IDH-I  NGDYLSDALAAQVGGIGIAPGANLGDEVAVFEATHGTAPKYAGKNKVNPGSVILSAEMMLRH 366
E-IDH    NGDYISDALAAQVGGIGIAPGANIGDECALFEATHGTAPKYAGQDKVNPGSIILSAEMMLRH 366
T-IDH    NGDILSDLTSGLIGGLGFAPSANIGNEVAIFEAVHGSAPKYAGKNVINPTAVLLSAVMMLRY 305

V-IDH-I  MGWLE-ADLLLKGMSGAIQAKTVTYDFERLMD-DATLVSCSAFGDCIIDHM 415
E-IDH    MGWTEAADLIVKGMEGAINAKTVTYDFERLMD-GAKLLKCSEFGDAIIENM 416
T-IDH    LEEFATADLIENALLYTLEEGRVLTGDVVGYDRGAKT---TEYTEAIIQNL 353
```

FIG. 6. Alignment of amino acid sequences of bacterial dimeric IDHs. V-IDH-I, E-IDH, and T-IDH indicate the sequences of *Vibrio* sp. strain ABE-1 IDH-I, *E. coli* IDH (35), and *T. thermophilus* IDH (21), respectively. Identical residues are shadowed.

```
                230       240       250       260
IDH-II   SKSALVEFFETEINKAKEEDVLLSLHLKATMM
A. v.    GKSALRKFIAAQIEDABK-GVLL-V-LKATMM
```

FIG. 7. Alignment of the partial amino acid sequences of monomeric IDHs. IDH-II, the deduced amino acid sequence from residues 230 to 261 of the *Vibrio* sp. strain ABE-1 IDH-II. A. v., *A. vinelandii* IDH; the sequence (10) of the peptide containing the methionyl residue which was modified by iodoacetic acid. The missing residues (dashes) in the *A. vinelandii* sequence were not determined. Identical residues are boxed.

acetate (18). As seen in Fig. 5, this increase was accomplished at the transcriptional level but not by modification after translation. It is interesting to note that the nucleotide sequence (from −45 to −12 [Fig. 2]) in the *icdI* promoter region resembles that (from −54 to −21) in the *E. coli aceB* promoter region, which has been suggested to interact with IclR (22), a repressor for acetate-inducible *ace* operon. Such a characteristic sequence is not present in the promoter region of *E. coli icd*. The amount of the *icdI* transcript was increased when pIS102-transformed *E. coli* was grown in an acetate-minimal medium (unpublished data). These results suggest that a functional isocitrate dehydrogenase kinase is absent in *Vibrio* sp. strain ABE-1 and that the transcriptional level of *icdI* gene is also elevated in *E. coli* cells.

The extremely thermolabile IDH-II, a monomeric IDH of *Vibrio* sp. strain ABE-1, has been suggested to function in the tricarboxylic acid cycle in this bacterium (13). We have recently shown (12) that antiserum raised against IDH-II specifically immunoneutralized the enzymatic activity of the other bacterial monomeric IDH in a sonic extract prepared from *A. vinelandii* or *V. parahaemolyticus*. Edwards et al. (10) reported that the *A. vinelandii* enzyme was inactivated by iodoacetic acid by the modification of a single methionyl residue, and they determined the amino acid sequence of a peptide containing this residue. Interestingly, the deduced amino acid sequence from residues 230 to 261 of IDH-II was found to be homologous to that of this peptide (Fig. 7). The modified methionyl residue of the *A. vinelandii* enzyme corresponds to methionyl residue 260 of IDH-II. However, there is no significant homology between the amino acid sequences derived from the ORFs of *icdI* and *icdII*, nor are there significant repeated amino acid sequences in the *icdII* gene. In addition, no significant matches were discovered in the DNA or protein data bases, when the sequences of the *icdII* gene and its protein were compared with other sequences. These findings rule out the possibility that the *icdII* gene arose from the *icdI* gene by gene duplication.

Since IDH-II is a thermolabile enzyme, expression of *icdII* in *E. coli* was examined at a low temperature (15°C) in this study. However, it will be interesting to investigate the effects of temperature on the transcriptional level of the gene, because the sequence CCAAT, which has been recently suggested to be a common motif seen in the low-temperature-inducible promoters of *E. coli* (26), is present 2 bp upstream from the −35 region of *icdII* (Fig. 2).

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