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Cloning, Sequence Analysis, and Expression in *Escherichia coli* of the Gene Encoding Monovalent Cation-activated Levodione Reductase from *Corynebacterium aquaticum* M-13

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The gene encoding (6*R*)-2,2,6-trimethyl-1,4-cyclohexanedione (levodione) reductase was cloned from the genomic DNA of the soil isolate bacterium *Corynebacterium aquaticum* M-13. The gene contained an open reading frame consisting of 801 nucleotides corresponding to 267 amino acid residues. The deduced amino acid sequence showed approximately 35% identity with other short chain alcohol dehydrogenase/reductase (SDR) superfamily enzymes. The probable NADH-binding site and three catalytic residues (Ser-Tyr-Lys) were conserved. The enzyme was sufficiently produced in recombinant *Escherichia coli* cells using an expression vector pKK223-3, and purified to homogeneity by two-column chromatography steps. The enzyme purified from *E. coli* catalyzed stereo- and regio-selective reduction of levodione, and was strongly activated by monovalent cations, such as K^+, Na^+, and NH_4^+, as was the case of that from *C. aquaticum* M-13. To our knowledge, this is the first sequencing report of a monovalent cation-activated SDR enzyme.

Key words: stereoselective reduction; short-chain alcohol dehydrogenase; cation activation

Optically active alcohols are versatile synthons in organic synthesis, especially in the preparation of natural and pharmaceutical products. However, in many reactions, obtaining the desired optically active alcohol with sufficient optical purity is still difficult, even though chiral metal complexes have been successfully used as catalysts for a number of enantioselective synthesis.[1,2] Optically active hydroxy cyclohexanone derivatives such as (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone (actinol) are also useful chiral building blocks of naturally occurring optically active compounds, such as xanthoxin,[3] and zeaxanthin,[4] however, chemical synthesis of these compounds is not trivial. Microbial reductions of ketones have provided chemists with the opportunity to prepare chiral alcohols for the development of novel chemical synthesis.[5,6] Microbial reduction of (6*R*)-2,2,6-trimethyl-1,4-cyclohexanedione (levodione) by whole cells has been reported,[7,8] although no report has previously appeared on the levodione reducing enzyme.

In our previous paper,[9] we screened for microorganisms that can catalyze stereo- and regio-selective reduction of the carbonyl group at the C-4 position of levodione and found that the soil-isolated bacterium, *Corynebacterium aquaticum* M-13 was the best producer of the enzyme under the conditions tested (Fig. 1). We have also reported the purification and characterization of a levodione reductase from *C. aquaticum* M-13 that required NAD^+ or NADH as a catalyzed reversible oxidoreduction between actinol and levodione and which was found to be a novel

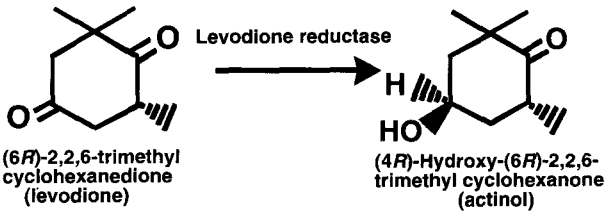

Fig. 1. Transformation of (6*R*)-2,2,6-Trimethyl-1,4-cyclohexanedione to (4*R*,6*R*)-4-Hydroxy-2,2,6-trimethylcyclohexanone by Levodione Reductase.

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Abbreviations: DTT, dithiothreitol; SDR, short chain alcohol dehydrogenase/reductase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; levodione, (6*R*)-2,2,6-trimethyl-1,4-cyclohexanedione; actinol, (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone. HPLC, high pressure liquid chromatography, NMR, nuclear magnetic resonance

monovalent-cation- activated enzyme.[9] The partial amino acid sequence analysis showed that levodione reductase belongs to the short chain alcohol dehydrogense/reductase (SDR) family.

In this paper, we report the cloning, sequence analysis, and expression in *Escherichia coli* of the gene encoding levodione reductase from *C. aquaticum* M-13, and the purification of the recombinant enzyme. The established expression system would supply sufficient enzyme for its application as a biocatalyst. To our knowledge, this is the first DNA and protein sequence report of levodione reductase.

Materials and Methods

Microorganisms and cultivation. Corynebacterium aquaticum M-13[9] was used as the source of chromosomal DNA. The strain was cultivated as described previously.[9] *E. coli* DH5α (*deo*R *end*A1 *gyr*A96 *hsd*R17 (rK^-, mK^+) *rec*A1 *sup*E44 *thi*-1 Δ(*lac*ZYA-*arg*F) U169 φ80 *lac* Δ ZM15 F^- λ^-) was used as a host for gene cloning. *E. coli* JM109 (*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1 Δ(*lac-pro*AB) F'[*tra*D36 *pro*AB^+ *lac*I^q *lac*Z ΔM15]) was used for gene expression and enzyme purification. *E. coli* cells were grown at 37°C in Luria-Bertani medium containing 1% Bacto-Tryptone (Difco-Laboratories, Detroit, Mich.), 0.5% Bacto-Yeast extract (Difco-Laboratories), and 0.5% NaCl (pH7.0). When necessary, ampicillin (100 μg/ml) was added to the medium.

Plasmids. The plasmids pYES2 (Stratagene, La Jolla, CA, USA) and pKK223-3[10] (Amersham Pharmacia Biotech, Buckinghamshire, England) were used as a vector for the construction of the genomic library and as an expression vector, respectively.

Enzymes and chemicals. Restriction enzymes were purchased from Takara Shuzo Co. Ltd. (Kyoto, Japan) and Toyobo Biochemicals (Osaka, Japan). DNA polymerases (Takara Ex Taq and Takara LA Taq) were purchased from Takara Shuzo Co., Ltd. Levodione and actinol were prepared as described previously.[4] All other chemicals used in this study were of analytical grade and commercially available.

Screening of a genomic DNA library. To amplify a levodione reductase DNA fragment from *C. aquaticum* M-13 chromosomal DNA by PCR, upstream and downstream primers were designed on the basis of the internal amino acid sequence.[9] The sequences of the primers were as follows: 1-19(+), 5'-GCNGTNGARTAYGGNMGITA-3', and 2-32(−), 5'-ATYTCNGGNGCYTCNCCRTA-3'. (where N is A, C, G or T; R is A or G; Y is C or T; M is A or C; and I is inosin.) One hundred ng of *C. aquaticum* M-13 chromosomal DNA partially digested by *Sau*3AI was used as the template. The PCR mixture (20 μl) contained 100 pmol of each primer, each dNTP at a concentration of 0.312 mM, and 2.5 U of Takara Ex Taq. The initial template denaturation step consisted of 5 min at 95°C. The amplification profile (30 sec at 95°C, 30 sec at 42°C, and 2 min at 72°C) was repeated for 25 cycles. The amplified DNA fragment, of about 180 bp, was purified by gel electrophoresis, then cloned into a pGEM-T vector (Promega, Madison, WI, USA). Its nucleotide sequence was analyzed with the dye terminator cycle sequencing kit and an ABI prism 377 DNA sequencer (PE Biosystems, Foster City, CA, USA). We conclude that the 172-bp fragment was a portion of the LVR gene, because the amino acid sequence of the internal peptide was found in the amino acid sequence deduced from the nucleotide sequence of this PCR product. This fragment was labeled with horseradish peroxidase using glutaraldehyde as a labeling improver, and used as a probe for colony hybridization.

Cloning of levodione reductase gene. The genomic DNA from *C. aquaticum* M-13 was isolated as described previously.[11] The genomic DNA was partially digested with *Sau*3AI. Fragments with a molecular size of about 3-5 kbp were separated and purified from agarose gel. The fragments were ligated into *Bam*HI-treated pYES2. The constructed genomic DNA library was screened by colony hybridization using an ECL direct nucleic acid labeling and detection system (Amersham Pharmacia Biotech, Buckinghamshire, England). The colony hybridization was done following the manufacturer's protocol.

Comparison of the amino acids sequence. The deduced amino acid sequence of *lvr* was compared with other protein sequences in the protein database (nr-aa, release 5/31/2000) by using the FASTA[12] program.

Expression of levodione reductase in E. coli. To obtain the complete coding sequence for the levodione reductase gene without the excessive flanking region, PCR amplification was done. The two synthetic primers used were LV- ORF (+), 5'-GGAGGCGAATTCATGACCGCAACCAGCTCC-3', (the underlined sequence is the position of an *Eco*RI site) and LV- ORF(−), 5'-GGGCTGCTGCAGTCAGTACGCGGCGGA-3', (the underlined sequence is the position of a *Pst*I site). Plasmid pYLVR2 was used as the template. The PCR mixture (20 μl) contained 5 pmol of each primer, each dNTP at a concentration of 0.2 mM, and 1 U of Takara LA Taq. The initial template denaturation step consisted of 1 min at 94°C. The amplification profile (20 sec at 98°C, 2 min at 70°C, and 4 min at 72°C) was repeat-

ed for 25 cycles. The PCR-generated DNA fragment was digested with *Eco*RI and *Pst*I, and then ligated into pKK223-3 cleaved with *Eco*RI and *Pst*I. The ligated plasmid was then used to transform *E. coli* JM109. After ampicillin selection, several clones were picked up, and the nucleotide sequence of the plasmid DNA was examined. The constructed plasmid was designated as pKKLVR.

Enzyme assay. Enzyme activity was measured by spectrophotometrically measureing the levodione-dependent decrease in the NADH content. The standard 2.5 ml assay mixture contained 5 μmol of levodione (final concentration, 2.0 mM), 0.80 μmol of NADH, 500 μmol of potassium phosphate buffer (pH7.0), and the enzyme. One unit of enzyme activity was defined as the amount of enzyme that catalyzed oxidation of 1 μmol of NADH per min. When the effects of monovalent cations were measured, the potassium phosphate buffer was replaced by an equimolar concentration of Tris-HCl buffer (pH7.4).

Purification of the enzyme expressed in E. coli. All purification procedures were done at 0–4°C in 10 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM dithiothreitol (DTT), unless otherwise specified.

E. coli JM109 (pKKLVR) cells were grown with shaking at 37°C for 18 h in LB medium containing 100 μg/ml ampicillin.

The washed cells (wet weight, 5 g), isolated from about 1.5 liters of culture broth, were suspended in 15 ml of the buffer, then disrupted with an ultrasonic oscillator for 30 min. After centrifugation, solid ammonium sulfate was added to the resulting supernatant at a concentration of 2 M. The supernatant obtained after centrifugation was put to a Butyl-Toyopearl 650 M (Tosoh, Tokyo, Japan) column (3.0 × 20 cm) equilibrated with the buffer containing 2 M $(NH_4)_2SO_4$. The enzyme was eluted with a linear gradient of 2 to 0 M $(NH_4)_2SO_4$ in 340 ml of the buffer at a flow rate of 1.5 ml/min. The activity- containing fractions, eluted at approximately 0.8 M $(NH_4)_2SO_4$ in the buffer, were pooled and dialyzed against 3 liters of the buffer for 8 h.

The enzyme solution was put on a MonoQ HR5/5 column (0.5 × 5 cm) connected to a FPLC system (Amersham Pharmacia Biotech, Buckinghamshire, England) and equilibrated with the buffer. The enzyme was eluted with a linear gradient of 0 to 1.0 M NaCl in 11 ml of the buffer at a flow rate of 1 ml/min. The activity-containing fractions, eluted at approximately 0.4 M NaCl in the buffer, were collected and used as the purified enzyme for characterization.

Amino acid sequence analysis. The N-terminal amino acid sequence was analyzed with a 476A pulsed liquid protein sequencer (PE Biosystems, Foster City, CA, USA) as described previously.[13]

Molecular mass measurement. The molecular mass of the enzyme was estimated using a MALDI-TOF mass spectrometer (Voyager Biospectrometry, PE Biosystems, Foster City, CA, USA) using 25-kV acceleration voltage. The samples were run in linear mode. Sinapic acid was used as the matrix.

Enzymatic preparation of actinol. Using levodione as the substrate the optical purity of the reduction product was analyzed by GC under the conditions described below. Enzymatic reduction of levodione was done as described previously.[9]

Analysis of levodione and actinol. Measurement of levodione and actinol contents was done with a Shimadzu model GC-14B GC with a flame ionization detector by using a type HR-20M capillary column (0.25 mm by 30 m; Shinwa Chemical Industries, Kyoto, Japan) at 160°C (isothermal) and He as the carrier gas at a flow rate of 1 ml/min. Under these conditions, levodione, actinol, and (4*S*,6*R*)-hydroxy-2,2,6-trimethylcyclohexanone (a diastereomer of actinol) were eluted at 6.8, 15.6, and 15.9 min, respectively. The enantiomeric excess (*e.e.*) was calculated from the peak areas of the stereoisomers.

Identification of levodione reducing product (actinol). Washed cells of *C. aquaticum* M-13 were incubated in 80 ml of reaction mixture containning 8 mmol of potassium phosphate buffer (pH 7.0), 69.9 μmol of NAD^+, glucose 22.2 mmol, 2,500 U of glucose dehydrogenase (Amano Enzyme Inc. Nagoya, Japan.), and 2.6 mmol levodione. The reaction mixture was incubated at 30°C for 47 h with shaking on a magnetic stirrer and then the mixture was vigrorously shaken with 80 ml of ethyl acetate. The ethyl acetate layer was concentrated to 1.6 ml and 50 μl of them were put on a silica gel- precoated thin layer plate (105717, 200 × 200 × 2 mm, Merck, Rahway, NJ, USA) and were developed with a solevent (n-hexane: ethylacetate = 7:3). Spots of levodione and actinol were identified by the Rf values and co-chromatography with authentic compounds. The spot of actinol was scraped off and was extracted with 10 ml of ethyl acetate. The ethyl acetate layer was analyzed to find the amount of actinol by gas chromatography. After the ethyl acetate layer was evaporated to dryness and diluted with 35% CH_3CN, the crude product was purified by high pressure liquid chromatography (HPLC) , LC-10A (Shimadzu Co, Kyoto, Japan) on a Cosmosil column (5C18-AR II, 20 × 250 mm, Nacalai Tesque, Kyoto, Japan.) to

give the actinol. The mobile phase was CH_3CN: H_2O = 35: 65 at flow rate of 5.0 ml/min, and the effluent was monitored by ultraviolet detection (210 nm). The chemical structure of the product was identified by proton nuclear magnetic resonance (NMR). NMR experiments were done on Mercury-300 (Varian, Inc., Palo Alto, CA, USA) (300 MHz at ^1H) and chemical shift assigned relative to the solvent signal. The product was dissolved in $CDCl_3$ containing 0.03% (v/v)TMS.

Results and Discussion

Isolation of levodione reductase gene

After the first colony hybridization, one positive clone out of 2800 colonies was obtained. This plasmid was designated as pYLVR1. The nucleotide sequencing of pYLVR1 showed that this *Sau*3AI fragment contains a portion of the levodione reductase gene, although this fragment did not contain the full length of the levodione reductase gene and lacked the 3′ coding region (data not shown). We found a *Sac*I site in the 5′ noncoding region of the gene, and subsequently re-screened a *Sac*I-digested genomic DNA library of *C. aquaticum* M-13 constructed in pYES2. The plasmid obtained after the second colony hybridization was designated as pYLVR2, and was found to contain the full length of levodione reductase gene.

Sequence analysis of the levodione reductase gene

The nucleotide sequence of the above-mentioned *Sac*I fragment (2007 bp) was analyzed and put into the DDBJ/EMBL/GenBank nucleotide sequence data base with the accession number AB042262. The levodione reductase gene (*lvr*) encodes a protein with 267 amino acid residues (calculated molecular mass of 27.92 kDa). The open reading frame (ORF) extends from the ATG initiation codon at position 943 to the TGA stop codon at position 1744, with a potential Shine-Dalgarno (SD) sequence, GGAGGC, from position 931 to position 936. In the 5′-flanking region of this ORF, no potential *E. coli* type promoter consensus sequence, such as TATAAT or TTGACA,[14] was found. No other ORF was found in this *Sac*I fragment. To diseover whether the promotor of *C. aquaticum* M-13 functions in *E. coli*, we constructed a plasmid that carried the region 150 bp upstream of the *lvr* start codon and *lvr* in the opposite orientation to the *lac* promotor of pUC18 and was introduced in *E. coli* JM109. The transformant had levodione reducing activity although it was weak (data not shown). This indicated its own promotor functions in E. coli.

A possible consensus sequence of the coenzyme binding site of the SDR family enzyme,[15,16,17] GXXXGXG (amino acid position 20 to position 26), and the amino acids residues reported to be important for enzyme activity of the SDR family enzyme,[16] Ser152, Tyr165, and Lys169, are fully conserved in the *lvr* sequence. The existence of the coenzyme-interacting Asp residue (at amino acid position 44), which regulates the specificity for NADH or NADPH,[16,17] agrees with the coenzyme specificity of this enzyme (NADH only).

Comparison of the lvr amino acids sequence

The levels of identity of the enzyme with other SDR family enzymes, including 3α,20β-hydroxysteroid dehydrogenase from *Streptomyces hydrogenans*,[18] 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase from *Sphingomonas paucimobilis* UT26,[19] glucose 1-dehydrogenase III from *Bacillus megaterium* IAM1030,[20] and 7β-hydroxysteroid dehydrogenase from *Eubacterium* sp.,[21] were 38.4, 37.0, 36.6, and 36.5%, respectively. The amino acid sequence alignment is shown in Fig. 2.

Purification of the enzyme

The levodione reductase was efficiently expressed in *E. coli* JM109 under the control of the *tac* promoter of the vector, pKK223-3. The enzyme activity of the cell-free extract of *E. coli* JM109 (pKKLVR) was 1.8 U/mg, while that of *C. aquaticum* M-13 was too low to measure the specific activity.[9] The purification of the recombinant levodione reductase is summarized in Table 1. The enzyme was purified to homogeneity with a 40.6% overall recovery. The purified enzyme gave a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 3).

It should be noted that the amount of levodione reductase produced was 3.0 mg/g of *E. coli* JM109 wet cells; this value was 600 times the amount produced in *C. aquaticum* M-13 cells (0.005 mg/g of wet cells).[9] This expression system may be useful for

Table 1. Purification of the Recombinant Levodione Reductase from *E. coli* JM109 (pKKLVR)

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)	Purification (fold)	Enantiomeric excess for (4*R*,6*R*) (%)
Cell extract	769	1360	1.80	100	1.0	91.2
2 M $(NH_4)_2SO_4$ supernatant	197	2540	12.9	186	7.17	89.7
Butyl Toyopearl	31.3	1290	41.2	94.7	22.9	89.0
MonoQ HR5/5	15.8	554	34.9	40.6	19.4	89.7

Enzyme activity was measured as reported previously.[9]

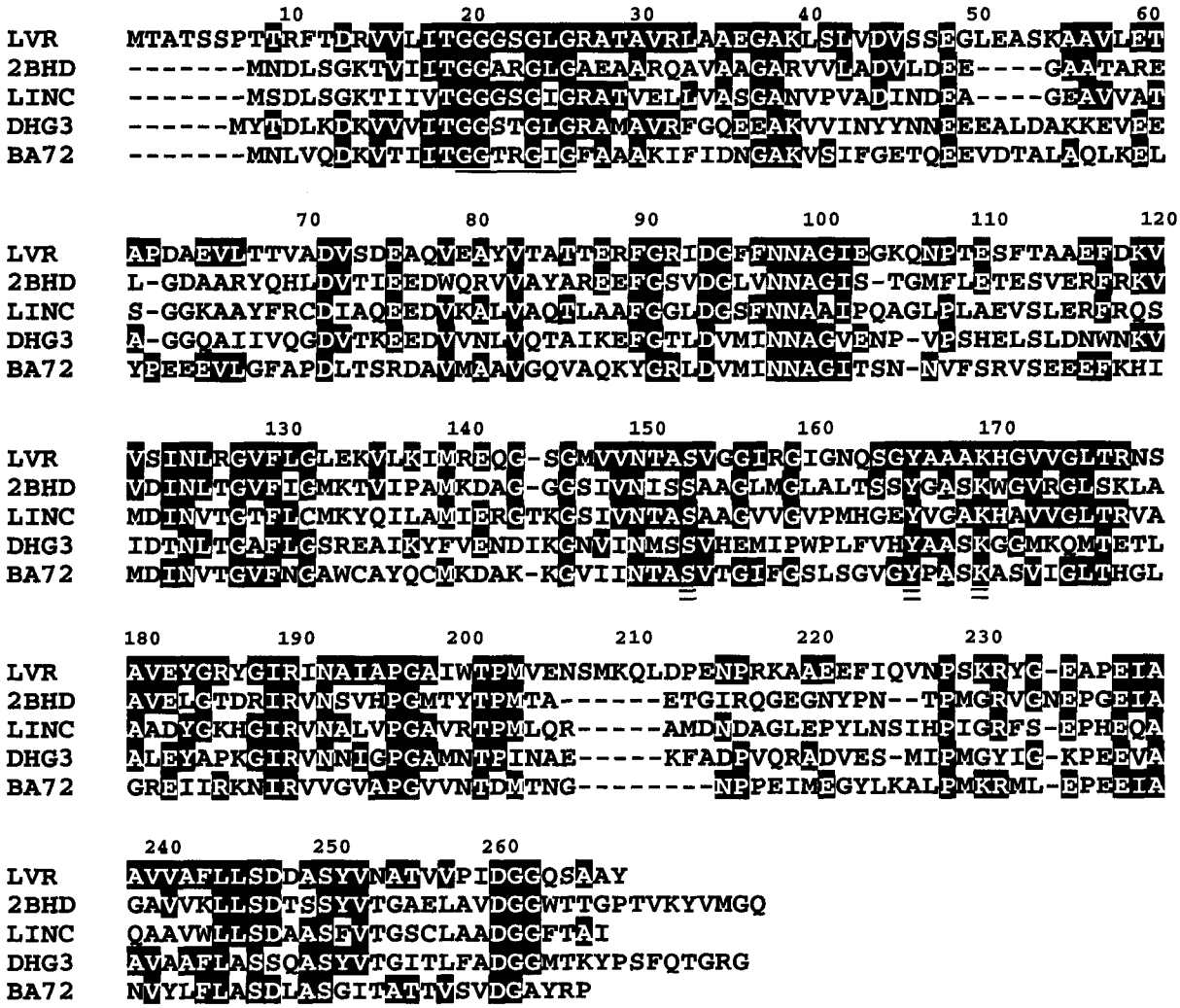

Fig. 2. Comparisons of the Deduced Amino Acid Sequences of the Levodione Reductase with the Amino Acid Sequences of Other Function-known SDR Family Enzymes.

2BHD, 3α,20β-hydroxysteroid dehydrogenase from *Streptomyces hydrogenans*[18] (Swiss prot No. P19992); LINC, 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase from *Sphingomonas paucimobilis* UT26[19] (Accession No. D14595); DHG3, glucose 1-dehydrogenase III from *Bacillus megaterium* IAM1030[20] (Accession No. D10625); and BA72, 7α-hydroxysteroid dehydrogenase from *Eubacterium* sp.[21] (Accession No. M22623). The alignment was constructed using the Clastal W software.[22] Identical residues are shown by white letters on a black background. A putative coenzyme binding region is underlined.[16,17] The amino acid residues reported to be important for enzymatic activity are double-underlined.[16]

practical production of the enzyme.

Molecular mass

The molecular mass of the enzyme deduced from the amino acid sequence (27.9 kDa) is in agreement with the range obtained by MALDI-TOF-MS analysis (27.5-28.0 kDa). This value was lower than that determined by SDS-PAGE (36 kDa, Fig. 3), or that of the enzyme purified from *C. aquaticum* M-13, which was previously reported by a SDS-PAGE (36 kDa).[9] The discrepancy may be due to the low p*I* of the protein (around 4.8 deduced from the amino acid sequence) or some unknown factors such as the conformation of the enzyme. Similar phenomena were observed in glucose dehydrogenase isozymes of *Bacillus megaterium* IAM1030.[20]

Identification of levodione reducing product (actinol)

^{1}H-NMR and H-H COSY of the actinol was measured. The same feature was also observed in the chemical sift of an authentic actinol. δ: 4.22 (1H, m, HO-CH), 3.17 (1H, m, Me-CH), 2.17 (1H, ddd, C5-H), 2.02 (1H, dt, C3-H), 1.82 (1H, dd, C3-H), 1.74 (1H, dd, C5-H), 1.36 (3H, s, C2-CH_3), 1.05 (3H, s, C2-CH_3), 1.03 (3H, d, J=6.6, C6-CH_3).

Stereoselectivity for levodione reduction

The levodione reducing product formed by the recombinant enzyme was a (4*R*,6*R*)-enantiomer with approximately 90.0% *e.e.* that was eluted at the same retention time of an authentic actinol and the actinol formed by *C. aquaticum* M-13 by GC. This result in-

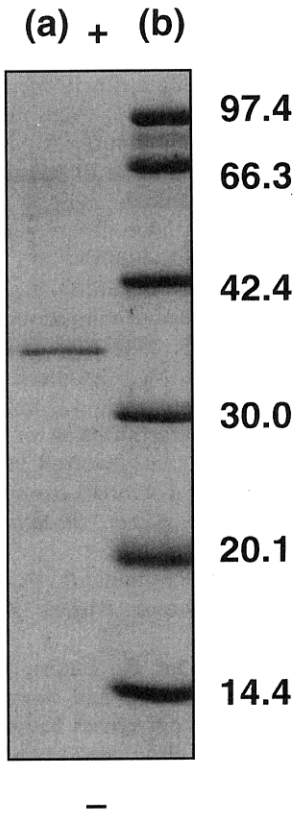

Fig. 3. SDS-PAGE of the Levodione Reductase from *E. coli* JM109 (pKKLVR).

(a) the purified enzyme after MonoQ chromatography (2 μg) (b) *M*r standards (from top): phosphorylase *b* (97,4 kDa), bovine serum albumin (66,3 kDa), aldolase (42,4 kDa), carbonic anhydrase (30,0 kDa), trypsin inhibitor (20,1 kDa), and α-lactalbumin (14,4 kDa). The gel was stained for protein with Coomassie Brilliant Blue R-250 and destained in methanol/acetic acid/water (7:6:47).

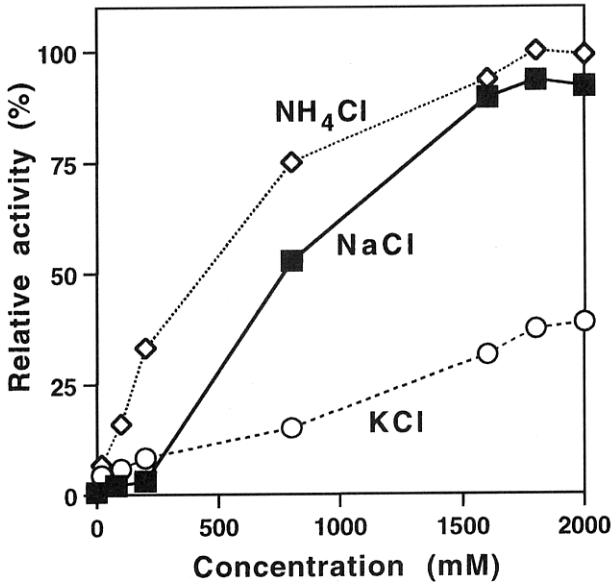

Fig. 4. Effects of K^+ (○), Na^+ (■), and NH_4^+ (◇) on the Enzyme Activity.

Tris-HCl (pH 7.4) was used as the buffer, and the enzyme activity with 2 M NH_4^+ was taken as 100%. Chloride was used as the anion in all mixtures.

dicated that the product was actinol. This value is slightly lower than that of the enzyme purified from *C. aquaticum* M-13, although the explanation for this discrepancy is unknown.

Effects of monovalent cations on the enzyme activity

The purified enzyme was eluted from the MonoQ HR5/5 column, which contained approximately 300 mM NaCl, showed 34.9 U/mg specific activity. The specific activity decreased to 2.6 U/mg after overnight dialysis against 10 mM Tris-HCl buffer (pH 7.4) with 0.1 mM DTT.

As in the case of the enzyme from *C. aquaticum* M-13, the decreased activity of the dialyzed enzyme was restored when a monovalent cation, such as NH_4^+, Na^+, Cs^+, K^+, or Rb^+, was added to the reaction mixture.[9] The activation by monovalent cations K^+, Na^+, and NH_4^+, is shown in Fig. 4. However, *C. aquaticum* M-13 is not a halophilic bacterium and scarecely grows in the presence of 1 M KCl. Glucose dehydrogenases from *Bacillus megaterium* IAM1030 were reported to be stabilized by addition of 2 M NaCl,[20] though activation by salts has not been previously reported. To our knowledge, this is the first sequencing report of a SDR family enzyme that is clearly activated by salts.

Other properties of the enzyme expressed in E. coli

The N-terminal amino acid sequence of the first 15 amino acid residues of the purified enzyme was found to match the deduced amino acid sequence of levodione reductase except for the first methionine. Substrate specificity and kinetic parameters of the recombinant enzyme were found to be the same or very similar to those of the results obtained for the enzyme from *C. aquaticum* M-13.[9] From these results, we conclude that the levodione reductase expressed in *E. coli* has the same properties as those of the enzyme from *C. aquaticum* M-13.

We report in this paper the cloning, sequence analysis, and expression in *E. coli* of levodione reductase. This is the first sequence report of a monovalent cation-activated SDR enzyme. Microbial production of actinol from levodione has been demonstrated previously by Nishii *et al.*,[7,8] however, in this case, a racemic mixture of 4-hydroxy-2,2,6-trimethylcyclohexanones was obtained as the reduction product and the enzyme involved in reduction of levodione has not been purified. We have established an overexpression system for levodione reductase in *E. coli* and the system provides a powerful means for obtaining optically pure actinol. We are now trying to clarify the mechanism of the activation by monovalent cations. As the first step, we have established techniques for obtaining purified levodione reductase in

amounts sufficient for crystallization. Analysis of the three-dimensional structure of this enzyme should reveal the mechanism of the activation by monovalent cations. Screening of the crystallization condition of this recombinant enzyme is currently under way.

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