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# Cloning, Sequencing, Heterologous Expression, Purification, and Characterization of Adenosylcobalamin-dependent D-Ornithine Aminomutase from *Clostridium sticklandii*[*]

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**D-Ornithine aminomutase from *Clostridium sticklandii* catalyzes the reversible rearrangement of D-ornithine to (2*R*,4*S*)-2,4-diaminopentanoic acid. The two genes encoding D-ornithine aminomutase have been cloned, sequenced, and expressed in *Escherichia coli*. The *oraS* gene, which encodes a protein of 121 amino acid residues with $M_r$ 12,800, is situated upstream of the *oraE* gene, which encodes a protein of 753 amino acid residues with $M_r$ 82,900. The holoenzyme appears to comprise a $\alpha_2\beta_2$-heterotetramer. OraS shows no significant homology to other proteins in the Swiss-Prot data base. The deduced amino acid sequence of OraE includes a conserved base-off/histidine-on cobalamin-binding motif, D*X*H*XX*G. OraE was expressed in *E. coli* as inclusion bodies. Refolding experiments on OraE indicate that the interactions between OraS and OraE and the binding of either pyridoxal phosphate or adenosylcobalamin play important roles in refolding process. The $K_m$ values for D-ornithine, 5′-deoxyadenosylcobalamin (AdoCbl), and pyridoxal 5′-phosphate (PLP) are 44.5 ± 2.8, 0.43 ± 0.04, and 1.5 ± 0.1 μM, respectively; the $k_{\text{cat}}$ is 6.3 ± 0.1 $s^{-1}$. The reaction was absolutely dependent upon OraE, OraS, AdoCbl, PLP, and D-ornithine being present in the assay; no other cofactors were required. A red-shift in UV-visible absorption spectrum is observed when free adenosylcobinamide is bound by recombinant D-ornithine aminomutase and no significant change in spectrum when free adenosylcobinamide is bound by mutant OraE-H618G, demonstrating that the enzyme binds adenosylcobalamin in base-off/histidine-on mode.**

One major finding from amino acid fermentation studies in *Clostridium* is the Stickland reaction; *i.e.* oxidation of one group of amino acids is coupled to reduction of another group of amino acids (1). In this reaction, alanine, leucine, isoleucine, valine, histidine, phenylalanine, and tryptophan can generally be used as electron donors, whereas proline, glycine, ornithine, and arginine serve as electron acceptors (2). The catabolism of ornithine in *Clostridium sticklandii*, a Gram-positive anaerobe, is of great interest, because it can be accomplished by two different pathways. Either ornithine can be reduced to 5-aminovaleric acid through the formation of L- and D-proline as intermediates or it can be oxidized to acetate, alanine, and ammonia. In the latter oxidation pathway, L-ornithine is first converted to D-ornithine by ornithine racemase, which has recently been purified and characterized by our group (3). This pathway is also found in *C. subterminales* (4).

D-Ornithine aminomutase (EC 5.4.3.5) catalyzes the reversible interconversion of D-ornithine to (2*R*,4*S*)-2,4-diaminopentanoic acid as the second step in the oxidation pathway of L-ornithine in *C. sticklandii*. The enzyme is one of a group of adenosylcobalamin-dependent enzymes that catalyzes unusual isomerizations in which a substituent group is interchanged with a hydrogen atom from an adjacent carbon atom. It comprises two strongly associating subunits, S and E, with molecular masses of 12,800 Da and 90,000 Da, respectively, as judged by SDS-PAGE.[1] Its molecular weight has been estimated by gel filtration to be about 200,000 (5), suggesting that it is a heterotetramer. The apoenzyme combines with adenosylcobalamin and pyridoxal phosphate to form the active holoenzyme.

AdoCbl is generally accepted to act as a radical initiator through the enzyme-promoted cleavage of its cobalt–carbon bond. The adenosyl radical then abstracts a hydrogen atom from either the substrate or a protein residue. In the case of carbon-skeleton mutases and aminomutases, a 1,2-rearrangement of the subsequently formed substrate radical is catalyzed. The crystal structures of the cobalamin-binding domains of methionine synthase, methylmalonyl-CoA mutase, and glutamate mutase have been determined. In each case, the pseudonucleotide tail of cobalamin has swung away from the corrin and is replaced as a ligand to cobalt by the imidazole group of a histidine residue (6–8). This key histidine residue is present in a conserved motif, D*X*H*XX*G, which is also found in other AdoCbl-dependent carbon-skeleton mutases and aminomutases (9).

PLP plays a pivotal role in the migration of the amino group between adjacent carbon atoms. The migrating amino group is believed to form a Schiff base with PLP (10). Like acyl-CoA mutases, the migration probably proceeds through a cyclic transition state (11). This mechanistic picture is strongly supported by a non-enzymatic mode study and quantum chemical calculations (12, 13).

The reactions catalyzed by D-ornithine and D-α-lysine amin-

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*The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY038595.*

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[1] The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; AdoCbl, 5′-deoxyadenosylcobalamin; AdoCbi, 5′-deoxyadenosylcobinamide; PLP, pyridoxal 5′-phosphate; PCR, polymerase chain reaction; IPTG, isopropyl-β-D-thiogalactopyranoside; DTT, dithiothreitol; bp, base pair(s); kb, kilobase(s).

TABLE I
*PCR primers*

Primer names and sequences. The restriction sites introduced via mutagenic primers, are *underlined*.

| Primer name | Sequence |
|---|---|
| 21 | ATG AAA (AC)GA GC(AT) GAT GAT TTT CAA CA |
| 23 | ATA TTT TC(AT) ACA TCT A(AG)T TTT TCA TT |
| 33 | AAT AGT TGA TCC ATT ACT GGA TTT AGG |
| 34 | TTG CAT TAA GTG AAG GCA AGT ACT GGG |
| 35 | CCA GCA CCT TTT CCC ATA AGC CCT |
| 36 | CCC TAA ATC CAG TAA TGG ATC AAC |
| 37 | GGG TCT AGA ATC GAA AAA GAT CTA CAG TTA AGA |
| 38 | CCA TAA TCC ACT CCA CCT GCT AAA |
| 39 | GGG GAA TTC CAT ATG AAA AGA GCA GAT GAT TTT CAA |
| 40 | CCC GGA TCC TTA TTT TAC TCC TCC TTT |
| 41 | GCG CGC CAT GGA AAA AGA TCT ACA GTT AAG A |
| 42 | GGG GGA TCC CCA TAA TCC ACT CCA CCT GCT AAA |
| 43 | ACC AAC TGA ACC CTC ATC TTC TCC TAC AG |
| 44 | GAA GAT GAG GGT TCA GTT GGT CTA AGA GA |

omutase are remarkably similar. Early studies indicated that D-$\alpha$-lysine aminomutase is a single-protein complex but that it catalyzes the rearrangement of two different substrates, D-$\alpha$-lysine and L-$\beta$-lysine. It is composed of a catalytic component, $E_1$, and a regulatory component, $E_2$. There are three non-identical subunits in the $E_1$ component with molecular masses of 30,000, 51,000, and 12,800 Da. Interestingly, both D-ornithine and D-$\alpha$-lysine aminomutase contain a small 12,800-Da subunit, and their amino acid compositions are virtually identical (5). The genes encoding D-lysine aminomutase in *C. sticklandii* have recently been cloned and sequenced (14). The *kamD* and *kamE* genes encode polypeptides of 57,261 and 29,191Da, respectively, and are adjacent on the clostridial chromosome. However, the recombinant enzyme, even in the absence of the small subunit reported by Baker and Stadtman (5), is still catalytically active. Therefore, the real role of the small subunit in D-$\alpha$-lysine or D-ornithine aminomutase remains unknown. We now describe the cloning and sequencing of *oraS* and *oraE*, the genes encoding components S and E of D-ornithine aminomutase, from *C. sticklandii*, together with the overexpression of OraS and OraE in *Escherichia coli*, and the purification, refolding, and initial characterization of the recombinant proteins.

## EXPERIMENTAL PROCEDURES

*Materials*—AdoCbl was obtained from Sigma Chemical Co. AdoCbi was the kind gift of Prof. Hui-Lan Chen (Department of Chemistry, Nanjing University, People's Republic of China). Q-Sepharose High Performance (HP) anion-exchange medium, SP-Sepharose HP cation-exchange medium, Sephacryl-S-200-HR gel filtration medium, Phenyl-Sepharose HP hydrophobic interaction gel medium, and a Mono-Q column were from Amersham Pharmacia Biotech. Restriction endonucleases *Xba*I, *Bam*HI, *Eco*RI, *Nde*I, *Hin*dIII, and *Nco*I; DNA-modifying enzymes; and Ex *Taq* DNA polymerase were purchased from TaKaRa. The kits used for non-radioactive labeling and detection (AlkPhos direct labeling system and CDP-Star detection system) were from Amersham Pharmacia Biotech. The LA PCR *in vitro* cloning kit was from TaKaRa. The *E. coli* strain BL21(DE3) codon plus was from Stratagene. All chemicals used were of molecular biology grade or higher.

*Bacterial Strain and Media*—*C. sticklandii* (ATCC 12662) cells were grown in a medium consisting of 0.6% Tryptone, 0.6% yeast extract, 10 $\mu$M $CoCl_2$, 10 $\mu$M $MnCl_2$, 10 $\mu$M $Na_2MoSO_4$, 100 mM $MgCl_2$, 100 mM $CaCl_2$, and 1% L-arginine hydrochloride in 40 mM potassium phosphate buffer (pH 7.5). The medium was used immediately after autoclaving to keep its oxygen content to a minimum. Provided the culture vessel was filled to the neck, an anaerobic atmosphere was unnecessary. Each batch of medium was inoculated with a 5% inoculum of an actively growing culture and was incubated at 37 °C in the dark for 12–16 h. The cell paste was frozen as soon as it was removed from the centrifuge and was stored at −20 °C until used.

*Purification of* D-*Ornithine Aminomutase from C. sticklandii*—All purification steps were performed on ice or at 4 °C. In a typical purification, 15 g of cells (wet weight) was resuspended in 60 ml of 50 mM potassium phosphate buffer, pH 7.0. The cells were ruptured in a volume of 30 ml by sonication. Cell debris was removed by centrifugation at 25,000 × *g* for 15 min, and the supernatant was brought to 25% saturation in ammonium sulfate by slow addition of solid. The precipitate was removed by centrifugation at 25,000 × *g* for 30 min, and the supernatant was directly applied to a Phenyl-Sepharose HP hydrophobic interaction column (2.6 × 25 cm) equilibrated in 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM DTT, 1 M $(NH_4)_2SO_4$, and 10% glycerol. After washing the column with 100 ml of the same buffer, the enzyme was eluted with a 1inear, descending gradient of ammonium sulfate in 1000 ml of buffer. The flow rate was 1 ml/min; 10-ml fractions were collected. Active fractions were pooled and concentrated to 15 ml by ultrafiltration in a stirred cell fitted with a PM-30 membrane. After overnight dialysis against 1 liter of 10 mM potassium phosphate buffer containing 10% glycerol, pH 6.4, the protein was loaded onto a 2.6- × 20-cm Q-Sepharose Fast Flow anion-exchange column equilibrated with the same buffer. Protein was eluted with a 600-ml gradient from 0 to 0.5 M KCl. The flow rate was 1 ml/min; 5-ml fractions were collected. Active fractions were pooled and dialyzed overnight against 1 liter of 10 mM potassium phosphate buffer containing 10% glycerol, pH 6.4. Final purification was achieved by fast-protein liquid chromatography using a Mono Q HR5/5 column. Protein was applied to the column equilibrated in 10 mM potassium phosphate, pH 6.4, and eluted with a 20-ml gradient from 0 to 0.5 M KCl. The flow rate was 1 ml/min; 0.4-ml fractions were collected. The protein solution was concentrated by ultrafiltration and stored at −20 °C in the presence of 50% glycerol.

*Probe Construction*—To determine the N-terminal sequences of the both components, S and E, of D-ornithine aminomutase, the protein solution from above was subjected to SDS-PAGE through a 5–20% gradient gel. Polypeptides were then transferred to a polyvinylidene difluoride membrane by electroblotting and stained with Coomassie Blue R-250 for 40 s. After partial destaining, the desired bands were cut out for N-terminal sequencing. The N-terminal sequences obtained were as follows: S component, MKRADDFQQRRAHLANLSDEELQTR; and E component, MEKDLQLRVNEKLDVENILKDLDKTT. Two oligonucleotides, nos. 21 and 23 (Table I), were designed based on the N-terminal sequences of the S and E components for use as primers in a degenerate PCR experiment. Genomic DNA was purified from *C. sticklandii* by phenol/chloroform extraction methods (15). The PCR reaction was carried out by using a PerkinElmer Life Sciences 480 thermal cycler as follows: 1 min at 94 °C, 1.5 min at 41 °C, and 3 min at 72 °C. After 35 cycles, a clear ~400-bp PCR product was obtained. The gel-purified PCR product was sequenced on both DNA strands to confirm that the PCR reaction had amplified the correct region of genomic DNA and was directly used as a probe.

*Gene Cloning and Sequencing*—Genomic DNA from *C. sticklandii* was restricted with *Xba*I or *Hin*dIII. The DNA fragments were separated by agarose gel electrophoresis, blotted onto a positively charged nylon membrane, and hybridized with the probe using the AlkPhos direct labeling system. After chemiluminescence detection with CDP-Star detection reagent, ~6- and 2.4-kb bands were detected within *Xba*I- and *Hin*dIII-restricted clostridial genomic DNA, respectively. The LA PCR *in vitro* cloning kit was directly used to clone the coding regions for the subunits of D-ornithine aminomutase. A 3.0-kb DNA fragment located on the downstream side of the probe DNA was amplified by two rounds of PCR reactions with the use of oligonucleotides 33 and 34 as primers and *Xba*I-restricted clostridial genomic DNA as template. Accordingly, a 1.2-kb DNA fragment located on the upstream side of the probe was amplified with the use of oligonucleotides 35 and 36 as primers and *Hin*dIII-restricted clostridial genomic DNA as template. All experimental steps and PCR conditions were carefully carried out according to the kit manufacturer's instructions. Both fragments amplified by PCR experiments were gel-purified and partially sequenced by automated methods. Based on the sequence obtained from the above two DNA fragments, the coding regions for the S and E subunits of D-ornithine aminomutase were amplified by PCR reaction using following pairs of oligonucleotides, 39/40 and 37/38, as primers and *Clostridial* genomic DNA as template. Amplification was achieved using 30 cycles at the following temperatures: 95 °C for 0.5 min, 50 °C for 1 min, and 72 °C for 4 min. Finally, the reaction was maintained at 72 °C for 5 min. The PCR products were gel-purified and sequenced on both strands using a PerkinElmer Life Sciences DNA autosequencer.

*Expression Construct*—The *oraS* gene was amplified by PCR using two primers, 39 and 40, which introduced *Nde*I and *Bam*HI sites at the 5′ and 3′ termini of the *oraS* gene, respectively. The PCR product was gel-purified using a Qiagen gel-extraction kit, restricted with *Nde*I and *Bam*HI, and ligated with *Nde*I/*Bam*HI-restricted pT7-7 vector. The ligation mixture was used to transform *E. coli* TG1 recO 1504::Tn5. The plasmid that carried the *oraS* gene in the correct orientation was designated poraSX. Similarly, the *oraE* gene was PCR-amplified using primers, 41 and 42, which introduced *Nco*I and *Bam*HI sites at the 5′and 3′ termini of the *oraE* gene, respectively. The PCR product was purified, restricted with *Nco*I and *Bam*HI, and ligated with *Nco*I/*Bam*HI-restricted pET-28a vector. The resulting plasmid containing the *oraE* gene was designated poraEX.

To facilitate the overexpression of the *ora* genes, poraSX and poraEX were used to transform *E. coli* BL21(DE3) codon plus. Cultures were grown at 37 °C by inoculating 5-ml overnight culture into 1 liter of LB medium containing the appropriate antibiotic, 100 mg/liter ampicillin for pOraSX or 30 mg/liter kanamycin for pOraEX. Incubation was continued until the culture reached an $A_{600}$ of between 0.8 and 1, at which point expression was induced by the addition of 200 mg/liter isopropyl-β-D-thiogalactopyranoside.

*Purification of OraS Protein*—All purification steps were performed on ice or at 4 °C. In a typical purification, 15 g of cells (wet weight) were resuspended in 50 ml of 50 mM potassium phosphate buffer, pH 7.0. The cells were ruptured in a total volume of 30 ml by sonication. Cell debris was removed by centrifugation at 25,000 × *g* for 15 min. The supernatant was filtrated through a 0.22-μm syringe filter and loaded onto a 2.6 × 20 cm SP-Sepharose HP cation-exchange column equilibrated with 10 mM potassium phosphate buffer, pH 7.0. Protein was then eluted with a 600-ml gradient ascending from 0 to 0.5 M KCl. The flow rate was 1 ml/min; 5.5-ml fractions were collected. Fractions containing OraS protein were pooled and concentrated to 7 ml by ultrafiltration in a stirred cell fitted with a PM-3 membrane. The concentrated protein solution was then applied to a Sephacryl-S200-HR gel filtration column (2.6 × 90 cm) equilibrated in 50 mM potassium phosphate buffer, pH 7.0. Fractions containing the major protein peak, which comprised pure OraS, were pooled and concentrated by ultrafiltration as described above. The protein solution was stored at −20 °C in the presence of 50% glycerol.

*Purification of OraE Protein from Inclusion Bodies*—OraE protein was expressed in an insoluble form at 37 °C. All purification steps were performed on ice or at 4 °C. In a typical purification, 15 g of cells (wet weight) were resuspended in 50 ml of 50 mM potassium phosphate buffer, pH 7.0, containing 100 mM NaCl, 1 mM DTT, and 1 mM EDTA. The cells were ruptured in a total volume of 30 ml by sonication. The insoluble fraction was collected by centrifugation at 25,000 × *g* for 15 min, and the supernatant was discarded. The pellet was washed twice with 25 ml of the same buffer supplemented with 0.5% Triton X-100 to remove lipophilic contaminants. Each wash was followed by centrifugation at 25,000 × *g* for 15 min. The pellet was solubilized in 25 ml of 0.1 M potassium phosphate buffer, pH 7.0, containing 6 M guanidine hydrochloride, 10 mM DTT, and 1 mM EDTA. The solution was stirred at room temperature for 2 h and cleared by centrifugation at 25,000 × *g* for 15 min. After this, the purity of the OraE was better than 90% as judged by SDS-PAGE. No steps were taken to further purify OraE before refolding. The supernatant was used as the stock solution for refolding experiments and stored at −70 °C. The protein concentration of OraE in the stock solution was estimated from a Coomassie Blue-stained 10% SDS-polyacrylamide gel.

*Refolding of OraE*—All steps were performed at 4 °C or on ice. About 3.8 mg of OraS in 2 ml of 50% glycerol and 9.1 mg of OraE in 3-ml stock solution were added dropwise to 100 ml of 0.1 M potassium phosphate buffer, pH 7.0, containing 1 M guanidine hydrochloride, 10% glycerol, and 1 mM DTT, with gentle stirring. After the solution was stirred for a further 20 min, about 400 μl of 12 mM PLP or 100 μl of 2 mM adenosylcobalamin was added. The solution was then dialyzed overnight against 3 × 2 liters of 0.1 M potassium phosphate buffer, pH 7.0, containing 10% glycerol and 1 mM DTT in the dark. The dialysate was then cleared by centrifugation at 25,000 × *g* for 15 min and concentrated to 20 ml by ultrafiltration in a stirred cell fitted with a YM 3 membrane. To remove excess OraS and other contaminating components, the refolded enzyme was then loaded onto a Q-Sepharose HP anion-exchange column (2.6 × 20 cm), equilibrated in 10 mM potassium phosphate buffer, pH 7.0, which contained 10% glycerol and 1 mM DTT. The column was first washed with 50 ml of the same buffer, and proteins were then eluted with a 500-ml linear gradient of 0–0.5 M KCl. The flow rate was 2 ml/min; 6-ml fractions were collected. Active fractions were pooled and concentrated to 8 ml by ultrafiltration as described above. The protein solution was stored at −20 °C in the presence of 50% glycerol.

*Mutant Construction*—The construction of mutant, *poraEX-H618G*, was carried out using recombinant PCR (16). Two overlapping, complementary oligonucleotides, 43 and 44, were designed to introduce the mutagenic sequence. A 1.9-kb and 300-bp region of the *oraE* gene was PCR-amplified using pmutEX as template and oligo-41/44 and -42/43 as primers. Both PCR products were gel-purified and assembled in a second-round PCR reaction using oligo-41 and -42 as primers and co-templates. The PCR product was purified, restricted with *Nco*I and *Bam*HI, and ligated with *Nco*I/*Bam*HI-restricted pET-28a vector. The resulting plasmid was designated poraEX-H618G. The procedures for expression and purification of the mutant protein were the same as that of the wild type. The mutant protein was refolded in the presence of OraS and PLP.

*Protein Concentration Determination*—The protein concentrations of purified OraS and refolded D-ornithine aminomutase were determined by Bradford assay using purified cobalamin-binding domain, glutamate mutase component S (MutS) as the standard (17).

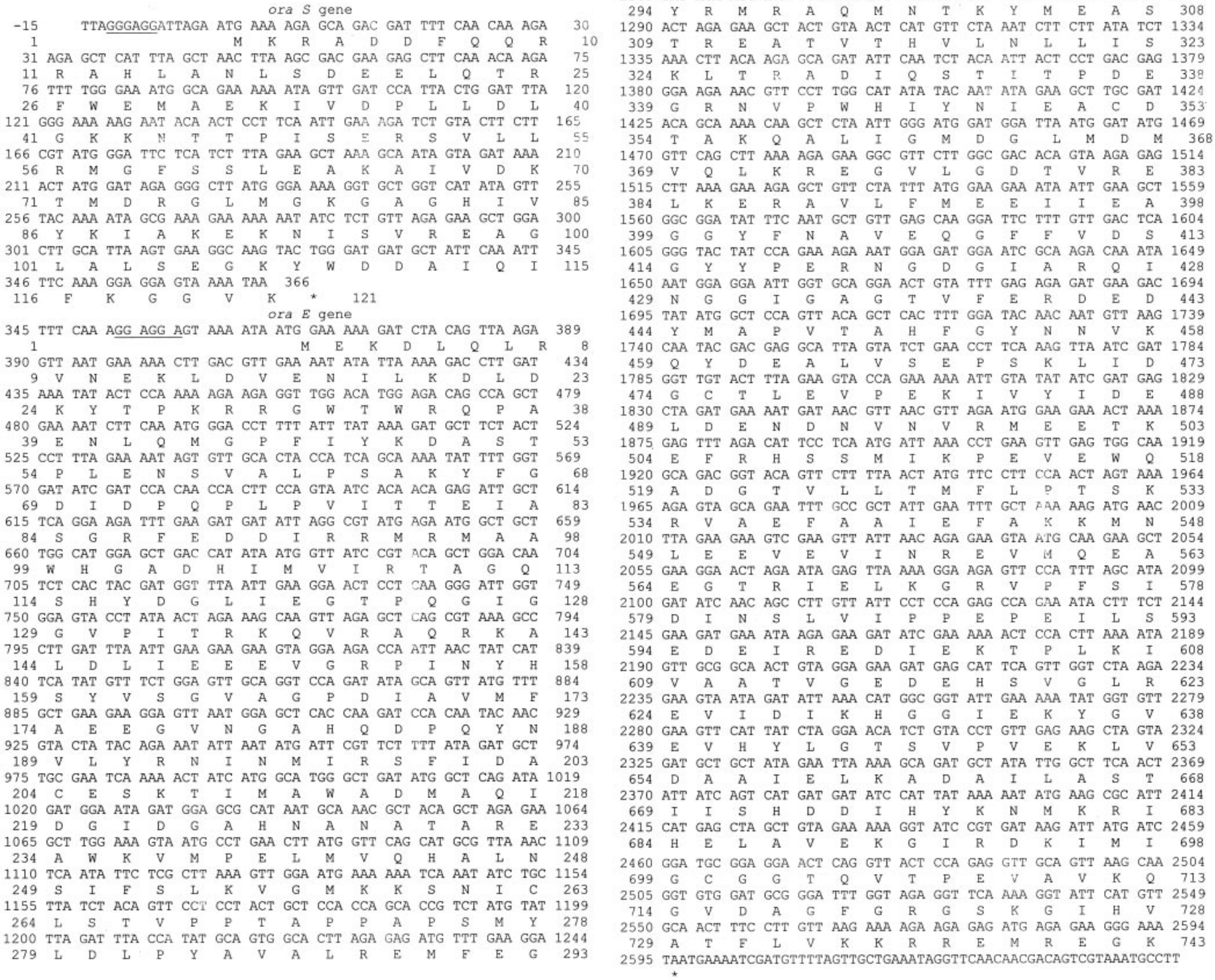
*Molecular Mass Determination*—The molecular mass of refolded recombinant D-ornithine aminomutase was estimated by gel filtration chromatography using a Amersham Pharmacia Biotech pre-packed Superdex 200 HR10/30 column equilibrated in 50 mM potassium phosphate buffer, pH 8.5, containing 150 mM NaCl. The analysis was performed on a Amersham Pharmacia Biotech AKTAbasic 10 system. The proteins were eluted isocratically with the same buffer at a flow rate of 0.5 ml/min. Calibration standards were (in daltons) blue dextran (2,000,000), thyroglobulin (669,000), apoferritin (443,000), β-amylase (200,000), glutamate mutase component E (107,000), and glutamate mutase component S (14,000).

*Enzyme Assay*—A rapid spectrophotometric method was used to assay D-ornithine aminomutase activity (18). The assay couples the formation of 2,4-diaminopentanoic acid to reduction of $NADP^+$ through the action of 2,4-diaminopentanoic acid dehydrogenase. The enzymatic activity was assayed by monitoring at 340 nm the production of NADPH. The assay solution was buffered by 50 mM Tris-Cl, pH 8.5. The reagents were added into the assay solution in the following order: coupling enzyme, 0.5 mM $NADP^+$, AdoCbl, PLP, and D-ornithine aminomutase. After mixing and incubating in the dark for 10 s, the reaction was initiated by addition of D-ornithine. After mixing by hand for about 10 s, the rate was steady for about 1.5 min. This assay was carried out at room temperature in dim light to protect the coenzymes. A computer program (Kalieda Graph, Abelbeck Software) was used to fit data to the Michaelis-Menten equation.

*UV-visible Spectrum*—For each measurement, 600-μl protein solutions containing 100 μM OraE or OraE-H618G and 250 μM OraS in 0.1 M Tris-Cl buffer, pH 8.5, were mixed with a 1.5-μl solution containing 2 mM adenosylcobinamide. After incubating in the dark for 2 min, spectra were recorded using a spectrophotometer (Hitachi U-3000).

## RESULTS

*Gene Cloning and Sequence Analysis*—From the N-terminal protein sequences obtained for components S and E, two oligonucleotides were designed to function as primers in a PCR experiment, the expectation being that both components would be transcribed as part of the same operon. Fortunately, this assumption proved correct. The S component of the enzyme is encoded upstream of the E component. The nucleotide sequence and deduced protein sequence of the *ora* genes are shown in Fig. 1. Although the stop codon of *oraS* and start



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                                         ora S gene
-15      TTAGGGAGGATTAGA ATG AAA AGA GCA GAC GAT TTT CAA CAA AGA   30
  1                        M   K   R   A   D   D   F   Q   Q   R    10
 31 AGA GCT CAT TTA GCT AAC TTA AGC GAC GAA GAG CTT CAA ACA AGA   75
 11  R   A   H   L   A   N   L   S   D   E   E   L   Q   T   R    25
 76 TTT TGG GAA ATG GCA GAA AAA ATA GTT GAT CCA TTA CTG GAT TTA  120
 26  F   W   E   M   A   E   K   I   V   D   P   L   L   D   L    40
121 GGG AAA AAG AAT ACA ACT CCT TCA ATT GAA AGA TCT GTA CTT CTT  165
 41  G   K   K   N   T   T   P   I   S   E   R   S   V   L   L    55
166 CGT ATG GGA TTC TCA TCT TTA GAA GCT AAA GCA ATA GTA GAT AAA  210
 56  R   M   G   F   S   S   L   E   A   K   A   I   V   D   K    70
211 ACT ATG GAT AGA GGG CTT ATG GGA AAA GGT GCT GGT CAT ATA GTT  255
 71  T   M   D   R   G   L   M   G   K   G   A   G   H   I   V    85
256 TAC AAA ATA GCG AAA GAA AAA AAT ATC TCT GTT AGA GAA GCT GGA  300
 86  Y   K   I   A   K   E   K   N   I   S   V   R   E   A   G   100
301 CTT GCA TTA AGT GAA GGC AAG TAC TGG GAT GAT GCT ATT CAA ATT  345
101  L   A   L   S   E   G   K   Y   W   D   D   A   I   Q   I   115
346 TTC AAA GGA GGA GTA AAA TAA  366
116   F   K   G   G   V   K   *    121
                                         ora E gene
345 TTT CAA AGG AGG AGT AAA ATA ATG GAA AAA GAT CTA CAG TTA AGA  389
  1                                  M   E   K   D   L   Q   L   R     8
390 GTT AAT GAA AAA CTT GAC GTT GAA AAT ATA TTA AAA GAC CTT GAT  434
  9  V   N   E   K   L   D   V   E   N   I   L   K   D   L   D    23
435 AAA TAT ACT CCA AAA AGA AGA GGT TGG ACA TGG AGA CAG CCA GCT  479
 24  K   Y   T   P   K   R   R   G   W   T   W   R   Q   P   A    38
480 GAA AAT CTT CAA ATG GGA CCT TTT ATT TAT AAA GAT GCT TCT ACT  524
 39  E   N   L   Q   M   G   P   F   I   Y   K   D   A   S   T    53
525 CCT TTA GAA AAT AGT GTT GCA CTA CCA TCA GCA AAA TAT TTT GGT  569
 54  P   L   E   N   S   V   A   L   P   S   A   K   Y   F   G    68
570 GAT ATC GAT CCA CAA CCA CTT CCA GTA ATC ACA ACA GAG ATT GCT  614
 69  D   I   D   P   Q   P   L   P   V   I   T   T   E   I   A    83
615 TCA GGA AGA TTT GAA GAT GAT ATT AGG CGT ATG AGA ATG GCT GCT  659
 84  S   G   R   F   E   D   D   I   R   R   M   R   M   A   A    98
660 TGG CAT GGA GCT GAC CAT ATA ATG GTT ATC CGT ACA GCT GGA CAA  704
 99  W   H   G   A   D   H   I   M   V   I   R   T   A   G   Q   113
705 TCT CAC TAC GAT GGT TTA ATT GAA GGA ACT CCT CAA GGG ATT GGT  749
114  S   H   Y   D   G   L   I   E   G   T   P   Q   G   I   G   128
750 GGA GTA CCT ATA ACT AGA AAG CAA GTT AGA GCT CAG CGT AAA GCC  794
129  G   V   P   I   T   R   K   Q   V   R   A   Q   R   K   A   143
795 CTT GAT TTA ATT GAA GAA GAA GTA GGA AGA CCA ATT AAC TAT CAT  839
144  L   D   L   I   E   E   E   V   G   R   P   I   N   Y   H   158
840 TCA TAT GTT TCT GGA GTT GCA GGT CCA GAT ATA GCA GTT ATG TTT  884
159  S   Y   V   S   G   V   A   G   P   D   I   A   V   M   F   173
885 GCT GAA GAA GGA GTT AAT GGA GCT CAC CAA GAT CCA CAA TAC AAC  929
174  A   E   E   G   V   N   G   A   H   Q   D   P   Q   Y   N   188
925 GTA CTA TAC AGA AAT ATT AAT ATG ATT CGT TCT TTT ATA GAT GCT  974
189  V   L   Y   R   N   I   N   M   I   R   S   F   I   D   A   203
975 TGC GAA TCA AAA ACT ATC ATG GCA TGG GCT GAT ATG GCT CAG ATA 1019
204  C   E   S   K   T   I   M   A   W   A   D   M   A   Q   I   218
1020 GAT GGA ATA GAT GGA GCG CAT AAT GCA AAC GCT ACA GCT AGA GAA 1064
219  D   G   I   D   G   A   H   N   A   N   A   T   A   R   E   233
1065 GCT TGG AAA GTA ATG CCT GAA CTT ATG GTT CAG CAT GCG TTA AAC 1109
234  A   W   K   V   M   P   E   L   M   V   Q   H   A   L   N   248
1110 TCA ATA TTC TCG CTT AAA GTT GGA ATG AAA AAA TCA AAT ATC TGC 1154
249  S   I   F   S   L   K   V   G   M   K   K   S   N   I   C   263
1155 TTA TCT ACA GTT CCT CCT ACT GCT CCA CCA GCA CCG TCT ATG TAT 1199
264  L   S   T   V   P   P   T   A   P   P   A   P   S   M   Y   278
1200 TTA GAT TTA CCA TAT GCA GTG GCA CTT AGA GAG ATG TTT GAA GGA 1244
279  L   D   L   P   Y   A   V   A   L   R   E   M   F   E   G   293
1245 TAT AGA ATG AGA GCA CAG ATG AAT ACA AAG TAT ATG GAA GCA TCT 1289
294  Y   R   M   R   A   Q   M   N   T   K   Y   M   E   A   S   308
1290 ACT AGA GAA GCT ACT GTA ACT CAT GTT CTA AAT CTT CTT ATA TCT 1334
309  T   R   E   A   T   V   T   H   V   L   N   L   L   I   S   323
1335 AAA CTT ACA AGA GCA GAT ATT CAA TCT ACA ATT ACT CCT GAC GAG 1379
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1380 GGA AGA AAC GTT CCT TGG CAT ATA TAC AAT ATA GAA GCT TGC GAT 1424
339  G   R   N   V   P   W   H   I   Y   N   I   E   A   C   D   353
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354  T   A   K   Q   A   L   I   G   M   D   G   L   M   D   M    368
1470 GTT CAG CTT AAA AGA GAA GGC GTT CTT GGC GAC ACA GTA AGA GAG 1514
369  V   Q   L   K   R   E   G   V   L   G   D   T   V   R   E   383
1515 CTT AAA GAA AGA GCT GTT CTA TTT ATG GAA GAA ATA ATT GAA GCT 1559
384  L   K   E   R   A   V   L   F   M   E   E   I   I   E   A   398
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459  Q   Y   D   E   A   L   V   S   E   P   S   K   L   I   D   473
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1830 CTA GAT GAA AAT GAT AAC GTT AAC GTT AGA ATG GAA GAA ACT AAA 1874
489  L   D   E   N   D   N   V   N   V   R   M   E   E   T   K   503
1875 GAG TTT AGA CAT TCC TCA ATG ATT AAA CCT GAA GTT GAG TGG CAA 1919
504  E   F   R   H   S   S   M   I   K   P   E   V   E   W   Q   518
1920 GCA GAC GGT ACA GTT CTT TTA ACT ATG TTC CTT CCA ACT AGT AAA 1964
519  A   D   G   T   V   L   L   T   M   F   L   P   T   S   K   533
1965 AGA GTA GCA GAA TTT GCC GCT ATT GAA TTT GCT AAA AAG ATG AAC 2009
534  R   V   A   E   F   A   A   I   E   F   A   K   K   M   N   548
2010 TTA GAA GAA GTC GAA GTT ATT AAC AGA GAA GTA ATG CAA GAA GCT 2054
549  L   E   E   V   E   V   I   N   R   E   V   M   Q   E   A   563
2055 GAA GGA ACT AGA ATA GAG TTA AAA GGA AGA GTT CCA TTT AGC ATA 2099
564  E   G   T   R   I   E   L   K   G   R   V   P   F   S   I   578
2100 GAT ATC AAC AGC CTT GTT ATT CCT CCA GAG CCA GAA ATA CTT TCT 2144
579  D   I   N   S   L   V   I   P   P   E   P   E   I   L   S  593
2145 GAA GAT GAA ATA AGA GAA GAT ATC GAA AAA ACT CCA CTT AAA ATA 2189
594  E   D   E   I   R   E   D   I   E   K   T   P   L   K   I   608
2190 GTT GCG GCA ACT GTA GGA GAA GAT GAG CAT TCA GTT GGT CTA AGA 2234
609  V   A   A   T   V   G   E   D   E   H   S   V   G   L   R   623
2235 GAA GTA ATA GAT ATT AAA CAT GGC GGT ATT GAA AAA TAT GGT GTT 2279
624  E   V   I   D   I   K   H   G   G   I   E   K   Y   G   V   638
2280 GAA GTT CAT TAT CTA GGA ACA TCT GTA CCT GTT GAG AAG CTA GTA 2324
639  E   V   H   Y   L   G   T   S   V   P   V   E   K   L   V  653
2325 GAT GCT GCT ATA GAA TTA AAA GCA GAT GCT ATA TTG GCT TCA ACT 2369
654  D   A   A   I   E   L   K   A   D   A   I   L   A   S   T   668
2370 ATT ATC AGT CAT GAT GAT ATC CAT TAT AAA AAT ATG AAG CGC ATT 2414
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684  H   E   L   A   V   E   K   G   I   R   D   K   I   M   I   698
2460 GGA TGC GGA GGA ACT CAG GTT ACT CCA GAG GTT GCA GTT AAG CAA 2504
699  G   C   G   G   T   Q   V   T   P   E   V   A   V   K   Q   713
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714  G   V   D   A   G   F   G   R   G   S   K   G   I   H   V   728
2550 GCA ACT TTC CTT GTT AAG AAA AGA AGA GAG ATG AGA GAA GGG AAA 2594
729  A   T   F   L   V   K   K   R   R   E   M   R   E   G   K   743
2595 TAATGAAAATCGATGTTTTAGTTGCTGAAATAGGTTCAACAACGACAGTCGTAAATGCCTT
      *
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FIG. 1. **The nucleotide and deduced protein sequence of the *ora* gene.** Potential ribosome binding sites are *underlined* in the DNA sequence.

codon of *oraE* are overlapped, a potential Shine-Dalgarno sequence is present upstream of both *oraS* and *oraE*. Downstream, *ora E* is followed by a gene, which encodes a protein of 59 amino acid residues with unknown function.

The *oraS* gene encodes a protein of 121 amino acid residues with $M_r$ 12,800, whereas the *oraE* gene encodes 753 amino acid residues with $M_r$ 82,900. The apparent molecular weight of recombinant D-ornithine aminomutase was determined to be about 201,000, which is in accord with previous results obtained from native enzyme (5). Therefore, the holoenzyme appears to be a $\alpha_2\beta_2$-heterotetramer. OraS shows no significant homology to other proteins in the Swiss-Prot data base. Comparison of the deduced amino acid sequence of *oraE* to those of known AdoCbl-dependent carbon-skeleton mutases and aminomutases reveals the presence of a conserved cobalamin-binding domain at the C terminus of OraE protein. Notably, the appearance of the conserved $^{614}$G*X*D*X*H*XX*G$^{621}$ motif in the middle of the cobalamin-binding domain suggests that D-ornithine aminomutase binds AdoCbl in a base-off/histidine-on mode. In addition, the gene sequence of *oraE*, perhaps unsurprisingly, has a high sequence similarity to that of *kamD* and *kamE*. The deduced amino acid sequence of *oraE* showed 28% identity and 39% similarity to *kamD* and 35% identity and 47% similarity to *kamE* (Fig. 2). It is interesting to note that the E component has excess Glu and Asp relative to Arg, Lys, and His, whereas the S component has excess Arg, Lys, and His relative to Glu and Asp.

*Expression and Purification of OraS*—The protocol described under "Experimental Procedures" gave good expression of OraS. Approximately 20 mg of purified protein was obtained per liter of culture. We developed a new purification based on chromatography on SP-Sepharose HP cation-exchange matrix. After disruption of cells by sonication, the crude cell extract was directly loaded onto a column of ion-exchange matrix. A large peak of nucleic acids and contaminating proteins eluted first followed by a smaller peak containing many other proteins. OraS eluted next in a well-resolved broad peak; this step resulted in protein that was nearly homogeneous (Fig. 3*A*). Finally, the minor contaminating component was removed by use of a Sephacryl S-200-HR gel filtration column (2.6 × 90 cm). The purification work of OraS was summarized in Table II. This method of preparation proved very reproducible, and OraS could be stored in a concentrated solution in the presence of 50% glycerol at −20 °C.

*Expression of OraE*—In contrast to OraS, our efforts to express OraE protein in *E. coli* were frustrated. The *oraE* gene

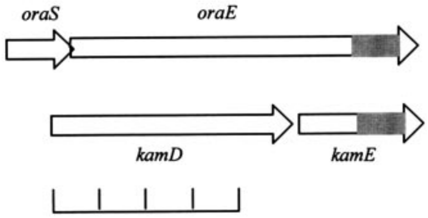

FIG. 2. **The relative positions on the chromosome of *C. sticklandii* of the genes for AdoCbl-dependent lysine aminomutase (KamDE) and D-ornithine aminomutase (OraSE).** The deduced amino acid sequence of *oraE* showed 28% identity and 39% similarity to *kamD*, and 35% identity and 47% similarity to *kamE*. The $\sim$100-residue region that encompasses the conserved cobalamin-binding domain in each enzyme is *shaded*. Each *line* on the *scale bar* represents 100 amino acid residues.

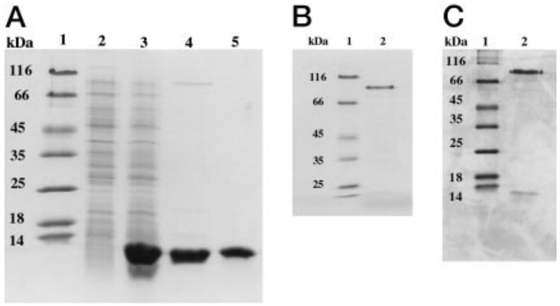

FIG. 3. **Shown are the results from SDS-PAGE analysis of samples taken after each step in the purification of the recombinant enzyme (gel stained with Coomassie Brilliant Blue).** *A*, purification of OraS (20% gel). *Lane 1*, maker; *lane 2*, crude cell extract before IPTG induction; *lane 3*, crude cell extract after IPTG induction; *lane 4*, pooled fractions after SP-Sepharose HP ion-exchange chromatography; *lane 5*, pooled fractions after Sephacryl S-200-HR gel filtration chromatography. *B*, purification of OraE from inclusion bodies (10% gel). *Lane 1*, marker; *lane 2*, insoluble protein isolated from induced cells. *C*, purification of refolded recombinant D-ornithine aminomutase (20% gel). *Lane 1*, marker; *lane 2*, pooled fractions after Q-Sepharose HP ion-exchange chromatography.

TABLE II
*Purification of OraS protein*

| Purification step | Protein concentration | Volume | Protein |
|---|---|---|---|
| | *mg/ml* | *ml* | *mg* |
| Crude extract | 9.06 | 25 | 226.5 |
| SP-Sepharose HP | 0.86 | 54 | 46.53 |
| Sephacryl S-200 | 0.79 | 50 | 39.45 |

was first subcloned into the pBluescript SK vector. The gene was placed under the control of the *lac* uv5 promoter. The resulting construct was used to transform *E. coli* TG1 recO 1504::Tn5. The expression of the gene was induced by addition of IPTG into exponentially growing cultures. The molecular weight of overexpressed protein was estimated to be about 78,000, a little lower than the molecular weight of the native enzyme from *C. sticklandii* as judged by SDS-PAGE. The expressed protein was exclusively found in the soluble fraction in the crude cell extract. However, the purified truncated OraE was inactive and unable to bind AdoCbl (data not shown). We therefore decided to seek another system to express the OraE protein.

The second system we used to express the OraE protein was the well-known pET vector. Two oligonucleotides were designed to amplify the coding sequence of the *oraE* gene in a PCR experiment and to incorporate a *Nco*I site at the start of the gene. This was to facilitate the subcloning of *oraE* gene into the expression vector, pET-28a, where it would be under the control of the bacteriophage T7 promoter. To obtain expression of OraE, the resulting construct, poraEX, was used to transform *E. coli* BL21(DE3) codon plus, which contains bacteriophage T7 RNA polymerase under the control of the *lac* uv5 promoter. The expression conditions were as described under "Experimental Procedures." The molecular weight of the expressed protein was the same as that of native enzyme, as judged by SDS-PAGE. However, after disrupting cells, the majority of OraE protein was found in the insoluble fraction. Varying the induction temperature and inducer concentration had little effect on the solubility of OraE (data not shown). Therefore, a procedure for purification of recombinant OraE was developed based on the protocols of Marston for the purification of proteins from inclusion bodies (19). The purified OraE dissolved in 6 M guanidine hydrochloride, 10 mM dithiothreitol, and 1 mM EDTA was nearly homogenous, as shown on by SDS-PAGE (Fig. 3*B*).

*Refolding and Purification of OraE*—In the absence of OraS, any attempt to reduce the occurrence of excessive precipitation of OraE during refolding was unsuccessful. This result suggests that the interaction between OraS and OraE plays an important role in the refolding process. Therefore, a molar excess of approximately 3-fold of OraS protein was included in the refolding experiment with OraE. We also found that, in the absence of AdoCbl and pyridoxal phosphate, the proteins demonstrated a tendency to precipitate irreversibly during refolding and subsequent concentration by ultrafiltration. The addition of neither 50% glycerol nor non-ionic detergents during ultrafiltration improved solubility. However, no precipitation was observed when either 200 nM AdoCbi or 4 $\mu$M pyridoxal phosphate was included in the dialysate during refolding and subsequent concentration. This result indicates that the binding of either AdoCbl or pyridoxal phosphate to the protein's corresponding motif might facilitate the refolding process and induce the protein to adopt a more stable conformation. Finally, minor contaminating proteins and excess OraS were removed by a Q-Sepharose HP anion-exchange column (Fig. 3*C*). Excess OraS protein eluted at the beginning of the gradient and refolded D-ornithine aminomutase eluted at the middle of the gradient in a well-resolved peak. The purification work of refolded recombinant D-ornithine aminomutase was summarized in Table III.

*Kinetic Analysis*—According to a previous report, the activity of D-ornithine aminomutase prepared from clostridia is not absolutely dependent upon PLP and AdoCbl (5). Therefore, accurate kinetic measurements are not feasible using the native enzyme from clostridia. The overexpression of OraE and OraS in *E. coli* has allowed protein to be obtained completely free of inhibiting cobamides that were always present in preparations from clostridia (14, 20–23). In addition, OraE was expressed in the form of inclusion bodies, which resulted in the absence of endogenous pyridoxal phosphate in refolded OraE. These properties of the recombinant enzyme have enabled us to make kinetic measurements on highly active pure enzyme.

The steady-state kinetic properties of the enzyme were investigated. The reaction was absolutely dependent upon OraE, OraS, AdoCbl, PLP, and D-ornithine being present in the assay; no other cofactors were required. The $K_m$ for AdoCbl was measured by using proteins that were refolded in the presence of PLP, and the $K_m$ for PLP by using proteins that were refolded in the presence of AdoCbl. Computer analysis gave $K_m$ values for D-ornithine, AdoCbl, and PLP of $44.5 \pm 2.8$, $0.43 \pm 0.04$, and $1.5 \pm 0.1$ $\mu$M, respectively; the $k_{\text{cat}}$ was $6.3 \pm 0.1$ s$^{-1}$ (Fig. 4). It is noteworthy that the activity measured is not affected by the addition of extra OraS protein to the assay solution.

*UV-visible Spectra and AdoCbl-binding Mode*—The analog of AdoCbl, adenosylcobinamide (AdoCbi), which lacks the nucleotide loop, was used to examine the AdoCbl-binding mode of

TABLE III

*Purification of refolded* D*-ornithine aminomutase*

Enzyme activity was determined in the presence of 25 μM AdoCbl, 0.5 mM D-ornithine, and 40 μM PLP. One unit of enzyme was that amount that catalyzed the formation of 1 μmol of 2,4-diaminopentanoic acid per minute in the assay.

| Purification step | Total protein | Total activity | Specific activity | Purification |
|---|---|---|---|---|
| | *mg* | *unit* | *unit/mg* | *-fold* |
| Refolded dialysate | 12.97 | 12.9 | 0.99 | 1 |
| Q-Sepharose HP | 2.01 | 6.9 | 3.43 | 3.46 |

the enzyme (24). When wild type apoenzyme was reconstituted with AdoCbi at pH 8.5, the absorption maximum of the corrinoid's UV-visible spectrum shifted from 458 to 525 nm, suggesting that the coordination state of the cobalt atom in AdoCbi was altered from five ligands to six ligands. However, no significant change was observed when AdoCbi was incubated with mutant OraE-H618G (Fig. 5). The resulting spectra demonstrate that D-ornithine aminomutase uses histidine 618 as a lower axial ligand to coordinate the cobalt atom. Therefore, we confirmed that AdoCbl is bound by D-ornithine aminomutase in the "base-off" mode, consistent with the results of sequence comparison.

## DISCUSSION

We have cloned, sequenced, and overexpressed the genes encoding D-ornithine aminomutase from *C. sticklandii*. The cloning and sequencing of the *oraS* and *oraE* genes will enable the enzyme's mechanism to be investigated in detail by site-directed mutagenesis techniques. In addition, overexpression of both genes in *E. coli* has both simplified the protein purification work and allowed the enzyme to be prepared in quantities sufficient for future studies.

The reactions catalyzed by AdoCbl-dependent D-lysine and D-ornithine aminomutase from *C. sticklandii* are strikingly similar, and comparison of the deduced amino acid sequences of *kamDE* and *oraE* genes is very informative. According to previous reports, KamDE itself, even in the absence of the small subunit, is sufficient for aminomutase catalysis (14). As shown in Fig. 2, the polypeptide chain lengths of OraE (743 residues) and the combined KamD and KamE protein (778 residues) are very close, and exhibit high sequence similarity. Therefore, one might expect that OraE alone would be catalytically active. However, we cannot obtain pure OraE in its native form to examine its catalytic properties.

The genes encoding D-ornithine aminomutase, *oraE* and *oraS*, are adjacent on the clostridial chromosome. They share overlapping start and stop codons, which might lead to transcription coupling so as to produce equal amounts of the two proteins. The formation of the $\alpha_2\beta_2$-heterotetramer complex seems quite stable, which results in the observation that the activity of D-ornithine aminomutase is not affected by addition of extra OraS. In addition, it elutes as a single well-resolved peak from a Superdex 200 HR 10/30 gel filtration column. The molecular weight of the enzyme is about 201,000, which is consistent with the molar ratio of OraE to OraS being 1:1.

As described above, truncated but inactive OraE is expressed in a soluble form. This result suggests that proper folding of the C-terminal part of OraE is crucial to its solubility and activity. Because the cobalamin-binding domain is located in this region, we suggest that inclusion of AdoCbl or PLP during refolding might facilitate the correct folding of the cobalamin-binding domain. It is interesting to note that, without the presence of OraS, OraE is unable to be refolded properly. However, the role of OraS in the D-ornithine aminomutase reaction remains obscure. Although reconstitution of OraS with KamDE might shed some light on the role of OraS in catalysis, some clues might be provided by recent crystallographic studies of diol dehydratase. In diol dehydratase, the cobalamin molecule is bound between the α and β subunits, and the substrate-binding site is located at the α subunit. Another small subunit, γ, doesn't make any contacts with the substrate or AdoCbl but is tightly associated with the α subunit. It seems likely that the role of the small subunit γ is to stabilize the catalytic subunit, α (25).

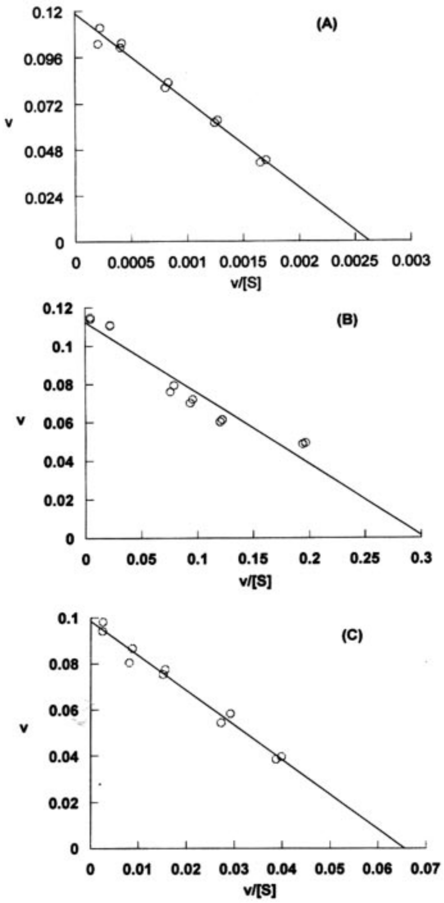

FIG. 4. **The Eadie-Hofstee plot of kinetic data for OraSE.** *A*, determination of apparent $K_m$ for D-ornithine. The concentrations of D-ornithine were 500, 250, 100, 50, and 25 μM. The assays were performed in the presence of 25 μM AdoCbl and 120 μM PLP. *B*, determination of apparent $K_m$ for AdoCbl. The concentrations of AdoCbl were 25, 5, 1, 0.75, 0.5, and 0.25 μM. The assays were performed in the presence of 0.5 mM D-ornithine and 120 μM PLP. *C*, determination of apparent $K_m$ of PLP. The concentrations of PLP were 40, 10, 5, 2, and 1 μM. The assays were performed in the presence of 0.5 mM D-ornithine and 25 μM AdoCbl.

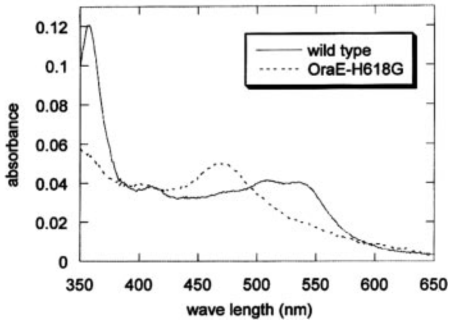

FIG. 5. **UV-visible absorption spectra of enzyme-bound AdoCbi.** *A*, wild type; *B*, mutant OraE-H618G.

The replacement of the lower axial ligand to cobalt by a conserved histidine residue in one class of $B_{12}$-dependent enzymes is well-documented (26). From the UV-visible spectrum of the enzyme-AdoCbi complex, we confirmed that D-ornithine aminomutase binds AdoCbl in the same way. No known PLP binding motif appears in OraE or KamDE. A lysine residue is thought to involve in PLP binding through a Schiff base linkage. Except for the cobalamin-binding domain, two lysine residues, $Lys^{303}$ and $Lys^{372}$, are conserved between OraE and KamDE, which might participate in the binding of PLP. To identify the PLP-binding site in D-ornithine aminomutase, further experiments are now in progress in our group. The consensus P-loop sequence (AVLNTGKT) and three-cysteine motif (CNYCSGLC) present in KamDE don't appear in OraE or OraS.

Although OraE protein is expressed in the form of inclusion bodies, this allows it to be obtained free of endogenous corrinoids and PLP and, thus, facilitates accurate kinetic characterization of the enzyme. This allowed us to measure the $K_m$ for AdoCbl of D-ornithine aminomutase, which has not been reported before. The value of $K_m$ for PLP we measured by coupled assay is about five times higher than that measured with the native enzyme by the same method (27). The proposed 1,2-rearrangement mechanisms of acyl-CoA mutases and aminomutases are quite similar. Both of them are thought to proceed through a cyclic transition state (12, 13, 28). Partly due to the involvement of two different coenzymes, or the different chemical nature of the migrating group, the turnover rates of aminomutases are lower than those of acyl-CoA mutases (29). In this paper, we report the initial characterization of D-ornithine aminomutase from *C. sticklandii*. Future work in our group will focus on the determination of the quaternary structure of the holoenzyme and catalytic mechanism of this 1,2-rearrangement reaction.

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