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Expression, crystallization and preliminary X-ray crystallographic study of ethanolamine ammonia-lyase from *Escherichia coli*

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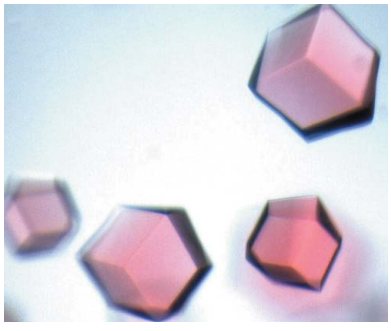


Ethanolamine ammonia-lyase (EAL) catalyzes the adenosylcobalamin-dependent conversion of ethanolamine to acetaldehyde and ammonia. The wild-type enzyme shows a very low solubility. N-terminal truncation of the *Escherichia coli* EAL β-subunit dramatically increases the solubility of the enzyme without altering its catalytic properties. Two deletion mutants of the enzyme [EAL($\beta\Delta$4–30) and EAL($\beta\Delta$4–43)] have been overexpressed, purified and crystallized using the sitting-drop vapour-diffusion method. Crystals of EAL($\beta\Delta$4–30) and EAL($\beta\Delta$4–43) diffracted to approximately 8.0 and 2.1 Å resolution, respectively.

1. Introduction

Ethanolamine ammonia-lyase (EAL) catalyses the formation of acetaldehyde and ammonia from 2-aminoethanol or of ammonia and propionaldehyde from 2-amino-1-propanol (Bradbeer, 1965; Carty *et al.*, 1974). The reaction is initiated by cleavage of the cobalt–carbon bond of adenosylcobalamin (AdoCbl) to form the cob(II)alamin–5′-deoxyadenosyl radical pair (Toraya, 2003; Brown, 2005; Banerjee, 1999). The 5′-deoxyadenosyl radical abstracts an H atom from the C1 C atom of the substrate to form a substrate radical, followed by the migration of an amino group from C2 to C1 of the substrate. AdoCbl-dependent enzymes accelerate the cleavage rate of the cobalt–carbon bond by $>10^{11}$ compared with AdoCbl in solution. EAL is composed of six pairs of α-subunits (EutB protein; M_r = 49 000) and β-subunits (EutC protein; M_r = 31 000) and its total molecular weight is ∼480 000 (Faust & Babior, 1992; Akita *et al.*, 2010; Wallis *et al.*, 1979; McRee, 1999). At present, no X-ray structure of complete EAL is available, although the structure of the EutB homohexamer (α_6) from *Listeria monocytogenes* 4b F2365 has recently been released in the Protein Data Bank (PDB code 2qez; Joint Center for Structural Genomics, unpublished data). This α-only structure provides little information about the functional aspects of the enzyme since neither the β-subunit of the enzyme nor cobalamin is included in the structure. Purified wild-type EAL from *Escherichia coli* has been shown to form inactive precipitates at high concentrations, but recently we found that N-terminal truncations of the β-subunit with a Cys34Ser mutation dramatically improved its solubility as well as its stability (Akita *et al.*, 2010). In order to obtain the complete structure including both subunits, cobalamin and substrate, we performed the expression, purification and crystallization of N-terminally truncated mutants of EAL and preliminary crystallographic data analysis of EAL crystals. The aim of the structural work on EAL is to investigate how the enzyme activates the cobalt–carbon bond of AdoCbl and catalyses the reaction by a radical mechanism.

2. Experimental procedures

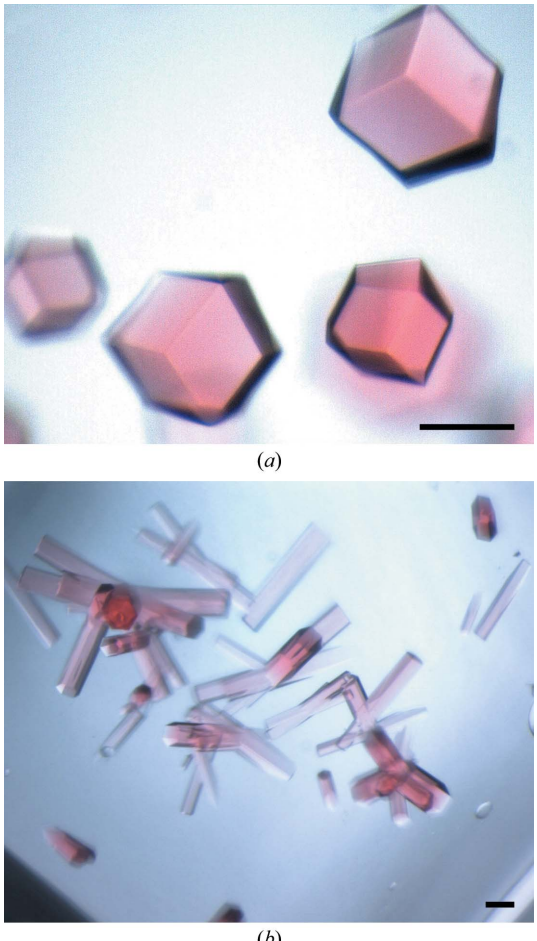
2.1. Construction of expression plasmids

The expression plasmid for EAL($\Delta\beta$4–30), which consists of the α-subunit and a His_6-tagged β-subunit lacking residues Lysβ4–Alaβ30, was constructed from pUSI2ENd(EAL) (Akita *et al.*, 2010), an expression plasmid for wild-type EAL from *E. coli*. Almost complete pUSI2ENd(EAL) lacking the region encoding Lysβ4–

Alaβ30 was amplified by PCR using *PfuTurbo* DNA polymerase (Agilent Technologies/Stratagene) and the 5′-phosphorylated primers b-4-30d6H_f (5′-**CATCATCATCATCATCAC**ACCACCAACTGTGCGGCACCGGTGACC-3′) and b-4-30d_r (5′-**GCTGCT**TTGATCCATGATATGTTATCTCCGCGTCATCAGAAGAAC-3′), where the inserted 6×His tag and linker sequence are indicated in bold. The resulting blunt-ended PCR product was self-ligated with Ligation High premixed T4 DNA Ligase reagent (Toyobo) to generate a circular plasmid, pUSI2ENd[EAL($\Delta\beta$4–30)]. pUSI2ENd[EAL($\Delta\beta$4–43)], which generates the Lysβ4–Cysβ43 deletion mutant, was obtained from pUSI2ENd[EAL($\Delta\beta$4–30)] using the QuikChange Multi Site-Directed Mutagenesis kit (Agilent Technologies/Stratagene) with the 5′-phosphorylated primer b-43d (5′-CAAAGCAGCCATCATCATCATCACGCGCTGGATTTAGGTTCCGCTGAAGCA-3′). Each plasmid was transformed into *E. coli* JM109 cells.

2.2. Expression and purification

The cells were grown at 303 K in 6 l LB medium containing 100 mg l^{-1} ampicillin. When the $OD_{600\,nm}$ reached 0.6, expression was induced by the addition of isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.5 m*M* and was then continued for 5 h at 303 K. The cells were harvested by centrifugation and suspended in a buffer solution containing 50 m*M* potassium phosphate pH 8.0, 20 m*M* imidazole, 10 m*M* ethanolamine pH 8.0, 5 m*M* 2-mercaptoethanol and 1 m*M* phenylmethanesulfonyl fluoride and disrupted by sonication. Cell lysates were centrifuged at 27 000*g* and applied onto an Ni–NTA agarose (Qiagen) column which had been equilibrated with the same buffer. The column was washed with five bed volumes of the buffer containing 40 m*M* imidazole and the bound protein was eluted by increasing the imidazole concentration in the buffer to 250 m*M*. The eluted protein was concentrated to ∼3 ml using an Amicon stirred pressure cell (Millipore) with a disc membrane (10 kDa cutoff). Cyanocobalamin powder (Sigma) was added to the protein solution to a final concentration of 2 m*M*, which was followed by incubation at 303 K for 30 min. Cyanocobalamin-bound enzyme was further purified and buffer-exchanged by size-exclusion chromatography on a Sephacryl SS-500 column (60 × 2.6 cm) which had been equilibrated with the final buffer [10 m*M* Tris–HCl buffer pH 8.0, 200 m*M* KCl, 10 m*M* ethanolamine, 1 m*M* dithiothreitol and 20 µ*M* cyanocobalamin]. Fractions containing the enzyme were pooled and concentrated to 20 mg ml^{-1} for crystallization.



(*a*)

(*b*)

Figure 1
Crystals of EAL($\beta\Delta$4–30) (*a*) and EAL($\beta\Delta$4–43) (*b*). The scale bar corresponds to 100 µm.

Table 1
Data-collection statistics.

Values in parentheses are for the outer shell.

	EAL($\beta\Delta$4–30)	EAL($\beta\Delta$4–43)
Beamline	Photon Factory, BL-17A	SPring-8, BL38B1
Wavelength (Å)	1.0000	1.0000
Space group	*R*3	$P6_3$
Unit-cell parameters (Å)	$a = b = 326$, $c = 200$	$a = b = 242.76$, $c = 76.46$
Resolution (Å)	50.00–8.00 (8.28–8.00)	50–2.10 (2.15–2.10)
No. of observations	29938	988418
No. of unique reflections	8263 (846)	145836 (9228)
Completeness (%)	99.7 (100.0)	97.2 (93.1)
R_{merge}†	0.102 (0.792)	0.096 (0. 413)
Multiplicity	3.6 (3.9)	6.8 (6.3)
$\langle I/\sigma(I)\rangle$	39.0 (1.66)	17.9 (4.35)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th observation.

2.3. Crystallization

All crystallization trials of EAL($\beta\Delta$4–30) and EAL($\beta\Delta$4–43) were performed without proteolytic cleavage of the His tag. EAL($\beta\Delta$4–30) and EAL($\beta\Delta$4–43) were initially screened at 277 K using the commercially available sparse-matrix kits Crystal Screens 1 and 2, Crystal Screen Lite, PEG/Ion and PEG/Ion 2 from Hampton Research and Wizards I, II and III and Precipitant Synergy Expanded 192 from Emerald BioSystems. 2 µl protein solution and an equal volume of reservoir solution were mixed on a Greiner 96-well CrystalQuick sitting-drop plate (Greiner Bio-One, Germany) and equilibrated against 100 µl well solution. Crystallization hits were obtained for EAL($\beta\Delta$4–30) using Precipitant Synergy (33% concentration) solution No. 28 and were obtained for EAL($\beta\Delta$4–43) using Crystal Screen Lite solution No. 41 and Precipitant Synergy (33% concentration) solution No. 51. The final optimized well solution for these initial hits were as follows: 6.0%(*v*/*v*) MPD (2-methyl-2,4-pentanediol), 3.0%(*w*/*v*) PEG 8000, 0.1 *M* calcium chloride and 0.1 *M* sodium acetate pH 6.0 for EAL($\beta\Delta$4–30) and 6.0–7.0%(*w*/*v*) PEG 6000, 24–26%(*v*/*v*) glycerol, 5.0%(*v*/*v*) 2-propanol and 0.1 *M* HEPES pH 7.0 or 6.0–7.0%(*w*/*v*) PEG 4000, 24–26%(*v*/*v*) glycerol, 1.0%(*v*/*v*) MPD and 0.1 *M* imidazole pH 6.3 for EAL($\beta\Delta$4–43).

2.4. Data collection and crystallographic analysis

The EAL($\beta\Delta$4–30) crystals were soaked in mother liquor containing 30% glycerol as a cryoprotectant for a few seconds and then flash-cooled in a nitrogen stream at 100 K. The EAL($\beta\Delta$4–43) crys-

tals were picked up from the protein drop with a cryoloop and directly flash-cooled. Diffraction images for the EAL($\beta\Delta$4–30) crystals were collected on the BL-17A beamline at the Photon Factory (Tsukuba, Japan) using an ADSC Q270 CCD detector system (camera distance 388.0 mm) and those for the EAL($\beta\Delta$4–43) crystals were collected on the BL38B1 beamline at SPring-8 using a Rigaku Jupiter CCD detector system (camera distance 200.0 mm). 120° of data were collected from a single crystal with oscillation ranges of 1.0° for EAL($\beta\Delta$4–30) and 0.3° for EAL($\beta\Delta$4–43). Diffraction data sets were indexed, integrated and scaled with the *HKL*-2000 program suite (Otwinowski & Minor, 1997). Details of the diffraction experiments are summarized in Table 1.

3. Results

The crystals of EAL($\beta\Delta$4–30) grew to typical dimensions of 0.15 × 0.15 × 0.15 mm (Fig. 1*a*). These crystals only diffracted to 8.0 Å resolution under cryogenic conditions using synchrotron radiation. The EAL($\beta\Delta$4–30) crystals belonged to the rhombohedral space group *R*3, with unit-cell parameters $a = b = 326$, $c = 200$ Å (Table 1). This low resolution might arise from the osmotic shock caused by soaking in cryoprotectant solution or from cryocooling. A diffraction experiment at a noncryogenic temperature is needed to inspect the quality of the original crystal.

The EAL($\beta\Delta$4–43) crystals grew as hexagonal rods with typical dimensions of 0.1 × 0.1 × 0.3 mm (Fig. 1*b*). This type of crystal diffracted better to a resolution of up to 2.1 Å. The EAL($\beta\Delta$4–43) crystals belonged to the hexagonal space group $P6_3$, with unit-cell parameters $a = b = 242.76$, $c = 76.46$ Å (Table 1). A Matthews coefficient (Matthews, 1968) calculation showed that the EAL($\beta\Delta$4–43) crystals could contain 2–4 copies of the $\alpha\beta$ unit per asymmetric unit. Structure determination by the molecular-replacement method was initiated using the α-subunit of *L. monocytogenes* 4b F2365 EAL (PDB code 2qez), which shows 75% sequence identity to *E. coli* EAL, as the initial structural model. The *Phaser* program (McCoy *et al.*, 2007) found a clear solution containing two copies of the $\alpha\beta$ unit per asymmetric unit, resulting in a Matthews coefficient V_M of 4.09 Å^3 Da^{-1}, which corresponds to a solvent content of 70%. Initial electron-density maps based on the molecular-replacement solution were sufficiently clear for model building of the α-subunits but not of the β-subunits. To construct an overall model, structure determination using the anomalous diffraction method is currently under way.

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References

Akita, K., Hieda, N., Baba, N., Kawaguchi, S., Sakamoto, H., Nakanishi, Y., Yamanishi, M., Mori, K. & Toraya, T. (2010). *J. Biochem.* **147**, 83–93.
Banerjee, R. (1999). Editor. *Chemistry and Biochemistry of B_{12}*. New York: John Wiley & Sons.
Bradbeer, C. (1965). *J. Biol. Chem.* **240**, 4675–4681.
Brown, K. L. (2005). *Chem. Rev.* **105**, 2075–2149.
Carty, T. J., Babior, B. M. & Abeles, R. H. (1974). *J. Biol. Chem.* **249**, 1683–1688.
Faust, L. P. & Babior, B. M. (1992). *Arch. Biochem. Biophys.* **294**, 50–54.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
McRee, D. E. (1999). *J. Struct. Biol.* **125**, 156–165.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Toraya, T. (2003). *Chem. Rev.* **103**, 2095–2127.
Wallis, O. C., Johnson, A. W. & Lappert, M. F. (1979). *FEBS Lett.* **97**, 196–199.