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# Crystallization and preliminary X-ray analysis of Aes, an acetyl-esterase from *Escherichia coli*

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Aes belongs to the family of hormone-sensitive lipases and has acetyl-esterase activity. It is also known to control maltose uptake through interaction with MalT, the central regulator of the *Escherichia coli* maltose system. Aes was crystallized as an N-terminally $His_6$-tagged protein both in the native form and with selenomethionine substitution. Crystals grew in both cases in space group *R*32 to dimensions of about 0.2 × 0.15 × 0.05 mm (native $His_6$-Aes) and about 0.5 × 0.3 × 0.1 mm (SeMet-$His_6$-Aes). A native data set has been obtained at 2.4 Å resolution; the selenomethionine-substituted Aes crystals diffracted to 3.0 Å resolution.

## 1. Introduction

Aes, a soluble 37 kDa protein consisting of 319 amino-acid residues, is an acetyl-esterase from *Escherichia coli*. It belongs to the family of hormone-sensitive lipases (HSL; Hemila *et al.*, 1994; Peist *et al.*, 1997; Kanaya *et al.*, 1998). The regulatory domain of members of the HSL family is the G*X*S*X*G motif containing the active-site serine residue (Yeaman, 1990; Hemila *et al.*, 1994; Osterlund, 2001). In the case of Aes, Gly163, Asp164, Ser165 and Gly167 are the components of the G*X*S*X*G motif, with Ser165, Asp262 and His292 as the catalytic triad (Kanaya *et al.*, 1998) and Asp164 as yet another critical residue for enzymatic activity (Haruki *et al.*, 1999).

In addition to its function as an esterase, Aes acts as a down-regulator of MalT, the central transcriptional activator of the *E. coli* maltose regulon (Boos & Shuman, 1998; Joly *et al.*, 2002). It was recently demonstrated that Aes down-regulates MalT activity through direct protein–protein interaction *in vivo* (Joly *et al.*, 2002). MalT is the transcriptional activator of the maltose regulon from *E. coli* and is the prototype of a new family of transcription factors (Valdez *et al.*, 1999). Its activity is controlled by multiple regulatory signals. ATP and maltotriose are two effectors of the activator that positively control multimerization of MalT, a critical step in promoter binding (Boos & Shuman, 1998). It was observed that Aes competes with maltotriose for MalT binding (Joly *et al.*, 2002). The binding site for Aes is located in the DT1 region, the N-terminal domain of MalT, which also contains the ATP-binding site. MalK, the ABC component of the maltodextrin-transport system, and MalY are already known to down-regulate MalT activity *in vivo* (Schreiber & Richet, 1999). Recent studies indicate that Aes and MalY have similar modes of interaction (Joly *et al.*, 2002).

The crystal structures of three bacterial proteins that are members of the mammalian hormone-sensitive lipase (HSL) family have already been determined by X-ray analysis. Wei *et al.* (1999) solved the structure of brefeldin A esterase (BFAE) of *Bacillus subtilis* at 2.5 Å resolution (PDB code 1jkm). BFAE is a homologue of the mammalian hormone-sensitive lipase (HSL) and reveals an amino-acid sequence identity of 25.3% to Aes in a 245-amino-acid overlap (LALIGN server). The second structure of a member of the HSL family which was solved was EST2, a thermophilic carboxylesterase from *Alicyclobacillus acidocaldarius*, at 2.6 Å resolution (PDB code 1evq; De Simone *et al.*, 2000). Sequence comparison of Aes and EST2 shows an amino-acid sequence identity of 29.3% in a 232-amino-acid overlap (LALIGN server). The third structure known is that of AFEST, a carboxylesterase from *Archaeoglobus fulgidus* at 2.2 Å resolution (PDB code 1jji; De Simone *et al.*, 2001), which shares an amino-acid sequence identity of 31.3% with Aes in a 252-amino-acid overlap (LALIGN server).

We have expressed, purified and crystallized Aes as an N-terminally $His_6$-tagged protein in the space group *R*32. Using the selenomethionine-substitution method (Doublié, 1997), we were able to obtain preliminary phases for Aes.

Sorrentino *et al.* (2003) have just published another method for purification and crystallization of Aes from *E. coli*. They have purified Aes as a native protein without any tags and crystallized it in the space group $P2_12_12_1$.

## 2. Materials and methods

### 2.1. Expression and purification

$His_6$-Aes was overexpressed in *E. coli* BRE 1162 cells in the vector pAS1 (Peist *et al.*, 1997). Cells were grown at 310 K to an $OD_{600}$ of 1.0 in 4 × 1 l LB/amp$^{100}$ (Luria Broth medium supplemented with 100 mg ampicillin per litre of culture). Protein expression was induced with 0.3 m*M* isopropyl-$\beta$-D-thiogalactosidase (IPTG) and carried out at 303 K for 4 h. Cells were then harvested at 5000 rev min$^{-1}$ at 277 K. The cell pellet was resuspended in 25 ml buffer *A* (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 20 m*M* imidazole) and the cells were broken with a French press in two cycles at 138 MPa. The lysate was spun down for 20 min at 11 000 rev min$^{-1}$ and the supernatant was then spun down again for 30 min at 31 000 rev min$^{-1}$. The supernatant was then loaded onto a 15 ml Ni–NTA column (Qiagen) and washed with buffer *A*. $His_6$-Aes was then eluted from the column with a linear gradient of buffer *B* (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 500 m*M* imidazole). $His_6$-Aes elutes at about 180 m*M* imidazole. The purity of the elution fractions was tested on a 12.5% SDS–PAGE and the samples containing $His_6$-Aes were pooled. Saturated ammonium sulfate solution at 277 K was added dropwise to the protein solution until the concentration of ammonium sulfate reached 60%. $His_6$-Aes was precipitated while stirring for 30 min at 277 K. The solution was then spun down at 10 000 rev min$^{-1}$ for 30 min at 277 K and the pellet was resuspended in 6 ml of ice-cold dialysis buffer (50 m*M* ammonium acetate pH 6.0). $His_6$-Aes was then dialysed against 25 m*M* ammonium acetate pH 6.0 overnight at 277 K to remove the ammonium sulfate. During this dialysis step, $His_6$-Aes precipitated and was recovered by centrifugation at 14 000 rev min$^{-1}$ for 10 min at 277 K. Precipitated $His_6$-Aes was resuspended in 4 ml buffer *C* (25 m*M* ammonium acetate pH 7.5, 150 m*M* NaCl, 150 m*M* imidazole) and the sample was then spun down at 14 000 rev min$^{-1}$ for 10 min at 277 K to remove insoluble protein. The supernatant was filtered through a 100 kDa filter (Vivaspin) and the flowthrough was then concentrated in a 10 kDa filter (Amicon) to 5 mg ml$^{-1}$. The final yield was 1 mg $His_6$-Aes per litre of culture. Purification of the SeMet-$His_6$-Aes was performed according to the protocol described above for the native $His_6$-Aes with the following exceptions: cells were grown in M63 minimal medium (Miller, 1972) to an $OD_{600}$ of 0.6 and a selected set of amino acids was then added to the medium: lysine, phenylalanine and threonine at a concentration of 100 mg l$^{-1}$, leucine, isoleucine and valine at 50 mg l$^{-1}$ and selenomethionine at 60 mg l$^{-1}$. 15 min later, 0.3 m*M* IPTG was added to start the overexpression of SeMet-$His_6$-Aes. Purified SeMet-$His_6$-Aes was concentrated in a 10 kDa filter (Vivaspin) and the flowthrough was discarded. The final yield was 10 mg SeMet-$His_6$-Aes per litre of culture. For crystallization, SeMet-$His_6$-Aes was used at a concentration of 100 mg ml$^{-1}$.

### 2.2. Crystallization and data collection

Initial screening was performed using Crystal Screen I (Jancarik & Kim, 1991) and Crystal Screen II (Hampton Research) at 291 K in 96-well sitting-drop plates (Hampton Research). Small crystals of dimensions 0.05 × 0.05 × 0.02 mm were obtained with Crystal Screen I, condition No. 22. Further refinement yielded crystals of dimensions 0.2 × 0.2 × 0.03 mm in 24-well hanging-drop plates (Hampton Reasearch) with 1 ml reservoir solution [100 m*M* Tris–HCl pH 8.5, 200 m*M* sodium acetate, 18%(*w*/*v*) PEG 4000] per sample within 3–4 d (Fig. 1). The protein drop contained 3 µl protein (5 mg ml$^{-1}$) and 3 µl reservoir solution. Prior to data collection, single crystals were soaked in cryoprotectant solution [reservoir solution mixed in a 1:1 ratio with buffer *C* containing 50%(*v*/*v*) PEG 400] for 10 min and were then transferred into liquid nitrogen. Crystallization of SeMet-$His_6$-Aes was performed according to the protocol for the native Aes with the following exceptions: the reservoir solution contained 14%(*w*/*v*) PEG 4000 instead of 18% and the protein concentration was 100 mg ml$^{-1}$ instead of 5 mg ml$^{-1}$. SeMet-$His_6$-Aes crystals grew out of the protein precipitate to dimensions of about 0.5 × 0.2 × 0.1 mm within a week (Fig. 2).

Data collection from the native Aes crystals was carried out to a resolution of 2.4 Å at the EMBL Outstation DESY in Hamburg, Germany on beamline X13 equipped with a MAR CCD imaging-plate detector. X-ray diffraction data from the SeMet-$His_6$-Aes crystals were obtained to a resolution of 3.0 Å at the beamline X06SA at the SLS, Villigen, Switzerland. Raw data were processed using *XDS* (Kabsch, 1993).

## 3. Results and discussion

We have expressed Aes in *E. coli* BRE1162 strain as an N-terminally $His_6$-tagged protein. $His_6$-Aes was purified to near-homogeneity as determined by SDS–PAGE analysis (data not shown) and crystallized at 5 mg ml$^{-1}$ with the hanging-drop method. The crystals had dimensions of about 0.2 × 0.15 × 0.05 mm (Fig. 1). A native data set was collected to 2.4 Å resolution and the raw data were processed with the program *XDS* (Kabsch, 1993). The space group was determined to be *R*32, with one molecule per asymmetric unit (Table 1). It is inter-

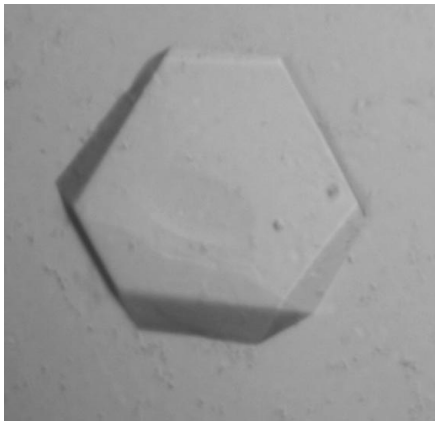

**Figure 1**
A native $His_6$-Aes crystal of dimensions 0.2 × 0.2 × 0.05 mm grown in space group *R*32 (see §2).

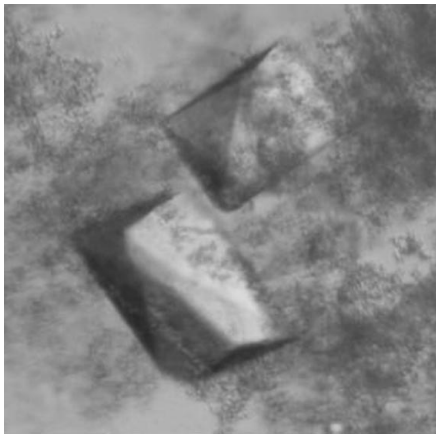

**Figure 2**
Two SeMet-$His_6$-Aes crystals of dimensions 0.5 × 0.35 × 0.25 mm grown out of precipitated protein (see §2).

**Figure 3**
A native $His_6$-Aes crystal of dimensions 0.6 × 0.06 × 0.06 mm grown in space group $P4_x$ (see §3).

**Table 1**
X-ray data-collection statistics for native and SeMet-$His_6$-Aes crystals.

| | | SeMet-$His_6$-Aes | | |
|---|---|---|---|---|
| | Native $His_6$-Aes | λ peak | λ inflection | λ remote |
| Protein concentration (mg ml$^{-1}$) | 5 | 100 | | |
| Crystallization conditions | 100 m*M* Tris–HCl pH 8.5, 200 m*M* sodium acetate, 18%(*w*/*v*) PEG 4000 | 100 m*M* Tris–HCl pH 8.5, 200 m*M* sodium acetate, 14%(*w*/*v*) PEG 4000 | | |
| Unit-cell parameters (Å, °) | $a$ = 113.0, $b$ = 113.0, $c$ = 147.8, $\alpha$ = 90, $\beta$ = 90, $\gamma$ = 120 | $a$ = 113.6, $b$ = 113.6, $c$ = 151.1, $\alpha$ = 90, $\beta$ = 90, $\gamma$ = 120 | | |
| Space group | *R*32 | *R*32 | | |
| Resolution (Å) | ∞–2.4 (2.5–2.4) | ∞–3.0 (3.5–3.0) | | |
| Wavelength (Å) | 0.8017 | 0.9797 | 0.9798 | 0.9763 |
| Unique reflections | 13774 | 13805 | 14273 | 14542 |
| Completeness (%) | 95.5 (97.2) | 94.7 (96.1) | 97.9 (98.6) | 99.8 (100) |
| $I/\sigma(I)$ | 7.4 (2.8) | 10.1 (4.9) | 7.5 (3.0) | 8.0 (2.9) |
| $R_{\rm merge}$ (%) | 11.5 (54.9) | 6.9 (18.4) | 8.6 (29.0) | 9.9 (38.7) |

esting to note that $His_6$-Aes produced crystals of several different shapes under the same conditions (data not shown). The best diffracting crystal is shown in Fig. 1. We were also able to crystallize $His_6$-Aes in the space group $P4_x$ with eight molecules per asymmetric unit and unit-cell parameters $a$ = 111.25, $b$ = 111.25, $c$ = 281.85 Å, $\alpha$ = 90, $\beta$ = 90, $\gamma$ = 90°, diffracting to 2.25 Å resolution at beamline BW7B at DESY/Hamburg (Fig. 3). In this case, 1 m*M* PMSF and 3 m*M* gadolinium sulfate were added to the 15 mg ml$^{-1}$ protein solution prior to crystallization. The 1 ml reservoir solution of the hanging-drop sample contained 20%(*v*/*v*) PEG 600 and 0.2 *M* imidazole malate pH 5.5. Gadolinium sulfate did not bind to Aes and molecular replacement with the search models 1jji, 1jkm and 1evq was not successful; therefore, we have expressed and purified Aes with selenomethionine substitution to near-homogeneity as determined by SDS–PAGE analysis (data not shown). For crystallization of SeMet-$His_6$-Aes, we used the conditions that previously gave the native Aes crystals in the space group *R*32 (§2) because in this case there was only one molecule per asymmetric unit (see Table 1). Since the amino-acid sequence of Aes contains ten methionines, space group $P4_x$ with eight molecules in the asymmetric unit would result in 80 selenomethionines per asymmetric unit, making phase determination difficult. We were able to obtain SeMet-$His_6$-Aes crystals in space group *R*32 with one molecule per asymmetric unit (Table 1) using the same conditions as for the native Aes (see §2) but with a protein concentration of 100 mg ml$^{-1}$; SeMet-$His_6$-Aes gave well ordered crystals of dimensions 0.5 × 0.3 × 0.1 mm (Fig. 2) that diffracted to 3.0 Å resolution. MAD data sets were collected at peak, inflection and remote high wavelengths and raw data were processed using *XDS* (Kabsch, 1993). We are currently working on the improvement of the crystal quality to obtain X-ray diffraction data at higher resolution.

Since the structure of mammalian hormone-sensitive lipase (HSL) has not yet been determined, structural analyses of bacterial homologues such as Aes are expected to facilitate understanding of the structure and function of mammalian HSL. We would also like to find out how Aes differs structurally from other members of the HSL family that share a high amino-acid sequence similarity with Aes (36.5% identity in a 96-amino-acid overlap in the case of the human HSL, as given by the LALIGN server) but do not interact with MalT. Our ultimate goal is to gain insight into the structure–function relationship of hormone-sensitive lipases and to elucidate the mechanism by which Aes down-regulates MalT activity.

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