Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Expression, purification, crystallization and preliminary X-ray data of *Escherichia coli* galactoside acetyltransferase

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Received 22 June 1999
Accepted 13 September 1999

Crystals of galactoside acetyltransferase from *Escherichia coli* have been prepared from solutions of ammonium sulfate containing acetyl-CoA. These crystals diffract to at least 2.7 Å resolution, belong to space group $C222_1$ and contain one copy of the trimeric enzyme in the asymmetric unit.

1. Introduction

The galactoside acetyltransferase (GAT; E.C. 2.3.1.18) of *Escherichia coli* is encoded by the *lacA* gene of the *lac* operon (Zabin *et al.*, 1962) and catalyzes transfer of an acetyl group from acetyl-CoA to the 6-hydroxyl group of certain galactosides (Zabin *et al.*, 1959; Musso & Zabin, 1973). Although the role of the enzyme is not well understood, it has been proposed that this enzyme may play a role in the detoxification of lactopyranosides (Andrews & Lin, 1976), since acetylated lactopyranosides are not taken up by the cell (Wilson & Kashket, 1969). GAT is now known to be a member of the hexapeptide acyltransferase superfamily of enzymes (Bairoch, 1993), which are characterized by an amino-acid sequence motif containing imperfect tandem-repeated copies of a hexapeptide repeat amino-acid sequence motif that encode a structural domain termed a left-handed parallel β-helix (LβH; Dicker & Seetharam, 1992; Vaara, 1992; Raetz & Roderick, 1995). The crystal structures of several hexapeptide acyltransferases have been determined, including UDP *N*-acetylglucosamine acyltransferase (Raetz & Roderick, 1995), tetrahydrodipicolinate *N*-succinyltransferase (Beaman *et al.*, 1997) and a xenobiotic acetyltransferase (Beaman *et al.*, 1998). Although all of these enzymes are trimeric and exhibit general similarities in the arrangement of the LβH domains that form their characteristic trimeric structure, the active sites of these three enzymes appear to be different. Structural studies of GAT may serve to identify the biological role of this enzyme and to characterize the catalytic properties of hexapeptide acyltransferases, in particular with respect to the structurally invariant LβH scaffold.

2. Results and discussion

2.1. Overexpression and preparation

The *lacA* gene was cloned from *E. coli* K12 Q100 genomic DNA by the polymerase chain reaction and inserted into a ptac-85 expression vector (Marsh, 1986). The sequence of the inserted gene was confirmed by sequencing. This plasmid was used to transform competent *E. coli* TG1 cells (Boehringer Mannheim) for overexpression of the enzyme. 4 l of culture were grown to an OD_{600} of 0.6, induced with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) and shaken for an additional 6 h at 310 K.

The harvested cells were washed twice with buffer (25 m*M* Tris–HCl pH 7.8, 1 m*M* EDTA and 0.5 m*M* β-mercaptoethanol), sonicated and a clarified extract was prepared by centrifugation. This extract was applied to a DEAE-Sepharose Fast Flow column equilibrated with 25 m*M* Tris–HCl buffer pH 7.8

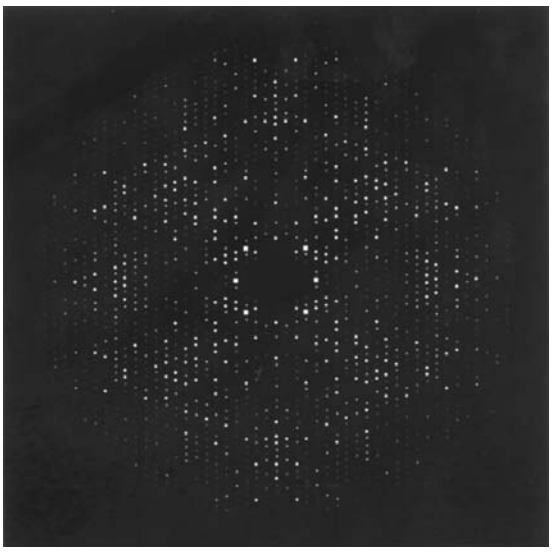

Figure 1
Pseudo-precession photograph of the (*hk*0) zone of *E. coli* GAT. The limiting resolution depicted is 2.7 Å. Produced using *PRECESS* (Furey & Swaminathan, 1990).

Table 1
X-ray data-collection statistics.

Space group	$C222_1$
Unit-cell parameters (Å)	a = 65.7, b = 182.5, c = 121.2
Data collection	
Resolution (Å)	20–2.7
No. of observed reflections	86059
No. of unique reflections	20153
$R_{\rm merge}$† (%)	8.8
$R_{\rm merge}$‡ (last shell) (%)	22.1
Completeness (%)	98.6
Completeness (last shell) (%)	94.3

† $R_{\rm merge}$ (%) = $100 \times \sum |I_i - \langle I \rangle| / \sum I_i$, where I_i is an individual intensity observation, $\langle I \rangle$ is the mean intensity for that reflection and the summation is over all reflections. ‡ The last shell is 2.80–2.70 Å.

containing 1 m*M* β-mercaptoethanol and eluted with a gradient of 0–1 *M* KCl in the same buffer. Fractions containing the desired protein, as judged by SDS–PAGE, were pooled and ammonium sulfate was added to a final concentration of 0.35 *M*. After centrifugation, the supernatant was loaded onto a phenyl-Sepharose column equilibrated with 25 m*M* Tris–HCl buffer pH 7.8 containing 0.35 *M* ammonium sulfate and 1 m*M* β-mercaptoethanol and eluted with a 0.35–0 *M* descending ammonium sulfate gradient. Fractions containing GAT were collected and the volume of protein solution was reduced by ultrafiltration. About 4 ml of protein solution was applied to a Superdex 200 pg gel-filtration column and eluted isocratically in a buffer of 25 m*M* Tris–HCl pH 7.8 containing 1 m*M* β-mercaptoethanol. The activity of the purified GAT was confirmed at 298 K using a standard assay mixture containing 25 m*M* Tris–HCl pH 7.8, 100 m*M* NaCl, 0.1 m*M* EDTA, 1 m*M* 5,5′-dithiobis(2-nitrobenzoic acid), 0.4 m*M* acetyl-CoA, 10 m*M* IPTG and was monitored at 412 nm (Alpers *et al.*, 1965). The purified protein (∼50 mg) was pooled and frozen at 103 K.

2.2. Crystallization and data measurement

In view of the fact that ammonium sulfate has previously been used to form small crystals of GAT from crude extracts for purification (Zabin, 1963), ammonium sulfate-based screens were adopted to investigate potential crystallization conditions for the production of large single crystals. Although initial screens using a commercially available ammonium sulfate screen kit failed to produce visible crystals, tests were carried out using a variety of additives combined with a fixed concentration of ammonium sulfate (2 *M*) at different pH values. These experiments showed that after two week incubations at room temperature, 0.2 *M* of some organic acids (*e.g.* tartrate, citrate, malonate, maleate and fumarate) at pH 8.0 caused GAT to form very small irregular crystals in the presence of acetyl-CoA.

Based on these observations, crystallization conditions were optimized using the hanging-drop vapor-diffusion method in tissue-culture plates at ambient temperature. 5 µl of 14 mg ml^{-1} GAT in 25 m*M* Tris–HCl pH 7.8, 1 m*M* β-mercaptoethanol and 10 m*M* acetyl-CoA were mixed on a cover slip with 2 µl of a precipitant solution containing 2.3 *M* ammonium sulfate, 0.1 *M* HEPES pH 7.4, 0.17 *M* L(+)-tartaric acid and were inverted over a reservoir containing 0.5 ml of the precipitant solution. Crystals of GAT appeared after about 4 d and were transferred to a storage solution of 2.4 *M* ammonium sulfate, 0.1 *M* HEPES pH 7.4, 0.18 *M* L(+)-tartaric acid and 10 m*M* acetyl-CoA.

The largest crystals of GAT measure 0.7 × 0.5 × 0.3 mm. A preliminary data set was measured to 2.7 Å resolution using a Siemens area detector mounted on a Rigaku RU200 rotating-anode X-ray generator operating with fine focus at 50 kV and 80 mA and was reduced with *XDS* and *XSCALE* (Kabsch, 1988; Table 1; Fig. 1). The symmetry of diffraction intensities and the pattern of systematic absences identified the space group as $C222_1$, with unit-cell parameters a = 65.7, b = 182.5, c = 121.2 Å.

2.3. Rotation function

Since structurally characterized members of the hexapeptide acyltransferase superfamily of enzymes are usually trimeric, a self-rotation function was calculated for the $\kappa = 120^\circ$ rotation angle using the *GLRF* rotation-function program (Tong & Rossmann, 1990) in order to determine the orientation of molecular threefold symmetry axes. The plotted output of this program (Fig. 2) identifies the inclination of a prospective threefold axis (peak height 11σ) with an orientation defined by the spherical polar angles (φ, ψ) = (46, 55°). A single trimeric molecule (3 × 22799 Da) present in the asymmetric unit of these crystals (181652 Å^3) yields a value of V_M of 2.66 Å^3 Da^{-1} and a reasonable estimated solvent content of 54% (Matthews, 1974). These crystals of *E. coli* GAT are suitable for a three-dimensional structure determination, which is in progress.

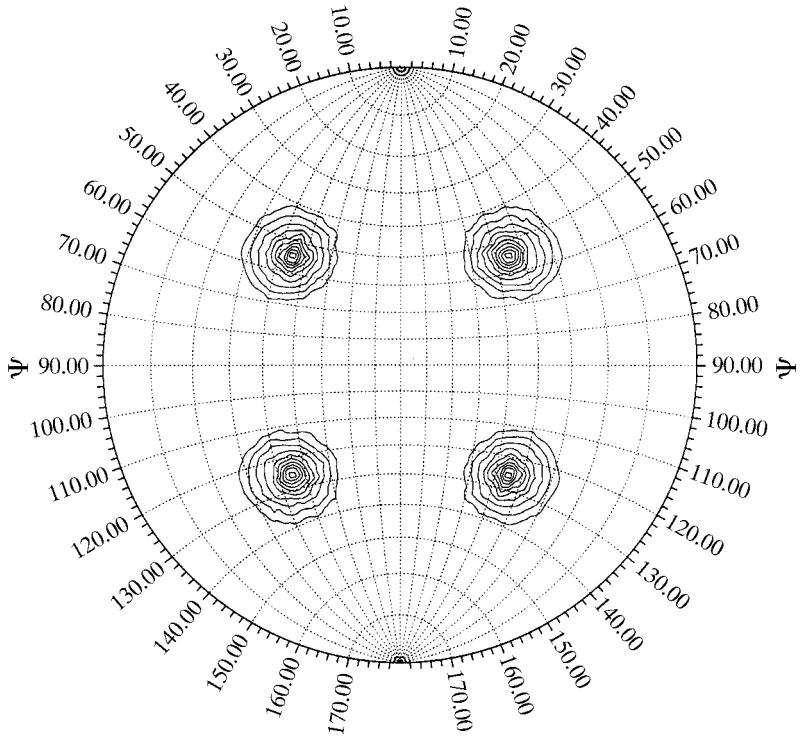

Figure 2
Self-rotation function ($\kappa = 120^\circ$) of *E. coli* GAT. The contour levels are drawn at integral increments of the standard deviation (σ) beginning at 1.0σ. The circumference of the figure corresponds to $\varphi = 0^\circ$. Produced using *GLRF* (Tong & Rossmann, 1990).

This work was supported by grants AI38328 and AI42154 from the National Institutes of Health. The authors gratefully

acknowledge helpful discussions with Drs Todd W. Beaman and Laurence R. Olsen and Mr Renjian Zheng.

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