Structural basis for reduced activity of 1-aminocyclopropane-1-carboxylate synthase affected by a mutation linked to andromonoecy

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1. Introduction

1-aminocyclopropane-1-carboxylate synthase (ACS), which catalyzes the first committed and in general rate-limiting reaction in the biosynthesis of the plant hormone ethylene [1], is a pyridoxal 5'-phosphate (PLP)-dependent enzyme, encoded from a multigene family present in every plant species [2]. ACS functions as a homodimer [3] but was shown, in vitro and in planta to be able to form functional and non-functional heterodimers of differentially expressed isoforms. ACS has been thoroughly studied from a biochemical and plant biological point of view [3–5]. Six crystal structures of the enzyme have been described: four of them are structures of Malus domestica (apple) ACS-1 (MdACS-1): an unliganded form at 2.4 Å [6], two complexes with the inhibitors i-aminooxyvinylglycine (AVG) [7] and i-vinylglycine (VG) [8], respectively and a structure mimicking the binding of the natural substrate S-adenosyl-l-methionine (SAM) by using [2-(aminoxy)ethyl][5'-deoxyadenosin-5'-yl](methyl)sulfonium as a substrate analogue [9]. In addition, an unliganded and an AVG-inhibited structure from Solanum lycopersicum (tomato) ACS-2 (SiACS-2) were reported [10]. Recently, Boualem et al. [11] have linked ACS activity to andromonoecy, a sexual system where the plant carries both male and bisexual flowers. They identified a genetic variant in Cucumis melo (muskmelon) ACS-7 (CmACS-7) expressed in carpel primordia, carrying the A57V mutation. The CmACS-7 A57V variant results in strongly reduced activity of the enzyme and ultimately in andromonoecious plants. Boualem and colleagues proposed that the mutation interferes with the proper orientation of the substrate SAM in the active site. In a later work, Martin et al. [12] were able to link sex determination in C. melo to epigenetic changes in the promoter of a transcription factor (CmWIP1). They showed that CmWIP1 causes arrest of carpel development, is epistatic to CmACS-7 and represses its expression. We present here a straightforward structural basis for the reduced activity of the A57V mutant, based on our work on Malus domestica ACS, including a new structure of the unliganded apple enzyme at 1.35 Å resolution.

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Abbreviations: ACS, 1-aminocyclopropane-1-carboxylate synthase; PLP, pyridoxal 5'-phosphate; LLP, N0-pyridoxyl-lysine-5'-monophosphate; AVG, i-aminooxyvinylglycine; SAM, S-adenosyl-l-methionine; VG, i-vinylglycine; Md, Malus domestica; Cm, Cucumis melo; Sl, Solanum lycopersicum; DPI, dispersion precision indicator
2. Materials and methods

2.1. Crystallization and data collection

C-terminally truncated (V435STOP) MdACS-1 was prepared and purified as described previously [13]. Crystals were obtained at 293 K by vapour diffusion (sitting drop). 1 μl of protein solution containing 15 mg/ml enzyme, 50 mM HEPES, pH 7.9, 10 μM PLP and 1 mM DTT was mixed with 1 μl of reservoir solution containing 27% MPD (v/v) and 50 mM MES, pH 6.5. Data were collected at a wavelength of 0.889 Å at 100 K on a MAR 345 detector at the Swiss–Norwegian Beamline of the European Synchrotron Radiation Facility (Grenoble, France). A complete dataset was obtained from a single crystal, by scaling together a low resolution sweep (30–4.08 Å) and a high resolution one (16–1.35 Å). Diffraction images were indexed and integrated with XDS [14] in space group C2 (a = 103.7 Å, b = 61.1 Å, c = 76.8 Å, β = 123.1°), scaled with XSCALE and converted to amplitudes with XDSCONV and programs of the CCP4 suite [15]. 1% of the reflections were set aside as test reflections.

2.2. Phasing and refinement

Phases were obtained by molecular replacement with Phaser [16] using as search model the 1.6 Å structure of MdACS-1 in complex with AVG [7] (PDB code 1M7Y), deprived of the cofactor and of all ligands.

The structure was refined with Phenix [17]. After the first round of refinement, clear 2mFo-DFc and mFo-DFc electron density appeared in the active site, allowing for manual modelling of PLP, K273 internal aldimine (LLP273) in Coot [18]. However, in the following rounds of refinement it became apparent that, due to difference density near the NZ atom of K273 and the C4 atom of the PLP, a fraction of about 20% of free K273 and PLP was also present in the structure. Water molecules were automatically added with Phenix and Coot and manually verified in Coot. MolProbity [19] was used for structure validation. Statistics are shown in Table 1. The coordinates and structure factors were deposited with the Protein Data Bank [20] (PDB code 3PIU).

2.3. Analysis of the radiation damage driven breakage of the internal aldimine

Cleavage of the internal aldimine Schiff base was analysed by creating two datasets from frames 1–184 (30.0–122.0°) and 185–361 (122.5–210.5°), respectively, of the high-resolution sweep and the corresponding low-resolution frames. Independent Phenix refinements of the final model based on each dataset resulted in occupancies of 22% and 35%, respectively, for free K273. A Fo–Fo difference map computed in Phenix confirmed the presence of radiation damage.

2.4. Modelling of MdACS-1 A46V

Modelling of A46V MdACS-1 was carried out using Modeller [21] (version 9v8) with the 1.35 Å ACS structure as template. Free PLP and K273 were removed from the template, LLP273 was treated as a rigid body. Modeller was run with the following options: library_schedule = autosched.slow, max_var_iterations = 300, md_level = refine.very_slow and repeat_optimization = 10. From the five best models obtained, that with the least dislocated R407 side chain was used (see par. 3.2).

3. Results and discussion

3.1. Structure of ACS at 1.35 Å resolution

1.35 Å diffraction data of native ACS were collected in space group C2, with one subunit per asymmetric unit. A PDB search (16 Nov 2010) reveals that the structure is among the ten highest-resolution structures of PLP-dependent enzymes. The final model was refined to an R-value of 14.9% and an R-free of 17.1% and exhibits excellent geometry and stereochemistry. Well-defined electron density encompasses protein residues 18–433, with a missing loop corresponding to residues 253–259. Between residues 73–74 and 351–352 the electron density of the main chain is interrupted. The structure is in very good agreement with the previous 2.4 Å structure of unliganded ACS (PDB code 1B8G [6], r.m.s.d. of 0.44 Å with subunit A and 0.45 Å with subunit B, respectively), as well as with the 1.6 Å AVG-inhibited ACS structure (PDB code 1M7Y, [7], r.m.s.d. of 0.39 Å), as calculated with Phenix.

The internal aldimine Schiff base linking the C4' atom of the PLP cofactor and the side chain nitrogen of K273 in the N'-pyridoxyl-lysine-5'-monophosphate (LLP) adduct are well defined. However, they are not fully occupied, as the internal aldimine coexists with a small portion, about 20%, of free K273 (Fig. 1). The high resolution of the present structure allows for a more accurate length determination (2.61 ± 0.05 Å) of the hydrogen bond shared by the O3' atom of the cofactor and by the side chain of Y233. In 1B8G (subunit B) the bond length was 2.7 ± 0.3 Å. The coordinate errors (Cruikshank dispersion precision indicator (DPI) [22]) were calculated with Sfcheck [23]. The importance of this hydrogen bond for the pKₐ value of the internal aldimine was pinpointed by Eliot and Kirsch [24].

A difference density peak near the active site corresponds to an unknown ligand (Fig. 1), not compatible with the chemicals present in the crystallization drop. The ligand may be a moiety of a larger and partly disordered entity, resulting from the recombinant expression or from the addition of protease inhibitors. This was also the case in the lower-resolution structure (188G). Based on shape, size and biochemical considerations we tentatively modelled an acetate ion into the difference density. The acetate

Table 1

<table>
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<th>Data collection parameters</th>
<th>25.0–1.35 (1.40–1.35)²</th>
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<tr>
<td>Resolution (Å)</td>
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<tr>
<td>No. of reflections/no. of test reflections</td>
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<tr>
<td>Bond angles (°)</td>
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</table>

Data was collected from a single crystal.

² From XDS.

Calculated with Phenix.

Calculated with Phenix.
carboxylate sits exactly where the α-caroxylates of AVG and of VG are found in the ACS complexes with those two inhibitors (1M7Y and 1YNU, respectively). Upon further refinement and density inspection we modelled a propionate group (coordinates in S1.pdb), but did not include it in the final model. Adjacent to this peak and closer to the cofactor there is another, smaller, separated difference density peak that could not be attributed to any ligand or moiety.

Near the backbone oxygen of F129, an elongated difference density peak was tentatively attributed, by comparison with 1M7Y, to displaced N-terminal residues of ACS, but refinement was unsatisfactory and the peak was left uninterpreted, as was another one near D222.

3.2. Effects of A57V (A46V in M. domestica)

As shown by Boualem et al. [11], the A57V mutation in CmACS-7, an isoform expressed in flowers, causes andromonoecy and results in reduced enzyme activity. We modelled the corresponding A46V mutation in the active site of our 1.35 Å structure of MdACS-1. All active site residues are conserved between MdACS-1 and in CmACS-7 (Fig. S1). The point mutation changes the structure of the neighbouring active site residues only marginally. The side chain of V46 adopts a staggered conformation (χ1 = 60°) thus avoiding a steric clash with the guanidinium group of R407. In the modelled conformation, the CG1 atom of the bulky valine side chain partly occupies the binding pocket for the SAM α-carboxylate (Fig. 2). This finding provides a solid structural basis for the reduced activity of CmACS-7 A57V and supports the proposal by Boualem et al. that the mutation may cause an improper orientation of SAM in the active site. Since ACS catalyses in general the rate-limiting step of ethylene biosynthesis, the A57V mutation exerts a direct effect on the rate of ethylene production in C. melo [26], thus affecting the formation of hermaphrodite flowers by allowing stamen development in pistillate flowers [11,27]. Notably, in a previous random mutagenesis study of SlACS-2, the A54T mutant, corresponding to A57V of CmACS-7, was characterized as having approximately the same protein expression level as wt SlACS-2 but only 0–5% of the wt activity. Since the side chain of threonine is isosteric to that of valine, it is very likely to assume a staggered conformation equivalent to that of valine in CmACS-7 A57V. The side chain conformation of T54 of SlACS-2 is most likely further stabilized by a hydrogen bond shared by its hydroxyl group with the phenolic moiety of Y240.

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Appendix A. Supplementary data

References


