Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Alliin lyase (alliinase) from garlic (*Allium sativum*): crystallization and preliminary X-ray characterization

Linda J. W. Shimon,[a]* Aharon Rabinkov,[b] Talia Miron,[b] David Mirelman,[b] Meir Wilchek[b] and Felix Frolow[c]

[a]Department of Chemical Services, The Weizmann Institute of Science, Rehovot 76100, Israel, [b]Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel, and [c]Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel

Correspondence e-mail: linda.shimon@weizmann.ac.il

Received 25 March 2002
Accepted 11 June 2002

The enzyme alliinase has been isolated from garlic bulbs and crystallized. The crystals belong to space group $P2_1$, with unit-cell parameters $a = 70.191$, $b = 127.006$, $c = 108.085$ Å, $\beta = 93.384^\circ$. They diffract to 2.2 Å at liquid-nitrogen temperature. Analysis of the Patterson self-rotation function suggests that the crystals contain two dimeric molecules per asymmetric unit.

1. Introduction

Alliinase (Cys sulfoxide lyase, alliin lyase, C-S lyase; EC 4.4.1.4) from garlic is an enzyme that catalyses the conversion of alliin to allicin (Fig. 1). The product of this reaction, allicin, is responsible for the specific pungent smell of garlic. For generations, throughout the world, garlic, *Allium sativum* L. has been known for its remarkable medicinal properties, which include antimicrobial and antiparasitic as well as hypolipidemic and anticancer activities (Lawson & Koch, 1996). Most of these effects are attributed to allicin. First described by Stoll and Seebeck in 1951 (Stoll, 1951), alliinase was isolated and characterized (Nock & Mazelis, 1986; Kazarian & Goriachenkova, 1978; Rabinkov *et al.*, 1994) and it was shown that the enzyme is a dimer with two equal subunits and its activity is dependent upon the pyridoxal 5′-phosphate cofactor. The pH optimum of alliinase activity is 6.5 and its isoelectric point is between 6.0 and 7.0. Alliinase belongs to the family of mannose-rich glycoproteins with an estimated carbohydrate content of about 6%, glycosylated at Asn146 (Rabinkov *et al.*, 1995). Alliinase was cloned and sequenced. It was shown that a single subunit has a molecular mass of 51 500 and consists of 448 amino-acid residues (Rabinkov *et al.*, 1994; van Damme, 1992). In the present work, alliinase was purified to homogeneity from garlic bulbs and crystallized. The structural analysis of this protein will provide insight into its enzymatic activity and will elucidate its mode of action.

2. Materials and methods

2.1. Purification and crystallization

Alliinase was purified from garlic cloves as previously described (Rabinkov *et al.*, 1995) with an additional final step. Briefly, peeled garlic cloves were homogenized in the cold in a mincing machine in 0.02 *M* sodium phosphate buffer pH 7.2 containing glycerol (10%) and 0.02 m*M* pyridoxal 5′-phosphate (buffer *A*). The homogenate was filtered through two layers of cheesecloth and the filtrate was centrifuged at 20 000*g* for 30 min at 177 K. Polyethylene glycol 8000 was added to the supernatant [to 25%(*w*/*v*)] and the mixture was stirred slowly for 20 min at 277 K. The slurry was then sedimented at 20 000*g* for 15 min at 277 K. The pellet was resuspended in 120 ml of 0.02 *M* HEPES buffer pH 7.2 containing 1 m*M* $CaCl_2$, 1 m*M* $MnCl_2$ and 0.5 *M* NaCl (buffer *B*), subjected to centrifugation again at 20 000*g* for 20 min at 277 K and dialyzed against the same buffer. The supernatant was placed on a ConA-Sepharose (Pharmacia) column (2.2 × 50 cm) pre-equilibrated with buffer *B*. The column was washed with buffer *B*. Elution of alliinase was carried out with 0.1 *M* methyl α-D-mannoside in buffer *B*. Solid $(NH_4)_2SO_4$ was added to the eluate to a final concentration of 1 *M*. The pH of the solution was adjusted to 6.5. This preparation was placed on an octyl-Sepharose column (1.0 × 10 cm) and fractions containing non-adsorbed protein were collected, dialyzed against 0.02 *M*

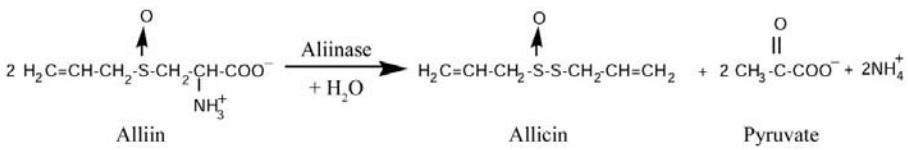

Figure 1
The reaction catalyzed by aliinase.

HEPES buffer pH 7.2, concentrated and stabilized by the presence of 20% sucrose.

2.1.1. Enzymatic assay. Alliinase activity was assayed according to the nitrothiobenzoate (NTB) method (Miron *et al.*, 1998).

2.1.2. Protein assay. Protein was assayed by the procedure of Lowry *et al.* (1951) with ovalbumin as a standard. Specific activity of the alliinase preparation obtained was 250 µmol min^{-1} of pyruvate per milligram of protein.

2.1.3. Crystallization screening. Preliminary crystallization screening was performed using commercially available kits from Hampton Research.

2.2. X-ray diffraction

A crystal was mounted in a fibre loop and flash-frozen in a liquid-nitrogen stream produced by an Oxford Cryostream low-temperature device. For data collection under cryogenic conditions, crystals were transferred to Hampton Paratone-N oil for a period of 10 min before being flash-frozen in liquid nitrogen. During measurement, the crystal was kept at 100 K. Data were collected on an R-AXIS IIc image-plate detector equipped with blue image plates mounted on a Rigaku RU-H3R generator with Cu $K\alpha$ radiation focused by Osmic confocal mirrors. A native data set to 2.2 Å resolution was collected as 1.0° oscillation frames, 30 min per frame, at a distance of 120 mm (Fig. 2). Diffraction data frames were processed with *DENZO* and *SCALEPACK* incorporated in the *HKL* package (Otwinowski & Minor, 1997).

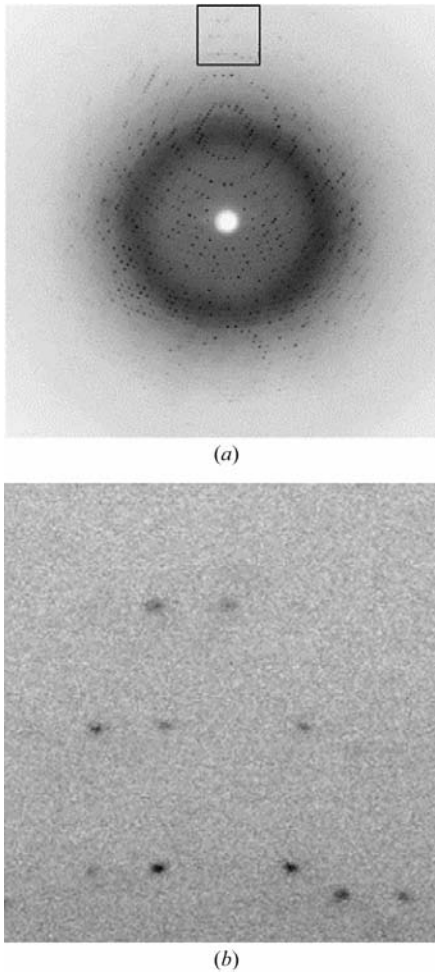

Figure 2
Diffraction pattern of a alliinase crystal as collected on R-AXIS IIc image-plate detector. The marked section of the diffraction pattern has been enlarged (below).

3. Results and discussion

3.1. Crystallization results

Several initial crystallization conditions appeared promising: the best of these preliminary conditions, one at high pH and one at low pH, 30% PEG 4000, 100 m*M* trisodium citrate pH 5.6 and 200 m*M* ammonium acetate (Crystal Screen, condition 9) and 30% PEG 4000, 100 m*M* Tris–HCl pH 8.5 and 200 m*M* magnesium chloride (Crystal Screen, condition 6), were taken as starting points for optimization. Initially, only poorly shaped and twinned crystals could be obtained which diffracted to a maximum resolution of 3.5 Å. However, eventually better formed and better diffracting monoclinic crystals were obtained and chosen for further characterization (Fig. 3). In the final conditions for crystallization at low pH, 4 µl of protein stock solution [10.0 mg ml^{-1} of protein, 20 m*M* HEPES pH 7.0, 20%(*w*/*v*) sucrose] was mixed with 2 µl of reservoir solution [28%(*w*/*v*) PEG 4000, 300 m*M* ammonium acetate, 100 m*M* sodium citrate buffer pH 5.6]. Initially, precipitate formed. However, after one week yellow crystals appeared and grew to a final size of about 0.1 × 0.05 × 0.05 mm over a period of 10 d.

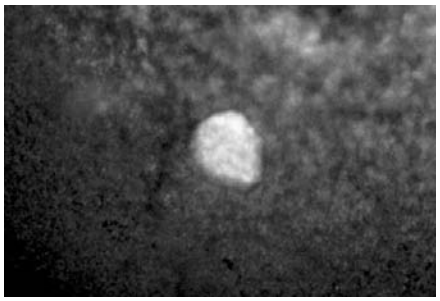

Figure 3
Crystal of *A. sativum* alliinase. The largest dimension is 0.1 mm.

Table 1
Data collection.

Resolution (Å)	R_{merge}	No. of unique reflections	Completeness (%)	Multiplicity	$\langle I\rangle/\sigma(I)$
20–4.39	0.058	12211	100.0	5.3	32.45
4.39–3.49	0.073	12159	100.0	5.0	22.71
3.49–3.05	0.095	12009	99.7	5.2	15.27
3.05–2.77	0.143	11992	98.9	4.5	8.98
2.77–2.57	0.209	11911	98.7	4.4	6.05
2.57–2.42	0.288	11832	98.8	4.4	4.23
2.42–2.30	0.406	11905	98.3	4.2	3.01
2.30–2.20	0.448	11403	94.9	3.6	2.38
Overall	0.051	95422	98.7	4.47	14.09

3.2. Diffraction

Diffraction-quality single crystals of alliinase from *Allium sativumm* L. were grown by the hanging-drop vapour-diffusion method. The yellow colour of the crystals reflected the presence of pyridoxal phosphate (vitamin B_6) cofactor. The crystals were stable when flash-frozen to 100 K and diffract to at least 2.2 Å at home X-ray laboratory facilities with a mosaicity of 0.4°. A full data set to 2.2 Å was collected. Indexing using *DENZO* gave monoclinic crystals with space group $P2_1$ and unit-cell parameters a = 70.191, b = 127.006, c = 108.085 Å, β = 93.384°. Data processing gave an R_{merge} of 0.075 (based on intensities). The data set is 98.7% complete to 2.2 Å (91.7% complete in the range 2.24–2.20) (see Table 1 for details). The low-pH and high-pH crystals were not isomorphous as judged by unit-cell parameters (the most variable dimension is a, with a maximum difference of 5%) and cross-crystal reflection intensity scaling and merging (overall R = 46%, R and scale factor strongly dependent on resolution). Assuming a calculated molecular weight of 52 500 Da and four monomers per asymmetric unit (see below), the V_M value is 2.31 $Å^3$ Da^{-1}, with a solvent content of 46.4%, which lies in the normal range for globular proteins (Matthews, 1974).

3.3. Molecular symmetry and heavy-atom derivatives

The Patterson self-rotation function was calculated with *POLARRFN* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The κ = 180° section of the self-rotation stereogram is presented in Fig. 4. Four independent axes are marked. Strong peaks, numbered 2 and 3 (about 86.6° apart in projection), suggest that the molecular content of the asymmetric unit can

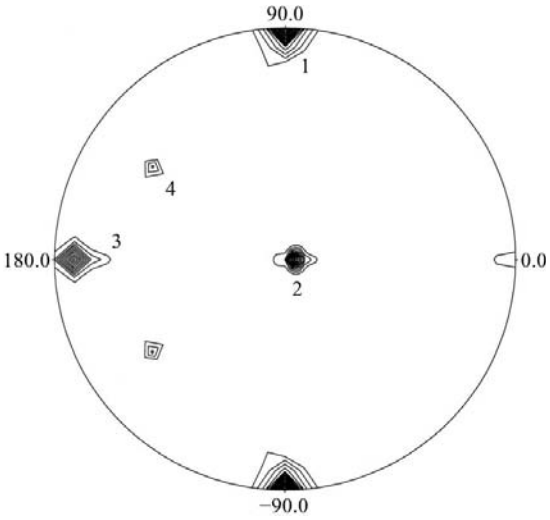

Figure 4
Self-rotation function, $\kappa = 180^\circ$ section. Peaks are scaled to the origin peak, which is represented by 100%; r.m.s. of the self-rotation function is 5.6% of the origin peak. Positions of the axes with the related φ angles are marked on the circumference. The peaks are numbered and are 67, 67 and 33% for peaks 2, 3 and 4, respectively. Contour levels are plotted for all peaks greater than 20% of the origin peak in intervals of 5%.

possess approximate 222 non-crystallographic symmetry, assuming that the third non-crystallographic twofold axis is parallel to the crystallographic axis. However, this assumption is ruled out by the absence of any significant pseudo-Harker peak on the native Patterson function. Another interpretation of the self-rotation function is that the two strong peaks, 2 and 3, are the non-crystallographic twofold axes of two dimers in an asymmetric unit of the crystal, in agreement with the two dimers per asymmetric unit inferred from the calculation of the Matthews coefficient. The nature of peak 4 is not clear; however, it may represent the third twofold axis between monomers in two dimers, which does not follow 222 symmetry.

Biochemical data have shown that alliinase is a homodimer in solution (Nock & Mazelis, 1986; Kazarian & Goriachenkova, 1978; Rabinkov *et al.*, 1994).

The search for suitable heavy-atom derivatives is currently under way using both crystalline forms. The low-pH form is suitable for uranyl and lanthanide species and the high-pH form is suitable for mercurials. In spite of non-isomorphism it might be possible to combine phases of both crystal forms through inter-crystal electron-density averaging (Cowtan & Main, 1998; Cowtan & Zhang, 1999; Kleywegt & Jones, 1994). An additional source of phasing improvement will be non-crystallographic symmetry averaging.

References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Cowtan, K. & Main, P. (1998). *Acta Cryst.* D**54**, 487–493.

Cowtan, K. D. & Zhang, K. Y. J. (1999). *Prog. Biophys. Mol. Biol.* **72**, 245–270.

van Damme, E. J. M., Smeets, K., Torrekens, S., van Leuven, F. & Perumans, W. J. (1992). *Eur. J. Biochem.* **209**, 751–757.

Kazarian, R. A. & Goriachenkova, E. V. (1978). *Biokhimya (Engl. Transl.)*, **43**, 1502–1508.

Kleywegt, G. J. & Jones, T. A. (1994). *Proceedings of the CCP4 Study Weekend. From First Map to Final Model*, edited by S. Bailey, R. Hubbard & D. Waller, pp. 59–66. Warrington: Daresbury Laboratory.

Lawson, L. D. & Koch, H. P. (1996). *Garlic: The Science and Therapeutic Application of Allium sativum L. and Related Species*, 2nd ed. Baltimore, USA: Williams & Wilkins.

Lowry, O. H., Rowebrough, N., Farr, A. L. & Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.

Matthews, B. W. (1974). *J. Mol. Biol.* **82**, 513–526.

Miron, T., Rabinkov, A., Mirelman, D., Weiner, L. & Wilchek, M. (1998). *Anal. Biochem.* **265**, 317–325.

Nock, L. P. & Mazelis, M. (1986). *Arch. Biochem. Biophys.* **249**, 27–33.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Rabinkov, A., Wilchek, M. & Mirelman, D. (1995). *FASEB J.* **9**, A1361.

Rabinkov, A., Zhu, X. Z., Grafi, G., Galili, G. & Mirelman, D. (1994). *Appl. Biochem. Biotechnol.* **48**, 149–171.

Stoll, A. S. E. (1951). *Adv. Enzymol.* **11**, 377–400.