Acta Cryst. (1996). D**52**, 864–865

Crystallization and preliminary X-ray analysis of a vanadium-dependent peroxidase from *Ascophyllum nodosum*

M. Weyand,[a] H.-J. Hecht,[a] H. Vilter[b]*† and D. Schomburg[a]*† *at* [a]*GBF, Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, 38124 Braunschweig, Germany, and* [b]*Zurmaienstrasse 16, 54292 Trier, Germany. E-mail: schomburg@gbf-brauschweig.de*

(*Received 17 November 1995; accepted 19 January 1996*)

Abstract

Peroxidase from the brown alga *Ascophyllum nodosum*, a non-heme vanadium-dependent haloperoxidase, has been purified to homogeneity and crystallized from ammonium sulfate solutions in a form suitable for X-ray diffraction analysis. The crystals have been grown by the vapour-diffusion technique using the sitting-drop method. X-ray diffraction studies show that the crystals belong to the tetragonal space group $P4_12_12$ or $P4_32_12$ with $a = b = 114.3$ and $c = 276.0$ Å. The crystals diffract to at least 2.4 Å resolution.

1. Introduction

Peroxidases are oxidoreductases acting on peroxide as electron acceptors (E.C. 1.11.1.X) (Webbs, 1992). They are present in diverse organisms, including animals (Dumontet & Rousset, 1983), plants (Krenn, Plat & Wever, 1988), fungi (Morris & Hager, 1966; Liu *et al.*, 1987), bacteria (van Pée & Lingens, 1985; van Pée, Sury & Lingens, 1987) and marine algae (Manthey & Hager, 1981; Vilter, 1984). Peroxidases are involved in the biosynthesis of plant hormones (Campa, 1991; Yang, 1967), lignin polymerization (Gross, 1977), lignin degradation (Tien & Myer, 1977) and biosynthesis of halogenated compounds (Neidleman & Geigert, 1985). The substrates of these haloperoxidases are peroxides (*e.g.* hydrogen peroxide), halide ions (chloride, bromide and iodide) and organic halogen acceptors.

Haloperoxidases are classified by their prosthetic group or cofactor. The first group consists of the well studied heme haloperoxidases (Dawson & Sono, 1987). No prosthetic group or metal is detectable in bacterial non-heme haloperoxidases (van Pée *et al.*, 1987; Wiesner, van Pée & Lingens, 1988; Krenn *et al.*, 1988), which represent the second group. The third group contains vanadium instead of heme (Vilter, 1984; De Boer, van Kooyk, Tromp, Plat & Wever, 1986).

Several three-dimensional structures of heme-containing haloperoxidases as well as a model of the reaction mechanism are found in the literature (Dawson & Sono, 1987; Zeng & Fenna, 1992). A three-dimensional structure of a cofactor-free bacterial bromoperoxidase was published (Hecht, Sobek, Haag, Pfeifer & van Pée, 1994). In contrast no structural information at atomic resolution of a haloperoxidase of the third group has been published yet, though structural information concerning the vanadium centre is known (Küsthardt, Hedman, Hodgson, Hahn & Vilter, 1993; Knüttel, Müller, Rehder, Vilter & Wittneben, 1992; Rehder, Holst, Priebsch & Vilter, 1991; Weidemann, Rehder, Kuetgens, Hormes & Vilter, 1989). To get information about the tertiary structure of an enzyme that catalyzes the oxidation of bromide and iodide ions with vanadium as prosthetic group, we crystallized a vanadium-dependent haloperoxidase from marine brown alga *Ascophyllum nodosum* (abbreviation AnI).

2. Materials and methods

AnI was purified in a large-scale process, consisting of a three-step liquid–liquid extraction, dialysis and hydrophobic interaction and anion-exchange chromatography (Vilter, 1995). The purified peroxidase AnI was concentrated by ultrafiltration using a CX 30 membrane with an Immersible-CX Low-Binding Ultrafilter System (Millipore). Before crystallization the protein solution was filtered with 0.2 µm Spartan13 filters (Schleicher & Schuell). Crystallization was achieved by the sitting-drop variant of the vapour-diffusion method using Cryschem crystallization plates. Initial conditions were established by the factorial experiment (Jancarik & Kim, 1991) using a Biomek automated laboratory station (Beckman, Mannheim, Germany). For X-ray analysis crystals were mounted in glass capillaries with a drop of mother liquor. Native data sets were collected using a Xentronics area detector and graphite-monochromated Cu $K\alpha$ radiation from a Siemens rotating anode operated at 50 kV and 100 mA. The diffraction limits were estimated from still frames with an exposure time of 60 s and detector swing-outs for 2.8 and 2.2 Å resolution. The native data set was collected on a Siemens four-axis goniometer with a crystal-to-detector distance of 280 mm and a 0.3 mm collimator. The crystal was mounted with the c axis parallel to the oscillation axis. Native data was measured at three settings, two high-resolution settings ($2\theta = 22°$) and one low-resolution

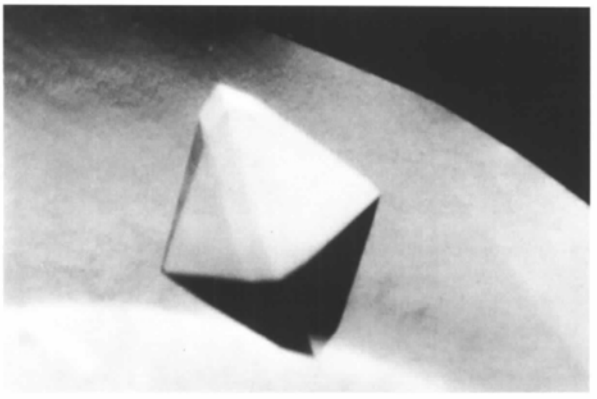

Fig. 1. Typical crystal of haloperoxidase from *Ascophyllum nodosum*.

† Correspondence concerning crystallography should be sent to D. Schomburg; correspondence concerning biochemistry should be sent to H. Vilter.

Table 1. *Native data set statistics of Ascophyllum nodosum peroxidase*

D (Å)	No. of unique reflections	No. of observed reflections	Percentage observed (%)	Multiplicity	R_{merge} (%) in shell
8.70	1679	1603	95.5	1.7	4.3
6.21	2788	2749	98.6	2.4	4.6
5.09	3501	3480	99.4	2.9	5.0
4.41	4089	4019	98.3	2.8	5.5
3.95	4587	4472	97.5	2.2	5.7
3.61	5030	4859	96.6	1.6	5.8
3.34	5428	5151	94.9	1.6	5.9
3.13	5809	5240	90.2	1.5	6.0
2.95	6139	4598	74.9	1.5	6.1
2.80	6448	3069	47.6	1.4	6.2
Total	45681	39240	85.9	1.9	5.0

setting ($\theta = 12°$) at constant φ angle. High-resolution settings were separated by a 30° rotation in the χ angle. The data for each setting is composed of 300 frames with a frame width of 10 min arc. The exposure time for a frame was 12 min for high-resolution data and 10 min for low-resolution data. All measurements were carried out at 283 K. The crystals were stable in the X-ray beam for more than one week. The data set was processed using the *XENGEN* 2.0 software (Howard *et al.*, 1987) and the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Self-rotation searches were calculated with *X-PLOR* (Brünger, 1992).

3. Results and discussion

Crystals grew from solutions containing ammonium sulfate as precipitant. The crystallization results described by Müller-Fahrnow, Hinrichs, Saenger & Vilter (1988) with PEG as precipitant could not be reproduced. Optimized conditions for crystallization are as follows: the reservoir solution consists of 1.9–2.1 *M* ammonium sulfate in 50 m*M* Tris–HCl buffer pH 8.0, and before mixing with the reservoir the sitting drop consists of 10 mg ml^{-1} AnI protein in 1.9 m*M* Tris solution. Crystals grow to their full size (0.4 × 0.4 × 0.3 mm) within two weeks at 298 K. The photograph (Fig. 1) shows the typical tetragonal bipyramidal habitus of the AnI peroxidase crystals indicating a tetragonal crystal class.

The crystals diffract to at least 2.4 Å resolution. The data measurements for isomorphous replacement presently available were limited to a resolution of 2.8 Å for technical reasons. The space group was determined by indexing as $P4_12_12$ or $P4_32_12$. Processing and scaling of the data yielded the refined lattice constants of $a = b = 114.3$, $c = 276.0$ Å and the symmetry R value of 5.0% [$R = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)_i$] (Table 1).

Assuming one homodimeric enzyme of molecular mass 120 kDa (Rüdiger & Vilter, 1993) in the asymmetric unit a crystal packing parameter (Matthews, 1968) of $V_M = 3.76$ Å^3 Da^{-1} was calculated. A self-rotation search shows only peaks at the positions of the crystallographic twofold axis. This suggests that the twofold non-crystallographic axis probably is parallel to one crystallographic axis. However, all peaks for the crystallographic axes are of nearly equal height and shape. Therefore, an identification of the non-crystallographic axis is not possible at present. Further native data collection and a search for heavy-atom derivative data are in progress.

References

Brünger, A. T. (1992). *X-PLOR. A System for Crystallography and NMR. Version* 3.1. Yale University Press, New Haven, Connecticut, USA.

Campa, A. (1991). *Peroxidases 1 in Chemistry and Biology*, Vol. 2, edited by J. Everse, K. Everse & M. B. Grisham, p. 25. Boca Raton: CRC Press.

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

Dawson, J. H. & Sono, M. (1987). *Chem. Rev.* **87**, 1255–1270.

De Boer, E., van Kooyk, Y., Tromp, M. G. M., Plat, H. & Wever, R. (1986). *Biochim. Biophys. Acta*, **869**, 48–53.

Dumontet, C. & Rousset, B. (1983). *J. Biol. Chem.* **258**, 14166–14172.

Gross, G. G. (1977). *Rec. Adv. Phytochem.* **11**, 141–148.

Hecht, H. J., Sobek, H., Haag, T., Pfeifer, O. & van Pée, K.-H. (1994). *Nature Struct. Biol.* **1**, 532–537.

Howard, A. J., L , Gilliland, G., Finzel, B. C., Poulos, T. L., Ohlendorf, D. H. & Salemme, F. R. (1987). *J. Appl. Cryst.* **20**, 383–387.

Jancarik, J. & Kim, S. H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Knüttel, K., Müller, A., Rehder, D., Vilter, H. & Wittneben, V. (1992). *FEBS Lett.* **302**, 11–14

Krenn, B. E., Plat, H. & Wever, R. (1988). *Biochim. Biophys. Acta*, **952**, 255–260.

Küsthardt, U., Hedman, B., Hodgson, K.O., Hahn, R. & Vilter, H. (1993). *FEBS Lett.* **329**, 5–8

Liu, T. N. E., M'Timkulu, T., Geigert, J., Wolf, B., Neidleman, S. L., Silva, D. & Hunter-Cevera, I. (1987). *Biochem. Biophys. Res. Commun.* **142**, 329–333.

Manthey, J. A. & Hager, L. P. (1981). *J. Biol. Chem.* **256**, 11232–11238.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Morris, D. R. & Hager, L. P. (1966). *J. Biol. Chem.* **241**, 1763–1768.

Müller-Fahrnow, A., Hinrichs, W., Saenger, W. & Vilter, H. (1988). *FEBS Lett.* **239**, 292–294

Neidleman, S. L. & Geigert, J. (1985). *Biohalogenation: Principles, Basic Roles and Application*, pp. 156–169. London: Ellis Horwood.

Rehder, D., Holst, H., Priebsch, W. & Vilter, H. (1991). *J. Inorg. Biochem.* **41**, 171–185

Rüdiger, A. & Vilter, H. (1994). Unpublished results

Tien, M. & Myer, S. B. (1977). *Appl. Environ. Microbiol.* **56**, 2540–2544.

Pée, K.-H. van & Lingens, F. (1985). *J. Gen. Microbiol.* **131**, 1911–1916.

Pée, K.-H. van, Sury, G. & Lingens, F. (1987). *Hoppe-Seyler Biol. Chem.* **368**, 5890–5894.

Vilter, H. (1984). *Phytochemistry*, **23**, 1387–1390.

Vilter, H. (1995). *Vanadium-Dependent Haloperoxidases.* In *Metal Ions in Biological Systems – Vanadium and Its Role in Life*, Vol. 31, ed. 1, ch. 10, edited by H. Sigel & A. Sigel, pp. 665–672. New York: Marcel Dekker.

Webbs, E. C. (1992). *Enzyme Nomenclature.* San Diego: Academic Press.

Weidemann, C., Rehder, D., Kuetgens, U., Hormes, J. & Vilter, H. (1989). *Chem. Phys.* **136**, 405–412

Wiesner, W., van Pée, K.-H. & Lingens, F. (1988). *J. Biol. Chem.* **263**, 13725–13732.

Yang, S. F. (1967). *Arch. Biochem. Biophys.* **122**, 122.

Zeng, J. & Fenna, R. E. (1992). *J. Mol. Biol.* **226**, 185–207.