Volume 239, number 2, 292–294 FEB 06484 November 1988

The first crystallization of a vanadium-dependent peroxidase

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Received 12 August 1988

Single crystals of a vanadium-containing peroxidase from the brown alga *Ascophyllum nodosum* have been grown by the vapour diffusion technique using polyethylene glycol 6000 along with sodium chloride as precipitant. The crystals belong to the monoclinic space group $P2_1$. X-ray diffraction extends to at least 2.4 Å. The cell dimensions $a=173.0$, $b=164.9$, $c=68.5$ Å, $\beta=94.5°$ indicate that there are four molecules of 100 kDa per asymmetric unit, suggesting that the native enzyme might occur as a tetramer.

Peroxidase; Vanadium(V); Crystallization; (Seaweed, *Ascophyllum nodosum*)

1. INTRODUCTION

Research carried out during the last decade has confirmed that vanadium is one of the transition metals with a biological task. It is involved in nitrogen fixation [1] and biosynthesis of halogenated compounds [2]. Nutrition experiments indicated the essential function of vanadium traces for higher animals [3]. Vanadium-containing blood cells from ascidians are presently under investigation [4], and vanadium-accumulating mushrooms have been described [5].

The marine brown alga *Ascophyllum nodosum* contains various peroxidases. One of them (A.n.I) has a molecular mass of approx. 100 kDa and catalyzes brominations and iodinations [6,7]. The enzyme and its active site have been characterized by several chemical and spectroscopic methods. In contrast to other peroxidases A.n.I does not belong to the hemoproteins as indicated by the lack of Soret band in the electron absorption spectrum [7,8]. It contains vanadium as prosthetic group instead of the heme. The removal and uptake of vanadate ions by the protein are reversible as shown by the activity tests [9,10].

The oxidation state (V) of the metal was detected by ^{51}V NMR and X-ray absorption spectroscopy [11–13]. In contrast to the tetrahedral vanadate ion, vanadium(V) in the native enzyme is surrounded by six to eight oxygen functions, some of which belong to carboxylate groups. The coordination sphere of the vanadium is changed and simultaneously decreased by reduction to vanadium(IV) [11].

We have crystallized A.n.I and intend to solve its three-dimensional structure in order to gain detailed insights into this unusual type of peroxidases.

2. MATERIALS AND METHODS

Highly purified peroxidase A.n.I was isolated in a large-scale process [14]. All salts and reagents used for crystallization trials were of analytical grade. PEG 6000 and 4000 were obtained from Merck (Darmstadt).

Crystals were grown by the vapour diffusion technique at room temperature with PEG/NaCl or ammonium sulfate. In the hanging-drop variant 7 μl of the protein solution were mixed with an equal volume of the reservoir solution and pipetted onto siliconized cover slides. They were sealed with silicon grease on tissue culture plates (Linbro, Flow Labs) containing

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

500 μl reservoir solution per well. After 24–48 h they were seeded with a freshly prepared seeding solution. The concentration of the protein solution was 10–20 mg/ml in 5 mM Tris-HCl buffer at pH 9.0.

3. RESULTS

Crystals of A.n.I were grown with ammonium sulfate or with PEG/NaCl. Ammonium sulfate as precipitant yielded very small, tetragonal-shaped crystals after a long time. The best single crystals used for X-ray experiments were grown in the presence of 500 mM NaCl and 15–18% PEG 6000 at pH 5.5 in 50 mM acetate buffer.

Table 1

Crystallographic data for A.n.I

Space group $P2_1$
a = 173.0 Å, b = 164.9 Å, c = 68.5 Å, β = 94.5°
V = 1950000 Å^3
Assuming 8 molecules per unit cell, we calculate
$\bar{V}$ = 2.4 Å^3/Da
$V_{solvent}$ = 49%

Changing the pH by 0.5 units leads either to unstable or intergrown crystals. Without seeding, phase separation occurred after 2 days followed by

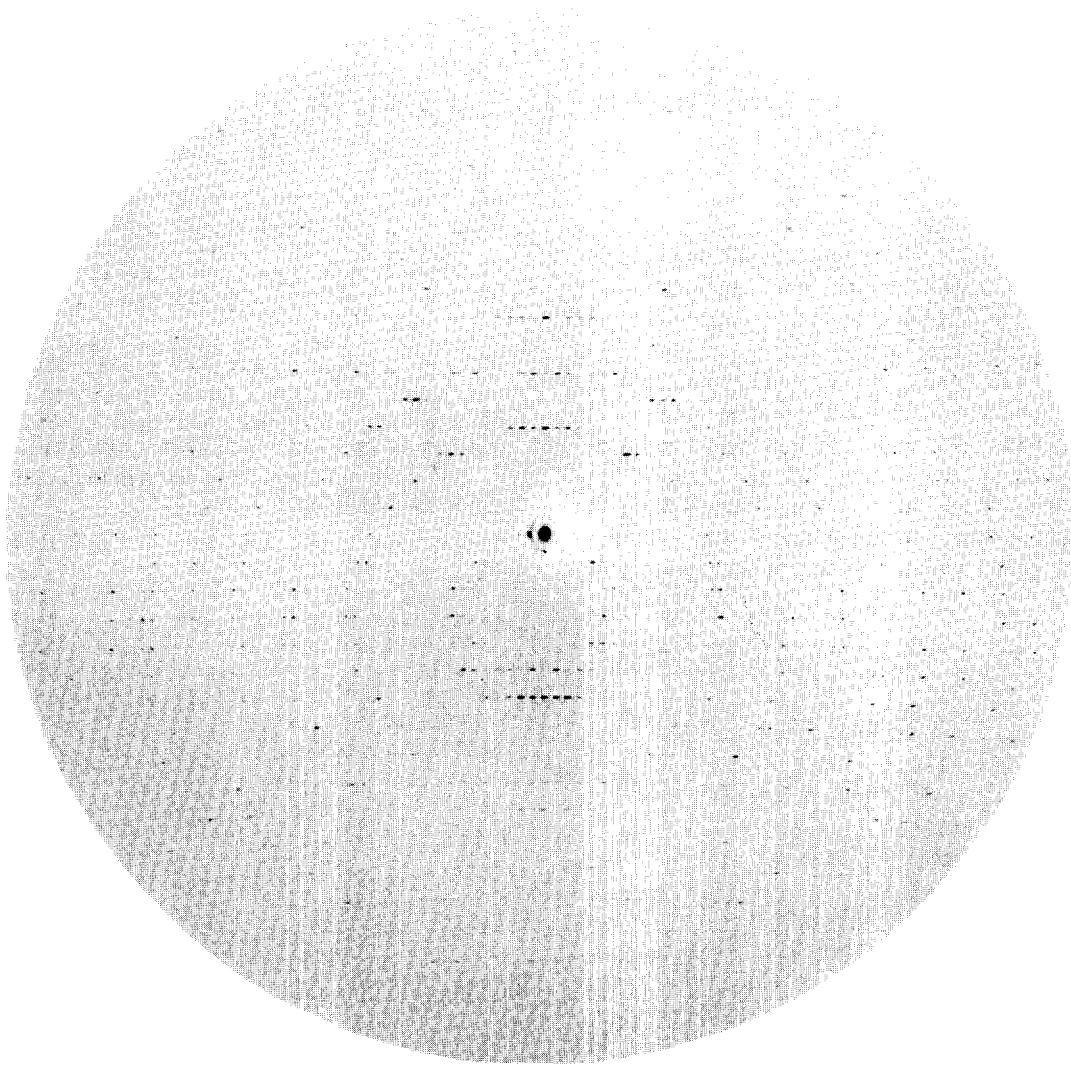

Fig.1. 1° oscillation photograph of the peroxidase A.n.I about b^* near the $0kl$ layer. Taken at the synchrotron beamline in Hamburg

the formation of microcrystals. To avoid this, the setups had to be seeded after 24–48 h.

The crystals reached their final size (0.5 × 0.15 × 0.15 mm^3) 4 days after seeding. They diffract beyond 2.4 Å and are reasonably stable without any radiation damage for 2 days. The crystal system and cell dimensions were determined from precession photographs (GX20 rotating anode operating at 40 kV, 70 mA). The monoclinic crystals belong to space group $P2_1$, indicated by systematic absences. Crystallographic data are given in table 1.

At present the amino acid sequence of A.n.I is unknown. Therefore, we have used the approximate value of the molecular mass, 100 kDa [7], for crystallographic calculations [15]. Heavy-atom derivatives are at present being screened. Fig.1 shows an oscillation photograph taken at the synchrotron beamline in Hamburg.

4. DISCUSSION

We report here the first crystallization of a vanadium-dependent enzyme. The unusually high number of four molecules per asymmetric unit could indicate that native A.n.I occurs as a tetramer. Further studies are needed to clarify this point which is of particular interest in view of several peroxidases, which were isolated and identified from other sources of the marine environments. They all contain vanadate(V) as prosthetic group, in contrast to the common haloperoxidases which depend on the heme group [16].

Acknowledgements: These studies were supported by the Deutsche Forschungsgemeinschaft (W.S., Sonderforschungsbereich 9, Teilprojekt A 7; H.V., Vi 91/1-2 Peroxidasen) and Fonds der Chemischen Industrie.

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