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Crystallization and preliminary crystallographic analysis of selenomethionine-labelled progesterone 5β-reductase from *Digitalis lanata* Ehrh

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Progesterone 5β-reductase (5β-POR) catalyzes the reduction of progesterone to 5β-pregnane-3,20-dione and is the first stereospecific enzyme in the putative biosynthetic pathway of *Digitalis* cardenolides. Selenomethionine-derivatized 5β-POR from *D. lanata* was successfully overproduced and crystallized. The crystals belong to space group $P4_32_12$, with unit-cell parameters a = 71.73, c = 186.64 Å. A MAD data set collected at 2.7 Å resolution allowed the identification of six out of eight possible Se-atom positions. A first inspection of the MAD-phased electron-density map shows that 5β-POR is a Rossmann-type reductase and the quality of the map is such that it is anticipated that a complete atomic model of 5β-POR will readily be built.

1. Introduction

Leaves from *Digitalis* plants are still the major source of cardenolides used for the treatment of cardiac insufficiency in humans. Cardenolides, also known as cardiac glucosides, are characterized by a typical steroid nucleus with its four rings connected in a *cis–trans–cis* configuration. Diversity within the cardenolides is generated through the attachment of sugar side chains of variable length to position C-3 of the cardenolide ring system. The biosynthesis pathway of cardenolides has been elucidated in principle with the use of radiolabelled precursors, but at present it is still not fully understood (Kreis *et al.*, 1998).

About 20 enzymes have been identified that contribute to the formation of cardenolides (Kreis *et al.*, 1998). Among those that have been further purified and characterized is the enzyme progesterone 5β-reductase (5β-POR), which catalyzes the conversion of progesterone into 5β-pregnane-3,20-dione (Gärtner *et al.*, 1990, 1994). Recently, we succeeded in cloning, overproducing and fully characterizing 5β-POR from *D. lanata* (Herl *et al.*, 2006). Recombinant 5β-POR specifically catalyzes the formation of 5β-pregnane-3,20-dione and displays some side specificity for substrates such as cortisol and cortexone (Herl *et al.*, 2006).

5β-POR is the first stereospecific enzyme of the cardenolide-biosynthesis pathway and its sequence is highly conserved among *Digitalis* family members (Herl *et al.*, 2006). However, only little sequence identity can be detected between 5β-POR and any other reductase of known three-dimensional structure. According to the MODBASE database (Pieper *et al.*, 2004), the enzyme GDP-mannose-4,6-dehydratase from *Escherichia coli* (PDB code 1db3; Berman *et al.*, 2002) serves as a good structural model (model score 0.96 out of 1.00), sharing a sequence identity of 19% with 5β-POR. From this, it can be safely assumed that 5β-POR is a member of the Rossmann-fold superfamily of NAD(H)-dependent enzymes (Rossmann & Argos, 1976; Branden & Tooze, 1999); however, only a detailed crystallographic analysis will generate the amount of atomic detail needed to fully understand the specificity and efficiency of this enzyme. Here, we report the production and crystallization of selenomethionine-labelled 5β-POR and show that a MAD data set collected from these crystals at 2.7 Å resolution is of sufficient quality to yield an electron-density map that can be readily interpreted.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the outer resolution shell.

Data set	Inflection point	Peak	Remote
Space group	$P4_32_12$		
Unit-cell parameters (Å)	a = 71.73, b = 71.73, c = 186.64		
Matthews coefficient (Å^3 Da^{-1})	2.6		
Molecules per ASU	1		
Solvent content (%)	53.1		
Wavelength (Å)	0.9799	0.9798	0.9082
Resolution range (Å)	20–2.7 (2.8–2.7)	20–2.7 (2.8–2.7)	20–2.7 (2.8–2.7)
Unique reflections	25557	25557	25554
Average redundancy	5.6	7.7	5.4
Completeness (%)	99.7 (100.0)	99.7 (100.0)	99.7 (100.0)
Average $I/\sigma(I)$	19.6 (3.1)	21.2 (3.5)	19.7 (2.9)
R_{merge} (%)	5.7 (66.8)	6.5 (74.6)	5.7 (69.0)
R_{meas} (%)	6.3 (73.7)	6.9 (79.9)	6.3 (76.5)
Figure of merit	0.56 and 0.63 (prior to and after density modification, respectively)		

2. Materials and methods

2.1. Protein production and purification

Recombinant 5β-POR from *D. lanata* was overproduced using the pQE expression system (Herl *et al.*, 2006). In this expression construct, the 398 residues of 5β-POR are fused to the N-terminus of a six-residue-long His tag. In order to produce selenomethionine-substituted 5β-POR, the plasmid carrying the gene was transformed into the methionine-auxotroph *E. coli* strain B834(DE3) and the bacteria were first grown in 650 ml minimal medium (Budisa *et al.*, 1995) containing regular methionine to an optical density at $OD_{600\,nm}$ of about 0.6. The cells were then harvested by centrifugation and the bacterial pellet redissolved in 600 ml minimal medium supplemented with 0.3 m*M* selenomethionine and 2 m*M* DTT. Protein expression was induced by the addition of 0.1 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) and the cells were grown for a further 96 h at 277 K. The cells were then harvested and the protein purified to homogeneity using Ni–NTA affinity chromatography as described previously (Herl *et al.*, 2006). The protein, which was fully functional in a standard enzyme assay (Fig. 1), was concentrated to 17 mg ml^{-1} in a buffer containing 2 m*M* Tris–HCl pH 7.5 and used as such in subsequent crystallization trials.

2.2. Crystallization

Initial crystallization conditions were obtained applying the sparse-matrix screening approach (Jancarik & Kim, 1991) by using a commercially available crystallization screen (Hampton Research). Although screening was first performed with unlabelled protein, we observed that the selenomethionine-labelled protein behaved quite similarly. Upon mixing 1 µl protein solution with 1 µl of a reservoir solution containing 30%(*w*/*v*) PEG 4000, 0.2 *M* ammonium acetate and 0.1 *M* sodium citrate pH 5.6, crystals were obtained within 30 min of dimensions up to 150 µm using the hanging-drop method and equilibrating the droplet against 700 µl reservoir solution. The crystals used for the diffraction experiments were obtained after further optimization of the crystallization conditions and were obtained using a reservoir solution containing 21.4% PEG 4000, 0.05 *M* ammonium acetate and 0.08 *M* sodium citrate pH 6.0. Upon mixing the protein and reservoir solution, the droplets immediately turn white owing to extensive precipitation. Crystals appear reproducibly in the cloudy droplets within 3 d (Fig. 2). The crystals are difficult to manipulate because they strongly adhere to the protein skin that forms within the crystallization droplets. Remarkable changes in the crystal properties occur as a function of time. Whereas freshly prepared crystals are as soft as butter and can be easily destroyed by slight mechanical pressure, several day-old crystals are rubber-like and extremely resistant against pressure. Freshly grown crystals also scatter X-rays to a significantly higher resolution than aged crystals.

2.3. Crystallographic data collection and analysis

A complete multiple anomalous dispersion (MAD) data set was collected at the Protein Structure Factory beamline BL14.1 of Free University Berlin at the BESSY Synchrotron, Berlin. For this purpose, a single selenomethionine-labelled crystal was soaked for 5 h in a cryoprotection solution consisting of 0.1 *M* sodium citrate pH 6.0, 0.1 *M* ammonium acetate, 20%(*w*/*v*) PEG 4000 and 30%(*v*/*v*) PEG 400 and subsequently flash-frozen in liquid nitrogen. Three individual data sets were collected at three different wavelengths (inflection point, peak and remote wavelength) using 0.5° rotation steps and covering total rotation ranges of between 90 and 130°. The different scans were processed individually with *XDS* (Kabsch, 1988) and then scaled together with *XSCALE* (Kabsch, 1988). The data sets obtained are highly complete and of reasonable quality and redundancy (Table 1). The crystal unit-cell parameters are such that only a single protein molecule fits into the crystallographic asymmetric unit. The calculated Matthews coefficient (Matthews, 1968) is 2.6 Å^3 Da^{-1} and the solvent content is 53.1%. Using the program *SOLVE*

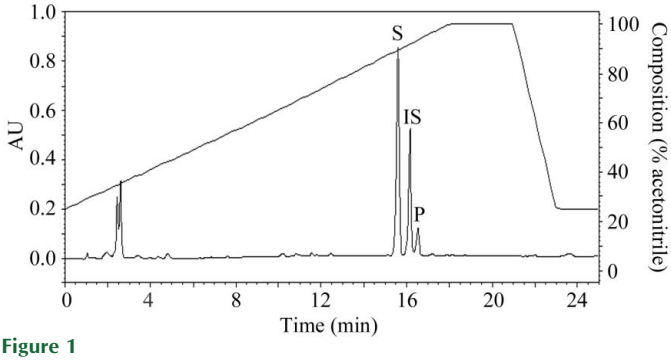


Figure 1
HPLC analysis of the selenomethionine-labelled 5β-POR enzyme activity: S, progesterone; P, 5β-pregnane-3,20-dione; IS, pregnenolone (internal standard).

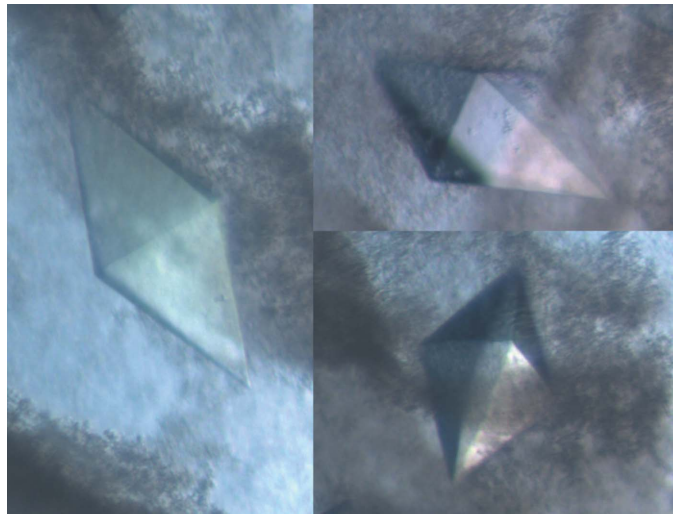
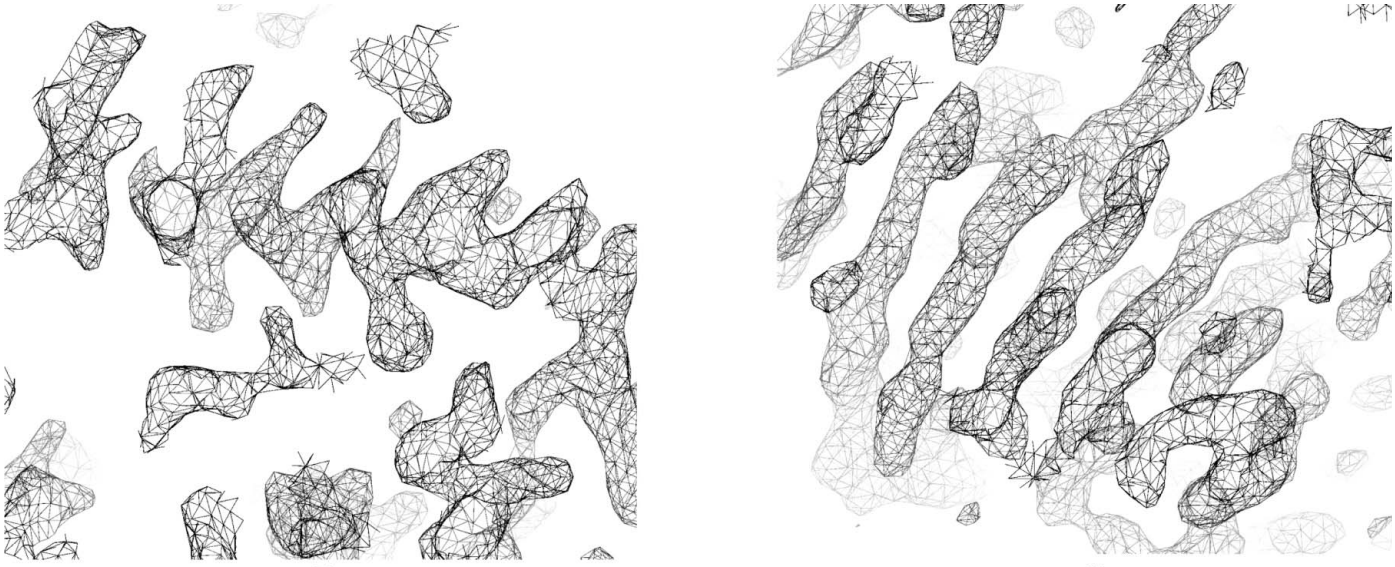


Figure 2
Crystals of 5β-progesterone reductase from *D. lanata* belonging to space group $P4_32_12$.



(*a*) (*b*)

Figure 3
Two representative portions of the solvent-flattened MAD-phased electron-density map of 5β-POR calculated at 2.7 Å resolution. (*a*) α-Helical and (*b*) β-sheet region of 5β-POR.

(Terwilliger & Berendzen, 1999), we were able to locate six of the eight possible Se atoms that are present in recombinant 5β-POR. Further processing of the data set with *RESOLVE* (Terwilliger, 2000) increased the figure of merit from 0.56 to 0.63. Inspection of the final electron-density map clearly identified space group $P4_32_12$ as the space group with the correct hand (Fig. 3).

3. Results and discussion

We succeeded in crystallizing selenomethionine-derivatized 5β-POR from *D. lantana*. The crystals belong to space group $P4_32_12$, contain a single 5β-POR molecule in the asymmetric unit and tend to diffract anisotropically. Whereas the diffraction pattern extends beyond 2.7 Å in direction of the crystal *c* axis, diffraction barely extends to 2.7 Å within the crystallographic *ab* plane. A MAD data set collected from a single selenomethionine-labelled crystal could be readily interpreted and yielded an electron-density map in which features such as α-helices and β-sheets are clearly discernable (Fig. 3). A preliminary inspection of the map hints that 5β-POR contains a Rossmann fold (Branden & Tooze, 1999) and that the quality of the MAD phases is such that a detailed atomic model can be built.

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References

Berman, H. M., Battistuz, T., Bhat, T. N., Bluhm, W. F., Bourne, P. E., Burkhardt, K., Feng, Z., Gilliland, G. L., Iype, L., Jain, S., Fagan, P., Marvin, J., Padilla, D., Ravichandran, V., Schneider, B., Thanki, N., Weissig, H., Westbrook, J. D. & Zardecki, C. (2002). *Acta Cryst.* D**58**, 899–907.
Branden, C.-I. & Tooze, J. (1999). *Introduction to Protein Structure.* New York: Garland.
Budisa, N., Steipe, B., Demange, P., Eckerskorn, C., Kellermann, J. & Huber, R. (1995). *Eur. J. Biochem.* **230**, 788–796.
Gärtner, D. E., Keilholz, W. & Seitz, H. U. (1994). *Eur. J. Biochem.* **225**, 1125–1132.
Gärtner, D. E., Wendroth, S. & Seitz, H. U. (1990). *FEBS Lett.* **271**, 239–242.
Herl, V., Fischer, G., Müller-Uri, F. & Kreis, W. (2006). *Phytochemistry*, **67**, 225–231.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kabsch, W. (1988). *J. Appl. Cryst.* **21**, 916–924.
Kreis, W., Hensel, A. & Stuhlemmer, U. (1998). *Planta Med.* **64**, 491–499.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Pieper, U., Eswar, N., Braberg, H., Madhusudhan, M. S., Davis, F. P., Stuart, A. C., Mirkovic, N., Rossi, A., Marti-Renom, M. A., Fiser, A., Webb, B., Greenblatt, D., Huang, C. C., Ferrin, T. E. & Sali, A. (2004). *Nucleic Acids Res.* **32**, D217–D222.
Rossmann, M. G. & Argos, P. (1976). *J. Mol. Biol.* **105**, 75–95.
Terwilliger, T. C. (2000). *Acta Cryst.* D**56**, 965–972.
Terwilliger, T. C. & Berendzen, J. (1999). *Acta Cryst.* D**55**, 849–861.