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Crystallization and preliminary X-ray diffraction studies of psoriasin

SIGRUN NOLSØE, SØREN THIRUP, MICHAEL ETZERODT, HANS CHRISTIAN THØGERSEN AND JENS NYBORG, *at Institute of Molecular and Structural Biology, University of Århus, DK-8000 Århus C, Denmark. E-mail: jnb@kemi.aau.dk*

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Abstract

Crystals of psoriasin, a protein related to the skin disease psoriasis, have been grown in two different crystal forms. Form I represents the protein in the Ca^{2+}-bound form, and form II represents the protein in the Zn^{2+}- and Ca^{2+}-bound form. The crystals of form I are orthorhombic belonging to the space group $P2_12_12_1$ with cell parameters $a = 52.15$, $b = 56.67$ and $c = 76.38$ Å and diffract to 2.4 Å. The crystals of form II are tetragonal and belong to the space group $P4_{1(3)}2_12$ with cell parameters $a = b = 51.86$, $c = 115.93$ Å and diffract to 2.0 Å.

1. Introduction

Psoriasin is a 11.45 kDa acidic protein that exhibits restricted tissue distribution and is highly up-regulated in psoriatic skin (Madsen, Rasmussen, Leffers, Honoré & Celis, 1992). It is a member of the S100 protein family, a family of small (9–12 kDa) homologous acidic proteins with sequence similarities. The exact function of these proteins is unknown but they are believed to mediate a variety of functions in eukaryotic cells, which include differentiation, cell-cycle progression, transmission of Ca^{2+} signals, and cytoskeletal membrane interactions (Hilt & Kligman, 1991; Zimmer, Cornwall, Landar & Song, 1995; Kretsinger, 1980). The gene coding for psoriasin has, together with the genes coding for nine other S100 proteins, been localized on a yeast artificial clone from human chromosome 1q21 (Schäfer, Wicki, Engelkamp, Mattei & Heizmann, 1995). According to the new nomenclature, based on the physical arrangement of the S100 proteins on chromosome 1q21, psoriasin has been termed S100A7.

S100 proteins contain two calcium-binding helix–loop–helix motifs usually referred to as EF hands (Kretsinger, 1980). The N-terminal EF hand differs from the canonical C-terminal EF hand in containing an insert of two amino acids in the calcium-binding loop (Hilt & Kligman, 1991). Though it contains some of the features of the S100 proteins, psoriasin is one of the most divergent members of the family, with only 26.8% identity to calcyclin (S100A6), and 29.1% identity to the intestinal calcium-binding protein (CALB3), on the amino-acid level. Psoriasin contains the C-terminal canonical EF hand but it does not contain the characteristic modified N-terminal EF hand of the S100 proteins. Sequence alignment of the S100 proteins shows that psoriasin has a deletion of three amino acids in the modified calcium-binding loop. Psoriasin has by Vorum *et al.* (1997) been shown to bind both calcium and zinc. Difference absorption spectra also show that the binding of each of the ions has been shown to be accompanied by conformational changes that probably involve the moving of tyrosine residues to more hydrophobic areas (Vorum *et al.*, 1997). This is consistent with reports on other S100 proteins. UV absorption spectroscopy studies of S100A1 and S100B show that the binding of Ca^{2+} and Zn^{2+} ions induce conformational changes. The conformational changes induced by Ca^{2+} ions are different from those induced by Zn^{2+} ions, and the binding sites for Ca^{2+} and Zn^{2+} are believed to be different (Baudier, Glasser & Gérard, 1986; Ogoma *et al.*, 1992; Baudier & Gérard, 1983). Chemical crosslinking and sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of the calcium-bound as well as the calcium-free protein shows, that psoriasin like some of the other S100 proteins is a dimeric molecule in solution (Etzerodt unpublished results; Potts *et al.*, 1995).

Here we report the crystallization and preliminary X-ray analysis of two different crystal forms of psoriasin: form I, crystallized in the presence of Ca^{2+} and form II, crystallized in the presence of Zn^{2+}.

2. Materials and methods

Recombinant psoriasin expressed in *Escherichia coli* BL21 cells, *in vitro* refolded and purified by a procedure described earlier by Hoffmann *et al.* (1994) was used with minor modifications in the present studies. The protein was stored in a buffer containing 10 m*M* Tris–HCl, 10 m*M* NaCl and 0.1 m*M* $CaCl_2$ (pH 7.7). Protein used for crystallization was concentrated to $\sim$15 mg ml^{-1} in a buffer containing 10 m*M* Tris and 10 m*M* NaCl (pH 7.7) in Centricon-10 cells (Amicon Inc., Beverly, MA 01915 USA) and flash frozen in liquid nitrogen. Some experiments were also made with the protein in the apo state. EDTA was added to the protein to a concentration of 25 m*M* EDTA, and the buffer was changed to 10 m*M* Tris, 10 m*M* NaCl and 0.1 m*M* EDTA (pH 7.7). The procedure of Jancarik & Kim (1991) was used for the initial screening for crystallization conditions. Promising conditions were further refined by exploring a matrix of conditions including various molecular weights of PEG, pH, and additives, as well as the concentration of PEG, the additives and the protein. Crystals were grown by the vapour-diffusion method with 4 µl sitting drops in Cryschem trays (Charles Supper Co., Massachusetts, USA) using a modified Gilson crystallization robot (Andersen & Nyborg, 1996).

A crystal of form I was mounted in a glass capillary, and data were collected at room temperature with a R-AXIS II image plate using Cu $K\alpha$ radiation $\lambda = 1.5418$ Å (Rigaku RU-200HB, rotating anode). A data set was collected on a single crystal 200 × 200 × 300 µm with a crystal-to-image-plate distance of 100 mm. The oscillation range was 2.5° and the time of exposure was 20 min for each frame. Diffraction was recorded over 180°.

Data on form II were collected at cryogenic temperature, $\lambda = 0.862$ Å at beamline BW 7B at EMBL in Hamburg. The crystals were mounted in loops directly from the mother liquor and flash frozen at 100 K in a stream of nitrogen. A data set was collected on a crystal 125 × 125 × 250 µm with a crystal-to-image-plate distance of 320 mm. The oscillation range used was between 1 and 2° and diffraction was recorded over 90°.

The program *DENZO* (Otwinowski, 1991) was used for indexing and integration of data. Programs from the *CCP*4

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package (Collaborative Computational Project, Number 4, 1994) were used for scaling, postrefinement and merging. Calculations of the self-rotation functions were performed using *AMoRe* (Navaza, 1994)

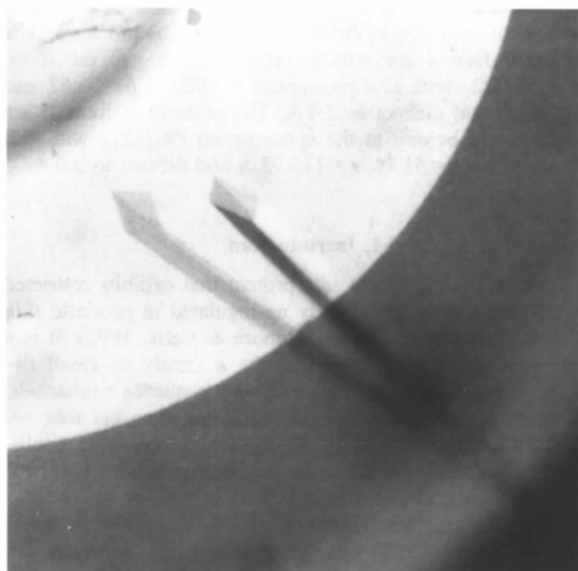
3. Results and discussion

Rod-shaped crystals (Fig. 1*a*) of psoriasin formed in 3–10 d by mixing 5.5 mg ml^{-1} protein in 10 m*M* Tris, 10 m*M* NaCl, pH 7.7 with reservoir solution containing 22%(*w*/*v*) PEG 4000, 5–10% glycerol, 5–10 m*M* $CaCl_2$ at a pH of 6.5 to 8.0. The best crystals were obtained at 293 K. The maximum size obtained was 200 × 200 × 500 μm. Crystals with the same morphology have been found in both PEG 4000 and PEG 8000 in the concentration range 22–27%(*w*/*v*). Bipyramidical crystals (Fig. 1*b*) formed in a week by mixing 14 mg ml^{-1} protein in the previously mentioned buffer with reservoir solution containing 5–12% PEG 4000, 10% glycerol and 20 m*M* $ZnSO_4$ at pH 6.7. Crystals formed at 277 K as well as at 293 K, but they grew to significantly larger dimensions at 277 K. The maximum size obtained was 125 × 125 × 250 μm. The presence of zinc sulfate proved to be essential for formation of crystal form II. No crystals nor precipitates were obtained from experiments with both Ca^{2+} and Zn^{2+} added to the crystallization solution under otherwise identical conditions. Refinement of the crystallization conditions has not been pursued in the presence of Ca^{2+} and Zn^{2+}.

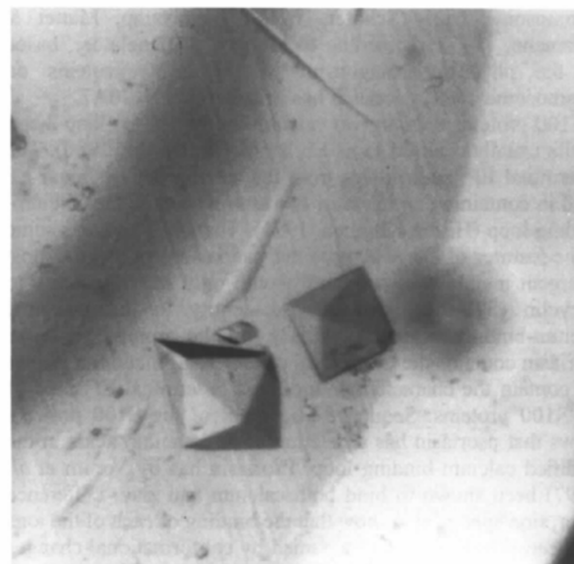
Psoriasin has, as mentioned previously, been shown to bind both zinc and calcium (Hoffmann *et al.*, 1994; Vorum *et al.*, 1996). Calcium is present (2 m*M* $CaCl_2$) throughout the refolding and purification procedure. On account of this, and the fact that recombinant psoriasin has been shown to bind calcium (Hoffmann *et al.*, 1994; Vorum *et al.*, 1996), the protein in both form I and form II probably binds one or several calcium ions per protein molecule. The crystals of form II crystallized in the presence of zinc, are therefore believed to represent the protein in a zinc- and calcium-bound form, and the crystals of form I crystallized in the presence of calcium to represent the protein in a calcium-bound form. The excact number of Ca^{2+} and Zn^{2+} ions bound under these conditions is not known, however. Crystallization experiments have also been performed with the apo-protein, where the Ca^{2+} ions were removed by addition of EDTA. However, the protein seemed to be very hydrophobic in the absence of calcium and only microcrystals, not suitable for X-ray diffraction were obtained.

A data set with 5946 unique reflections in space group $P2_12_12_1$ was collected from a single crystal of form I giving a data-set completeness of 99.8% at 2.8 Å. The R_{merge} of the data set is 10.0%. The crystal showed no significant decay upon exposure and diffracted to approximately 2.4 Å resolution. On the second form a data set with 10 574 unique reflections in $P4_{1(3)}2_12$ was collected also from a single crystal. The completeness of the data set was 94.8% at 2.0 Å. The R_{merge} of the second data set is 7.3% and the crystals diffract to approximately 1.8 Å resolution (Table 1). The assignment of screw axes was based on the criteria that the measured intensity of any systematically absent reflection did not exceed 3σ.

The refined unit-cell dimensions for form I are a = 52.15, b = 56.67 and c = 76.70 Å. The volume-to-mass ratio (V_m) for two molecules in the asymmetric unit is 2.47 Å Da^{-1} and the estimated solvent content is 49%. This agrees with the V_m and solvent content normally found in protein crystals (Matthews, 1968). The refined unit-cell dimensions for form II are $a = b = 51.86$, $c = 115.93$ Å and the unit-cell volume is 3.1×10^{-5} Å^3. This gives a possible number of molecules in the asymmetric unit of one or two, which gives a solvent content of 64 and 28%, respectively. The self-rotation function calculated using data from form I and form II, did not show any non-crystallographic twofold axis in either case. A native Patterson map, calculated for form II, did show a peak at a height of 6σ corresponding to 10% of the origin peak. The peak position is consistent with a non-crystallographic twofold axis parallel to a. Consequently the asymmetric unit contains two



(*a*)



(*b*)

Fig. 1. (*a*) Crystal form I. Crystals of psoriasin, crystallized in the presence of $Ca^{2+}$with dimensions 500 × 50 × 50 μm. (*b*) Crystal form II. Crystals of psoriasin, crystallized in the presence of Zn^{2+} with dimensions 100 × 100 × 200 μm.

Table 1. *Data-collection statistics for crystal form II*

Breakdown of intensities after scaling and merging as output from *AGROVATA*. The statistics are given in shells of resolution.

d_{min} (Å)	$R_{sym}(I)$	$\langle I/\sigma \rangle$	No. of unique reflections	Completeness (%)	Multiplicity (%)
6.06	0.041	12.5	441	96.1	3.4
4.39	0.040	15.6	685	97.9	3.4
3.61	0.039	14.8	834	96.3	3.1
3.14	0.049	12.6	944	95.0	2.9
2.81	0.064	11.0	1029	92.6	2.8
2.57	0.087	8.3	1154	94.6	2.9
2.39	0.117	6.1	1218	92.7	2.9
2.23	0.154	4.7	1351	95.8	2.9
2.11	0.182	4.0	1421	95.3	2.9
2.00	0.259	2.8	1497	95.3	3.0
Total	0.073	8.7	10574	94.8	3.0

molecules. This agrees well with the high diffraction quality of these crystals which would not be expected from crystals with high solvent content.

In conclusion, a structure for psoriasin in the calcium-bound form and in the zinc- and calcium-bound form may be obtained, either by the preparation of isomorphous heavy-atom derivatives, or by molecular replacement of other S100 proteins as ICaBP (Szebenyi & Moffat, 1986) or calcyclin (Potts *et al.*, 1995).

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