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Crystallization and initial X-ray analysis of rabbit mature sterol carrier protein 2

Thomas Choinowski, James H. Dyer, Bernhard Maderegger,† Kaspar H. Winterhalter, Helmut Hauser and Klaus Piontek*

Institut für Biochemie, Swiss Federal Institute of Technology (ETH), Universitätstrasse 16, CH-8092 Zürich, Switzerland

† Present address: Institut für Organische Chemie, Karl-Franzens Universität Graz, Heinrichstrasse 28, A-8010 Graz, Austria.

Correspondence e-mail: klaus.piontek@bc.biol.ethz.ch

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Sterol carrier protein 2 (SCP2) is a basic intracellular protein which facilitates the *in vitro* intermembrane transfer of cholesterol, phospholipids and glycolipids. SCP2 was expressed in *Escherichia coli*, purified to apparent electrophoretic homogeneity and crystallized. Single crystals were obtained by hanging-drop vapour diffusion using ammonium sulfate as precipitant. These crystals belong to space group $P4_12_12$ or its enantiomorph, with unit-cell parameters $a = b = 57.5$, $c = 86.5$ Å, and have one molecule in the crystallographic asymmetric unit. Intensity data to 1.8 Å resolution were collected from native SCP2 crystals using synchrotron radiation, were processed and scaled with an $R_{\rm linear} = 4.9\%$.

1. Introduction

The intracellular sterol carrier protein 2 (SCP2), also called non-specific lipid-transfer protein, has been associated with lipid trafficking and metabolism (Wirtz, 1997). This small basic protein of about 13 kDa is expressed in all mammalian tissues examined (Myers-Payne *et al.*, 1996; Wirtz, 1991) and its primary structure is highly conserved among species. It is encoded by a single gene which has two initiation sites (Seedorf *et al.*, 1994). The translation products are a 15 kDa pre-SCP2 and a 58 kDa SCPX protein. A 20-residue leader sequence is rapidly cleaved from the pre-SCP2 after translation, yielding the mature 13.2 kDa SCP2 (Seedorf *et al.*, 1994). SCPX is a fusion protein between a thiolase domain and SCP2, which is located at the C-teminus (Ossendorp *et al.*, 1991). SCP2 carries a C-terminal peroxisomal targeting signal and has been shown to be predominantly localized in peroxisomes (Ossendorp & Wirtz, 1993), while SCPX exclusively occurs in these organelles which are involved in the oxidative metabolism of fatty acids (Van Heusden *et al.* (1990). SCP2 activates the enzymatic *in vitro* conversion of 7-dehydrocholesterol to cholesterol (Noland *et al.*, 1980), cholesterol esterification, bile acid biosynthesis and the formation of progesterone (for a review, see Pfeifer *et al.*, 1993). Furthermore, SCP2 catalyzes the intermembrane transfer of cholesterol, phospholipids (Bloj & Zilversmit, 1977) and glycolipids (Zilversmit, 1984). In addition, it was found recently to bind *in vitro* fatty acids and fatty acyl coenzyme A, with K_d values in the μ*M* and n*M* ranges, respectively (Schroeder *et al.*, 1995; Frolov *et al.*, 1996; Stolowich *et al.*, 1997).

Since the discovery of SCP2 in 1968 (Wirtz & Zilversmit, 1968), an ever-increasing number of *in vitro* functions has emerged; the physiological role of SCP2, however, remains elusive. In contrast to the wide array of implicated functional roles for SCP2, very little is known about the structure underlying these functions. As a first step towards an understanding of the interaction of SCP2 with its various substrates, we have initiated the crystal structure determination of this unique and biologically interesting protein.

2. Materials and methods

Mature rabbit SCP2 cDNA was cloned into the pET 20b expression vector and the recombinant protein was expressed in *Escherichia coli* BL-21 cells. For the expression and purification of the protein, a modified protocol according to Weber *et al.* (1998) was used. Bacteria were grown at 310 K on standard LB medium (Life Technologies, Paisley, Scotland) containing 50 mg l^{-1} carbenicillin (Sigma, St Louis, USA). Protein expression was induced by addition of 2 m*M* isopropyl β-D-thiogalactopyranoside at optical density $OD_{600} \simeq 0.7$. Bacteria were harvested by centrifugation 3–4 h after induction, washed and resuspended in buffer *A* [10 m*M* Tris–HCl pH 8.0, 5 m*M* dithiothreitol (DTT), 2 m*M* EDTA and 0.005% sodium azide] supplemented with 2 m*M* phenylmethanesulfonylfluoride and 2 m*M* benzamidine–HCl as protease inhibitor. Bacteria were lysed and DNA sheared by several passages through a French pressure cell at 1.38×10^8 Pa. All following steps were performed at 277 K. The lysate was centrifuged for 30 min at 20000*g* and the supernatant was applied to a

Table 1
Summary of the processing statistics for SCP2 crystal form II.

Values in parentheses are for the highest resolution shell.

Resolution (Å)	18.0–1.8
Total number of observations	104415
Number of unique reflections	14102
Completeness (%)	99.9 (99.8)
I/σ	35.6 (5.6)
$R_{\rm linear}$ (I)†	4.9 (38.6)

† $R_{\rm linear} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h)$ for all i measurements.

DEAE–Sepharose column (Pharmacia, Uppsala, Sweden). SCP2 was eluted with buffer *A* in the flow-through and applied to a SP–Sepharose column (Pharmacia). After rinsing away unbound proteins with buffer *B* (10 m*M* Tris–HCl pH 7.0, 5 m*M* DTT, 2 m*M* EDTA, 0.005% sodium azide), bound SCP2 was eluted with a linear NaCl gradient (0.1–0.3 *M*) in buffer *B*. Fractions (10 ml) containing SCP2 were concentrated to about 3.0 ml by ultrafiltration using a YM3 membrane from Amicon (Amicon, Beverly, USA), with a nominal molecular-weight cutoff of 3000. This concentrate was loaded onto a Sephadex G-75 column (Pharmacia) and eluted with buffer *B*. Pure SCP2 fractions were concentrated as above. The purity of the resulting protein was examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), isoelectric focusing gel electrophoresis (IEF) and matrix-assisted laser desorption ionization mass spectrometry (MALDI–MS). For the IEF, special ampholytes in the p*I* range 9–11 (Fluka, Buchs, Switzerland) and 8–10.5 (Pharmacia) had to be used owing to the high p*I* (about 10) of rabbit SCP2. The protein was concentrated to about 10 mg ml^{-1}, stored at 277 K in buffer *B* and subsequently used for crystallization experiments. Protein concentration was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as reference protein.

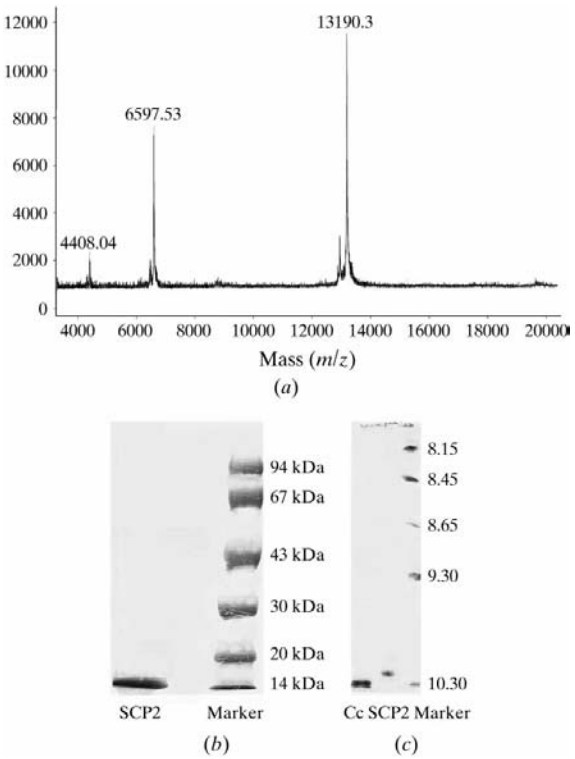


Figure 1
(*a*) The molecular mass of rabbit SCP2, purified as described in §2, was determined by MALDI–MS using time-of-flight mass analysis (Voyager Elite mass spectrometer from PerSeptive Biosystem). The peaks of decreasing intensity (from right to left) correspond to the molecular ions M^{1+}, M^{2+} and M^{3+}, respectively. The deviation of their mass from the theoretical value was about 0.2%. (*b*) SDS–PAGE (12% SDS) of purified rabbit SCP2 (left lane). Pharmacia protein standards were used as markers and the apparent molecular masses of these proteins in kDa are indicated on the right. (*c*) IEF gel of purified rabbit SCP2 (middle lane), horse heart cytochrome *c* (left lane) and protein standards (Pharmacia) as markers (right lane). The numbers on the right refer to the isoelectric points of the markers.

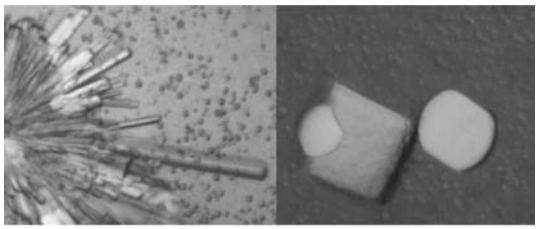
Initial crystallization conditions were established using the sparse-matrix screening approach of Jancarik & Kim (1991) within the Crystal Screen I kit (Hampton Research), in conjunction with the hanging-drop vapour-diffusion technique. Standard solutions were used at room temperature and 277 K. After optimization, a suitable cryo-protectant was found by soaking crystals in equilibrated crystallization liquor which contained increasing concentrations of various carbohydrates, glycerol, alcohols or PEGs. Crystals were flash-frozen in a N_2 stream at 103 K and tested on a rotating-anode generator using Cu $K\alpha$ radiation with an Stoe imaging plate. A partial diffraction data set with crystals obtained from the Crystal Screen I kit was collected on the BL1 beamline of the Swiss–Norwegian beamline (λ = 0.873 Å) at the ESRF in Grenoble. A data set from improved crystals was collected on the BW7A beamline (λ = 0.8345 Å) at the EMBL Outstation at DESY, Hamburg at 100 K using a MAR Research imaging-plate system. Oscillation pictures were taken for 40 s with an oscillation range of 1.0° and a crystal-to-detector distance of 290 mm. Indexing and processing of the diffraction data was performed with the *HKL* package (Otwinowski & Minor, 1997). Intensities were converted to structure-factor amplitudes using the program *TRUNCATE* (French & Wilson, 1978) within the *CCP*4 (Collaborative Computational Project, Number 4, 1994) suite of programs.

3. Conclusions

Expression of the 13.2 kDa rabbit SCP2 protein was achieved in *Escherichia coli* and the expressed protein was purified by ion-exchange chromatography. The yield was about 7 mg of purified protein from 1 l bacterial culture. The protein was of high purity as judged by SDS–PAGE, IEF and MALDI–MS (Fig. 1). A single band was found on both electrophoresis gels stained with Coomassie blue (Fig. 1). A single major peak was obtained with MALDI–MS, corresponding to the calculated mass of the protein within the estimated error. The additional minor band (Fig. 1) corresponding to a smaller mass probably results from proteolytic cleavage of one or two amino acids at the C- or N-terminus of the protein. From the above methods, the protein was estimated to be about 98% pure.

Small needle-like crystals (form I) were obtained at 277 K with 2.0 *M* ammonium sulfate as precipitant (Fig. 2). These crystals grew in clusters making it difficult to isolate single crystals. The 3.5 Å resolution obtained with these crystals (which lasted only about 15 min at 277 K in the beam) was improved to 3.0 Å at 105 K with synchrotron radiation using sucrose as a cryo-protectant. A few oscillation images could be collected, but the limited number of single crystals did not allow us to collect a complete data set. A crystal cell was established belonging to the tetragonal crystal system. Systematic extinctions of the 00*l* and *h*00 zone and merging of symmetry-equivalent intensities suggested the space group to be either $P4_12_12$ or its enantiomorph $P4_32_12$.

Improved crystallization conditions (2.0 *M* ammonium sulfate, 300 m*M* lithium sulfate and 100 m*M* citrate buffer pH 6.5 at 277 K) resulted in chunky crystals (form II, Fig. 2) which diffracted considerably better than the initial crystals. Like the needle-like crystals, these crystals only lasted for a few minutes at ambient temperatures when exposed to X-rays; therefore, cryo-conditions had to be used. A diffraction data set was collected from one single crystal treated



(*a*) (*b*)

Figure 2
Crystals of purified rabbit SCP2. (*a*) Crystal form I; the size of the largest single crystal is about 0.5 × 0.1 × 0.1 mm. (*b*) Crystal form II; these crystals appear in two morphologically different forms within 2–3 months, after a protein precipitate has been formed which gives rise to a cloudy appearance of the solution. The form with the square face and the form with the rounded edges are isomorphous and diffract to the same resolution limit. The size of the largest crystal is about 0.3 × 0.3 × 0.2 mm.

with 30% sucrose as cryo-protectant to 1.8 Å resolution utilizing synchrotron radiation. These crystals have practically the same unit-cell dimensions as the initial crystals ($a = b = 57.5$, $c = 86.5$ Å) and belong to the same space group, as deduced from systematic extinctions and scaling results. A calculated Matthews volume (Matthews, 1968) of 2.7 Å^3 Da^{-1} implies that there is one molecule per asymmetric unit, corresponding to a solvent content of about 54%. The processing and scaling statistics are summarized in Table 1 and show that the data are of high quality and are almost complete, even in the highest resolution shell.

About four years ago, other workers obtained SCP2 crystals which diffracted to about 3.5 Å resolution (K. Wüthrich, personal communication), but no X-ray structure has so far been reported. Presumably, the limited resolution and/or high-radiation sensitivity of the crystals did not allow the structure solution. We attribute our success in obtaining high-resolution data of good quality from SCP2 crystals to two factors. Firstly, the protein samples were of high purity, as judged by gel electrophoresis methods and MALDI–MS. Secondly, suitable cryo-conditions could be established, allowing us to collect diffraction data without observing significant radiation damage.

An NMR determination of the secondary structure of SCP2 has been performed showing that the protein contains a unique fold with a five-stranded β-sheet and three α-helices (Szyperski *et al.*, 1993). In contrast, the last 20 amino acids at the C-terminus appear to lack secondary-structural elements. To date, no tertiary structure of this class of protein or any other homologous protein is available which could allow us to approach the structure determination by the molecular-replacement method. Therefore, we are currently in the process of screening for heavy-atom derivatives in order to solve the crystal structure of SCP2 by the MIR method.

We gratefully acknowledge the opportunity to collect diffraction data at the EMBL Outstation, DESY, Hamburg, Germany and at the Swiss–Norwegian Beamline of the ESRF, Grenoble, France. Drs Wolfram Welte and Kay Diederichs (University of Konstanz, Germany) are thanked for allowing us to perform preliminary X-ray experiments on their rotating-anode/Stoe imaging-plate system.

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