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Two different crystal forms of sorcin, a penta-EF-hand Ca^{2+}-binding protein

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Sorcin is a 198 amino-acid Ca^{2+}-binding protein that belongs to the penta-EF-hand family. Its Ca^{2+}-binding domain (residues 33–198) has been crystallized in the absence of Ca^{2+} in two different crystal forms. Two complete data sets have been collected on a synchrotron source under cryocooling conditions from crystals grown using ammonium sulfate as precipitant: monoclinic crystals in space group $C2$, with unit-cell parameters a = 130.93, b = 103.85, c = 78.55 Å, β = 118.0°, diffracting to 2.1 Å, and tetragonal crystals in space group $P42_12$, with unit-cell parameters $a = b$ = 103.33, c = 79.15, diffracting to 2.7 Å. Crystals were also grown using PEG 6000 as precipitating agent. They also belong to space group $C2$, diffract to 2.8 Å and their unit-cell parameters are very similar to the first form. Structure determination by molecular replacement has been initiated. Structural information should be useful for elucidating the interaction of sorcin with membrane targets.

1. Introduction

Sorcin (soluble resistance-related calcium-binding protein) is a 21.6 kDa protein isolated from the cytosol of multidrug-resistant cells, as the name indicates, but expressed also in a number of normal tissues such as heart, muscle, brain and adrenal medulla. It is a member of the small penta-EF-hand family which comprises grancalcin, the small calpain subunit, the apoptosis-linked protein ALG-2 and peflin (Maki *et al.*, 1997). All members of the family are cytosolic proteins that bind reversibly to cell membranes at physiological Ca^{2+} concentrations and hence are endowed with inherent signalling capacity. In the case of sorcin, binding to cell membranes entails interaction with specific target proteins. These have been identified in cardiac and skeletal muscle as the ryanodine receptor (Meyers, Pickel *et al.*, 1995) and the pore-forming α1 subunit of voltage-dependent L-type calcium channels (Meyers *et al.*, 1998) and in adrenal medulla as annexin VII (Brownawell & Creutz, 1997). For the other members of the penta-EF-hand family the protein targets on cell membranes are not known.

Common structural characteristics of the penta-EF-hand proteins are a two-domain organization and a dimeric state of association of the calcium-free form, whereas the Ca^{2+}-bound protein has a marked tendency to precipitate. The N-terminal domain is rich in glycines, prolines and hydrophobic residues and is of variable length in different proteins. The C-terminal calcium-binding domain contains four canonical EF-hands with different affinities for the metal and a fifth EF-hand that plays a structural role forming the dimer interface, where it pairs up with the corresponding site of the second monomer in the dimer (Maki *et al.*, 1997; Blanchard *et al.*, 1997; Lin *et al.*, 1997).

Solution studies of sorcin and of two fragments comprising the complete sorcin Ca^{2+}-binding domain (SCBD; residues 33–198; Fig. 1) or the last three EF-hands only (fragment 90–198) allowed us to establish that Ca^{2+} binding to the high-affinity EF1–EF2 pair triggers the translocation process to membranes and that SCBD is like the intact protein in terms of calcium affinity and capacity to interact with the ryanodine receptor.

However, the Ca^{2+}-induced quenching of the intrinsic fluorescence that characterizes native sorcin does not take place in SCBD, indicating that in the fragment the two tryptophan residues (Trp99 and Trp105) are exposed to solvent in a Ca^{2+}-independent manner. In turn, these data suggest that in the native protein the Ca^{2+}-dependent conformational changes alter the relative positions of the N- and C-terminal domains rather than affecting the structure of the Ca^{2+}-binding domain itself (Zamparelli *et al.*, 2000). We also established that SCBD is not involved in the interaction with annexin VII, which requires the N-terminal domain of both partner proteins (Verzili *et al.*, 2000). The complexity of the interaction pattern emerging from these

studies prompted the present investigation of the three-dimensional structure of sorcin and of SCBD.

2. Materials and methods

2.1. Protein preparation and crystallization

Native sorcin was prepared as described in Meyers, Zamparelli *et al.* (1995). Crystals of sorcin appeared in two weeks from a 7 mg ml^{-1} protein solution in the presence of 16–20% saturated $(NH_4)_2SO_4$ and 100 m*M* MES buffer pH 5.8 at 294 K. The crystals were well formed tetragonal bipyramids and their maximum dimension approached 1 mm. However, their diffraction was poor (about 4.5 Å) and despite extensive experimentation with the crystallization conditions we were unable to obtain improved diffraction. We suspected that the poor diffraction was a consequence of the presence of the glycine-rich N-terminal fragment containing 32 amino acids. Thus, we proceeded to the study of SCBD.

SCBD was constructed on the basis of the cDNA inserted in plasmid pKT7 used for the expression of wild-type sorcin (Van der Bliek *et al.*, 1986). Five oligonucleotides coding for both strands of the 33–90 sequence were phosphorylated using standard procedures. The plasmid containing the wild-type sorcin gene was digested with *Nco*I and the cohesive ends of the resulting large DNA band were ligated to the annealed mixture of oligonucleotides with T4 DNA ligase. The SCBD gene was subcloned in plasmid pET28 (Novagen, Madison, WI, USA). DNA sequences were determined by the dideoxy sequencing method. Expression and purification of SCBD in *Escherichia coli* were performed as for native sorcin.

Crystallization was performed at room temperature by the hanging-drop vapour-diffusion technique (McPherson, 1990). All solutions used were Ca^{2+}-free.

The droplets were composed of 2 µl of protein and 2 µl of reservoir solution. Two different crystals forms, one monoclinic and one tetragonal, were obtained using $(NH_4)_2SO_4$ as precipitant.

MAYPGHPGAG GGYYPGGYGG APGGPSFPGQ TQDPLYGYFA SVAGQDGQID
ADELQRCLTQ SGIAGGYKPF NLETCRLMVS MLDRDMSGTM GFNEFKELWA
VLNGWRQHFI SFDSDRSGTV DPQELQKALT TMGFRLNPQT VNSIAKRYST
SGKITFDDYI ACCVKLRALT DSFRRRDSAQ QGMVNFSYDD FIQCVMTV

Figure 1
Amino-acid sequence of sorcin. The first 32 amino acids (underlined) are the glycine-rich N-terminal part that was removed. The remaining part corresponds to the calcium-binding domain and is designated SCBD.

Table 1
Crystallographic data-collection parameters of the crystal forms studied.

Protein	Unit-cell parameters				Crystal system/ space group	Resolution (Å)	Overall completeness (%)
	a (Å)	*b* (Å)	*c* (Å)	*β* (°)			
Sorcin	115	115	94	90	Tetragonal	4.5	Not measured
SCBD-A	103.33	103.33	79.15	90	Tetragonal $P42_12$	2.7	97.7
SCBD-B	130.93	103.85	78.55	118.0	Monoclinic *C*2	2.1	99.4
SCBD-C	133.20	105.82	78.15	117.0	Monoclinic *C*2	2.8	Not measured

The tetragonal crystals (SCBD-A) belong to space group $P42_12$ and diffract to 2.7 Å resolution. They grew from 21% saturated $(NH_4)_2SO_4$ in 100 m*M* MES buffer pH 5.6, 1 m*M* DTT and 1 m*M* NaN_3. The monoclinic *C*2 crystals (SCBD-B), which were better diffracting (to 2.1 Å), grew at 16% $(NH_4)_2SO_4$ and pH 5.4 (Fig. 2).

Crystals were also obtained using PEG 6000 as precipitant (SCBD-C). They belonged to the monoclinic space group *C*2, with unit-cell parameters similar to those of SCBD-B and diffracted to 2.8 Å resolution. The reservoir solution contained 9%(*w*/*v*) PEG 6000 in 100 m*M* NaOAc–HOAc pH 5.0, 1 m*M* DTT and 1 m*M* NaN_3.

The SCBD solution used for crystallization (10 mg ml^{-1}) consisted of 10 m*M* Tris–HCl pH 7.4, 200 m*M* NaCl, 2 m*M* EGTA; however, the solution that gave SCBD-B crystals lacked NaCl. All crystals grew within a few days.

2.2. Cryofreezing and data collection

Crystals of sorcin were first tested using a Rigaku R-AXIS IIC imaging-plate detector mounted on a Rigaku RU-200 rotating-anode X-ray (Cu *Kα*) generator equipped with focusing mirrors and operating at 50 kV and 100 mA. Initial data were collected at cryogenic temperature (100 K) (Rodgers, 1994) using 25% glycerol in the mother liquor as cryoprotectant; crystals diffracted to about 4.5 Å. Resolution did not improve significantly on measuring the data from larger crystals (1.0 × 0.4 × 0.2 mm) at the DESY synchrotron facility (Hamburg, Germany) and the crystals were not further analysed.

The three types of SCBD crystals were also analysed at our in-house X-ray source. They all diffracted to better than 3 Å. Further data were collected from crystals of SCBD-A and SCBD-B at 100 K (28% glycerol as cryoprotectant) at the synchrotron-radiation source at ELETTRA (Trieste, Italy) using a MAR CCD detector system. The radiation wavelength was set to 1.0 Å and 0.5° oscillation frames were collected over a range of 180°. The data were indexed with *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997). Subsequent calculations were performed using the *CCP*4 program package (Collaborative Computational Project, Number 4, 1994).

Table 2
Statistical data-collection parameters as a function of resolution.

(*a*) SCBD-A.

Resolution (Å)	$I > 3\sigma(I)$ (%)	χ^2†	R_{merge}‡	Completeness (%)
15–4.62	98.8	0.66	0.043	96.1
4.62–3.66	97.3	0.69	0.060	100.0
3.66–3.2	88.3	0.70	0.128	100.0
3.2–2.91	68.0	0.72	0.272	99.8
2.91–2.7	54.0	0.70	0.385	92.6
Overall	81.9	0.70	0.083	97.7

(*b*) SCBD-B.

Resolution (Å)	$I > 3\sigma(I)$ (%)	χ^2†	R_{merge}‡	Completeness (%)
15.0–3.49	99.0	1.09	0.030	99.9
3.49–2.78	92.1	1.32	0.044	99.7
2.78–2.43	78.3	1.47	0.078	99.7
2.43–2.21	66.7	1.48	0.143	99.6
2.21–2.05	56.0	1.54	0.248	98.1
Overall	78.4	1.30	0.036	99.4

† $\chi^2 = \sum_h \sum_i (I_{hi} - \langle I_h \rangle)^2 / [\sigma_h^2 N/(N-1)]$, where N is the number of observations. ‡ $R_{merge} = \sum_h \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i I_{hi}$, where I_{hi} is the ith observation of the reflection h and $\langle I_h \rangle$ is the mean intensity of the hth reflection.

3. Results and discussion

The crystallographic details of the crystals studied are presented in Table 1. The unit-cell parameters of SCBD-B grown from $(NH_4)_2SO_4$ and determined at the synchrotron resemble those of SCBD-C grown from PEG 6000 and determined with the conventional rotating-anode source. Table 2 summarizes the statistics for SCBD-A and SCBD-B crystals. As seen from Table 2, SCBD-B diffracted strongly to 2.1 Å resolution with an overall completeness of 99.4%, while the highest resolution shell is 98.1% complete. The unit-cell parameters for crystal form SCBD-B suggest that the asymmetric unit may contain four, five or six

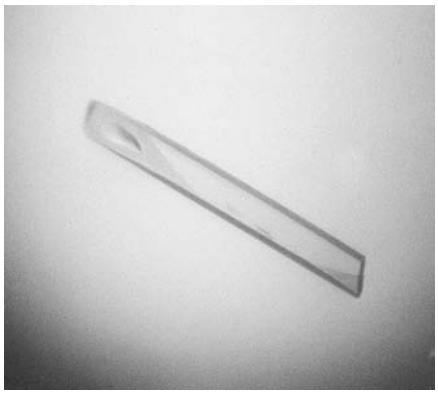

Figure 2
Crystals of SCBD-B (dimensions 0.7 × 0.2 × 0.1 mm).

molecules. The value of five is the least likely because of the dimeric nature of SCBD (Zamparelli *et al.*, 2000). The value of six molecules (three dimers) leads to a V_M (Matthews, 1968) of 2.08 Å^3 Da^{-1} (solvent content 41%), which is in agreement with the strongly diffracting crystals. However, the value of four molecules (two dimers) per asymmetric unit is the correct one as shown by the self-rotation function calculated using *POLARRFN* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). This leads to V_M of 3.12 Å^3 Da^{-1} and a solvent content of 61%. We are proceeding to the determination of the three-dimensional structure. The sorcin structure will add to those of the small calpain subunit (Blanchard *et al.*, 1997; Lin *et al.*, 1997) and grancalcin (Jia *et al.*, 2000) and foster the understanding of how penta-EF-hand proteins interact with membrane targets.

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References

Blanchard, H., Grochulski, P., Li, Y., Arthur, J. S. C., Davies, P. L., Elce, J. S. & Cygler, M. (1997). *Nature Struct. Biol.* **4**, 532–538.
Brownawell, A. M. & Creutz, C. E. (1997). *J. Biol. Chem.* **272**, 22182–22190.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Jia, J., Han, Q., Borregaard, N., Lollike, K. & Cygler, M. (2000). *J. Mol. Biol.* **300**, 1271–1281.
Lin, G., Chattopadhyay, D., Maki, M., Wang, K. K. W., Carson, M., Jin, L., Youen, P., Takano, E., Hatanaka, M., DeLucas, L. J. & Narayana, S. V. L. (1997). *Nature Struct. Biol.* **4**, 539–546.
McPherson, A. (1990). *Eur. J. Biochem.* **189**, 1–23.
Maki, M., Narayana, S. V. & Hitomi, K. (1997). *Biochem. J.* **328**, 718–720.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Meyers, M. B., Pickel, V. M., Sheu, S. S., Sharma, V. K., Scotto, K. W. & Fishman, G. I. (1995). *J. Biol. Chem.* **270**, 26411–26418.
Meyers, M. B., Puri, T. S., Chien, A. J., Gao, T., Hsu, P. H., Hosey, M. M. & Fishman, G. I. (1998). *J. Biol. Chem.* **273**, 18930–18935.
Meyers, M. B., Zamparelli, C., Verzili, D., Dicker, A. P., Blanck, T. J. J. & Chiancone, E. (1995). *FEBS Lett.* **357**, 230–234.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rodgers, D. W. (1994). *Structure*, **2**, 1135–1140.
Van der Bliek, A. M., Meyers, M. B., Biedler, J. L., Hes, E. & Borst, P. (1986). *EMBO J.* **5**, 3201–3208.
Verzili, D., Zamparelli, C., Mattei, B., Noegel, A. A. & Chiancone, E. (2000). *FEBS Lett.* **471**, 197–200.
Zamparelli, C., Ilari, A., Verzili, D., Giangiacomo, L., Colotti, G., Pascarella, S. & Chiancone, E. (2000). *Biochemistry*, **39**, 658–666.