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Crystallization and preliminary crystallographic studies of calgranulin C, a S100-like calcium-binding protein from pig granulocytes

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Abstract

Calgranulin C (CAGC) from pig granulocytes has been crystallized and X-ray diffraction data have been collected to 2.6 Å resolution. The crystals belong to the trigonal system, space group $P3_121$ or $P3_221$, cell parameters $a = b = 54.35\,(2)$, $c = 141.32\,(5)$ Å and probably contain two molecules in the asymmetric unit. CAGC is amongst the first reported typical S100-like calcium-binding protein to be crystallized and studied by X-ray crystallography.

1. Introduction

The S100 family is a group of low molecular weight (10–12 kDa) calcium-binding proteins thought to be involved, as calcium-dependent mediators, in cell-type differentiation, cell-cycle progression and signal transduction (Kligman & Hilt, 1988). Furthermore, deregulation of the expression of some S100 proteins, such as calcyclin (Potts *et al.*, 1995) and CAPL, has also been associated with tumor development and the induction of metastasis (Murao, Collart & Huberman, 1989). A distinctive structural feature of the S100 family is the presence of two Ca^{2+}-binding sites in the conformation of the classical *EF*-hand motives, with the N-terminal site showing an unusual 14-residue calcium-binding loop (Heizmann & Hunziker, 1991).

The three-dimensional structures of two members of the S100 family have been determined in solution by NMR techniques; calbindin, also known as D9K (Kordel, Skelton, Akke & Chazin, 1993), and calcyclin, known as CACY. The structure of D9K has also been determined by X-ray crystallography (Szebenyi & Moffat, 1986). In all cases, structures were determined in their calcium-free form and fold as a single globular domain, resembling that of calmodulin and troponin C. However, inspite of high sequence and structural similarity, there are some important functional differences between members of the S100 family. Calbindin D9K, for example, is an atypical S100 protein, as it does not undergo significant calcium-induced conformational changes, an observation which led to the suggestion that it functions as an intracellular calcium buffer (Skelton, Kordel, Akke, Forsén & Chazin, 1994). D9K has also not been shown to bind zinc as do other S100 proteins and does not dimerize *in vitro*.

The biochemical characterization of three similar S100-like calcium-binding proteins has recently been reported: calgranulin C, which is abundantly expressed in pig granulocytes (Dell'Angelica, Schleicher & Santomé, 1994), p6 from human neutrophils (Guignard, Mauel & Markert, 1995) and the recombinant human calcium-binding protein A3 (Fohr, Heizmann, Engelkamp, Schafer & Cox, 1996). In this paper we describe the crystallization and preliminary crystallographic data for a typical S100-like calcium binding protein, calgranulin C. The protein has been shown to bind two Ca^{2+} ions and one Zn^{2+} ion per molecule and there is evidence that upon calcium binding the protein exposes hydrophobic patches that probably mediate the interaction between calgranulin C and a specific effector protein.

2. Methods, results and discussion

Calgranulin C from pig granulocytes was purified as described (Dell'Angélica *et al.*, 1994), dialysed against water and lyophilized. Crystals were initially obtained using the vapour-diffusion technique in hanging drops (McPherson, 1982) at 291 K under the following conditions: 3.59 *M* ammonium sulfate per 100 m*M* sodium acetate buffer pH 5.0 in 1 ml of reservoir solution. The drop contained equal amounts (2.5 µl) of the reservoir solution and protein which was previously dissolved in a 1 m*M* zinc sulfate at a concentration of 10 mg ml^{-1}. Small crystals in the form of clusters were visible after 2 d. In order to reduce the rate of nucleation and growth and thus improve crystal quality, two distinct modifications to the experimental conditions were introduced: the preparation of drops at 277 K and the addition of 5 m*M* calcium chloride in the reservoir solution for experiments performed at 291 K. The growth of prismatic crystals was visible after 30 d at 277 K while at 291 K this varied between 7 and 10 d, with crystal sizes up to 0.3 × 0.5 × 0.5 mm. Preliminary experiments at synchroton sources indicate that the crystals diffract to better than 2 Å resolution.

Unit-cell dimensions $a = b = 54.35\,(2)$ and $c = 141.32\,(5)$ Å, were obtained by least-squares fitting using 337 setting reflections measured from two independent still images separated by a 90° rotation and collected with a Rigaku R-AXIS II image-plate area detector system using a rotating anode generator operating at 40 kV and 120 mA. Averaging of equivalent reflections unambiguously define the Laue symmetry as $3\bar{m}1$. Analysis of systematic absences indicated the condition limiting reflection indicated an $l = 3n$, therefore compatible with space groups $P3_121$ or $P3_221$.

The amino-acid sequence determined by Dell'Angélica *et al.* (1994) leads to a calculated M_r of 10 614 Da. Gel-filtration and cross-linking experiments suggest that calgranulin C is predominantly a dimeric species in solution. Therefore, assuming one dimer per asymmetric unit and a protein partial specific volume of 0.74 cm^3 g^{-1}, the crystal density is calculated as 1.15 g cm^{-3} and is associated with a solvent content of 56.7% by volume, with a calculated V_m value of 2.84 Å^3 Da^{-1}, values which lie within the range commonly observed for protein crystals (Matthews, 1968). Diffraction data sets were independently collected to 2.6 Å resolution both for crystals grown at 277 K without Ca^{2+} and at 291 K in the presence of 5 m*M* $CaCl_2$, in order to examine the possibility of differences as a

Table 1. *Data-collection statistics for the 2.6 Å native data set of calgranulin C*

Crystals 1 and 2 were grown in the absence and presence of added Ca^{2+} ions, respectively.

Resolution (Å)	Crystal 1 Reflections Measured	Crystal 1 Reflections Independent	Crystal 1 R_{merge}	Crystal 1 Cumulative % of reflections with $I > 3\sigma$	Crystal 2 Reflections Measured	Crystal 2 Reflections Independent	Crystal 2 R_{merge}	Crystal 2 Cumulative % of reflections with $I > 3\sigma$	Overall merge R_{merge}
8.00	1294	306	0.026	97.7	1103	297	0.046	98.3	0.193
5.70	2026	500	0.043	96.4	2288	525	0.042	98.3	0.025
4.67	2078	586	0.039	98.6	2542	616	0.042	98.7	0.031
4.05	2083	699	0.036	97.4	2768	732	0.040	97.8	0.038
3.62	1980	757	0.042	96.4	2911	806	0.042	98.6	0.039
3.31	2008	829	0.047	94.9	2993	888	0.045	98.0	0.040
3.06	2000	866	0.058	91.4	3022	937	0.047	96.7	0.043
2.87	1865	857	0.078	86.7	2922	924	0.054	96.5	0.055
2.70	2068	940	0.097	83.3	3099	1027	0.058	94.8	0.064
2.60	2046	898	0.125	77.9	3019	991	0.068	92.8	0.075
Totals	19448	7238	0.044	90.8	26677	7743	0.045	96.7	0.056

consequence of calcium binding to the protein. Diffraction data were collected with an R-AXIS II system using graphite monochromatized Cu $K\alpha$ radiation, at 277 K, and processing was performed with the software provided by the manufacturers of the data-collection system. Data-collection statistics for each crystal are given in Table 1. In order to evaluate the possibility of intensity differences between the two data sets, these were scaled and merged with programs from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The resulting statistics, also shown in Table 1, indicate significant differences only at very low resolution (below 8 Å), which can be accounted for by bulk solvent scattering of the different crystallization media. This suggests that the crystallized species may be identical. Considering the observed high affinity of the protein for Ca^{2+} ions (Dell' Angélica *et al.*, 1994), it is plausible to suppose that the purification procedures adopted did not remove the calcium ions bound to the protein, and that this is, therefore, the form captured in the solid state. Alternatively, ion binding may produce no conformational changes large enough to result in significant intensity differences compared to the apo protein crystal. However, in order not to discount the possibility of having Ca^{2+} ions bound even with low occupancy, the data sets were kept separate and difference Fourier maps, once the whole protein chain has been determined, will possibly solve this ambiguity.

As the density considerations indicate a dimer per asymmetric unit, a self-rotation function analysis was performed using the program *AMoRe* (Navaza, 1994), in order to determine the non-crystallographic symmetry elements. The data included were from crystal 1, in the absence of Ca^{2+}, within the resolution range 15–2.6 Å, and excluding expansion coefficients lower than 6. Fig. 1 is the stereographic projection of the $\chi = 180°$ self-rotation function map showing the peaks corresponding to the crystallographic twofold axes and a small but significant non-crystallographic twofold peak, with maximum correlation coefficient of 25.9%, indicated as *P*. This might suggest that if there is a dimer in the asymmetric unit, the non-crystallographic symmetry axis would either be along *P* or would have to be parallel to the crystallographic twofold or very close to it. A monomer in the asymmetric unit would correspond to a solvent content of 78.4% ($V_m = 5.68$ Å^3 Da^{-1}), uncommon for highly diffracting crystals. On the other hand, a trimer in the asymmetric unit would correspond to a solvent content of 35.1% ($V_m = 1.89$ Å^3 Da^{-1}), possible for crystals of small proteins but is not supported by the self-rotation function.

Structure determination is being sought *via* molecular replacement techniques with model structures selected from several calcium-binding proteins determined crystallographically or by NMR and available from the PDB. However, regulatory proteins that respond to calcium levels frequently show large conformational differences as a function of the nature and size of the calcium-binding loop and the interaction with both calcium and target proteins (Cook, Ealick, Babu, Cox & Kumar, 1991; Silva & Reinach, 1991; Meador, Means & Quiocho, 1993; Szebenyi & Moffat, 1986). The possibility of having a novel conformation in our crystals is not negligible, and a search for isomorphous derivatives may prove to be necessary in order to solve the present structure.

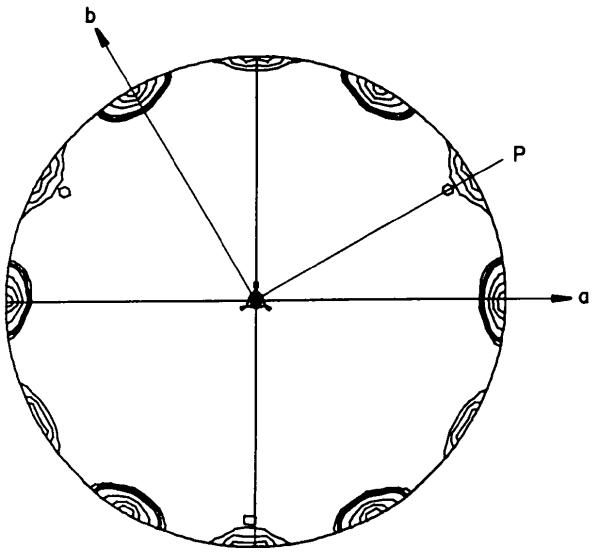

Fig. 1. Stereographic projection down the *c* axis of the $\kappa = 180°$ polar section of the self-rotation function calculated with data in the range 2.6–15 Å. Contours are made at the following values of correlation coefficient: 16, 20, 24, 40, 60, 80%.

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