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Crystallization and preliminary crystallographic studies of an apoptosis-linked calcium-binding protein ALG-2

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ALG-2 is an apoptosis-linked Ca^{2+}-binding protein. It is required for T-cell receptor-induced, Fas-induced and glucocorticoid-induced cell death. Structurally, ALG-2 contains five putative EF-hand Ca^{2+}-binding sites. Ca^{2+}-free ALG-2 was crystallized in two crystal forms by the hanging-drop vapour-diffusion method.

1. Introduction

Apoptosis is a regulated and integrated cellular process that causes a well orchestrated form of cell death. The core components in apoptosis machineries are highly conserved from nematodes to humans (Adams & Cory, 1998; Thorberry & Lazenik, 1998; Yuan *et al.*, 1993). Induction of apoptosis triggers an increase in Ca^{2+} concentration (McConkey *et al.*, 1989; Nakayama *et al.*, 1992) and intracellular chelators of Ca^{2+} inhibit cell death (Oshimi & Miyazaki, 1995). It is believed that calcium ions may play an important regulatory role in the progress of apoptosis. However, owing to the lack of structural information on the specific calcium ion dependent molecules which are directly associated with cell death, the detailed mechanistic involvement of calcium ions in apoptosis still remains unknown.

ALG-2 (apoptosis-linked gene 2 product) is the first calcium-binding protein shown to be directly linked to calcium-associated apoptosis by different stimuli (Lo *et al.*, 1999). Recently, two ALG-2 partners Alix and AIP1 were found and cloned (Missotten *et al.*, 1999; Vito *et al.*, 1999). The interactions between them and ALG-2 were calcium dependent and AIP1 was proved to cooperate with ALG-2 in executing the calcium-dependent requirements along the apoptosis pathway.

ALG-2 is a 22 kDa protein consisting of a single polypeptide chain of 191 amino-acid residues and containing five putative EF-hand calcium ion binding sites (Vito *et al.*, 1996). The calcium-free form of ALG-2 exists as a weak dimer in solution (Lo *et al.*, 1999; Lin *et al.*, 1997). However, in the presence of calcium ion ALG-2 turns out to be insoluble (Lo *et al.*, 1999), which suggests the structure of ALG-2 undergoes dramatic changes when it associates with calcium ions. Recently, the crystal structure of grancalcin, another member of the penta-EF-hand protein family, has been determined in the presence and the absence of calcium (Jia *et al.*, 2000). Only one calcium ion was found to bind to EF3 in one molecule of a dimer. It has been proved that EF1 and EF3 are strong binding sites in ALG-2, while the others have weaker calcium affinities. Sequence comparison shows that EF1 of ALG-2 has a single-residue insertion compared with grancalcin. Whether this site causes major conformational change in ALG-2 is not clear.

2. Methods and results

In order to elucidate the structural details of ALG-2, especially the conformational changes resulting from calcium-ion binding, we tried to crystallize ALG-2 in the calcium-free form and in the calcium-bound form using the hanging-drop vapour-diffusion technique. In order to increase the likelihood of crystallization, the 23 N-terminal residues beyond the first EF-hand were truncated. The protein-purification procedure was as described previously (Lo *et al.*, 1999). Several crystal forms have been obtained under different conditions. The ion-free form was crystallized using a well solution containing 20%(*w*/*v*) PEG 8000, 50 m*M* KH_2PO_4 and 10 m*M* EDTA at 277 K. These conditions are temperature sensitive. No crystals appeared at temperatures higher than 283 K. pH values between pH 3.0 and 9.0, however, had no effect on crystallization. In most cases only a shower of needle-shaped crystals appeared, but occasionally better crystals were obtained (form *A*). Furthermore, ALG-2 could also be crystallized with 50%(*v*/*v*) MPD, 2 m*M* EDTA and 100 m*M* Na_2HPO_4–KH_2PO_4 pH 6.5 well solution at 277 K. Under these conditions, when protein solution was mixed with the well solution the protein precipitated immediately. However, after about 3 d crystals appeared in the precipitant (form *B*). The size and quality of the

form *B* crystals were good enough for diffraction data collection.

The diffraction data were collected on a MAR Research image-plate system using a local X-ray source and at beamline X26C, NSLS with an 2 × 2 CCD detector (ADSC) at 298 K. The data were processed with the *DENZO* (Otwinowski, 1993) and *SCALEPACK* (Minor, 1993) programs. The best crystals of calcium-free ALG-2 in form *A* diffracted to 4 Å and belong to the hexagonal crystallographic system, with unit-cell parameters $a = b = 85.5$, $c = 79.4$ Å. The form *B* crystals diffracted to 2.5 Å. The space group is *P*222, with unit-cell parameters $a = 47.5$, $b = 56.0$, $c = 74.6$ Å. The structure determination of ALG-2 in the calcium-free form is being carried out by our group. Recently, microcrystals of ALG-2 containing calcium ions were also obtained. A preliminary X-ray diffraction experiment proved the crystals were proteins and not salts. ALG-2 structures should help us to reveal the structural details of the mechanism of calcium ion associated apoptosis at the molecular level.

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