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Crystallization and preliminary X-ray analysis of maize ZBP14 protein, a member of a new family of zinc-binding proteins.

By BING XIAO,* KAREN ROBINSON, ALASTAIR AITKEN and MIRIAM HIRSHBERG,
Laboratory of Protein Structure, National Institute for Medical Research, Mill Hill, London NW7 1AA, England

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Abstract

A preliminary X-ray crystallographic study of a novel zinc-binding protein from maize is presented. Native and several heavy-atom derivative sets of data have been collected on synchrotron sources, to a resolution of 2.1 Å. The space group was found to be orthorhombic, $P2_12_12_1$, with unit-cell dimensions of a = 92.64, b = 129.45 and c = 196.31 Å

Introduction

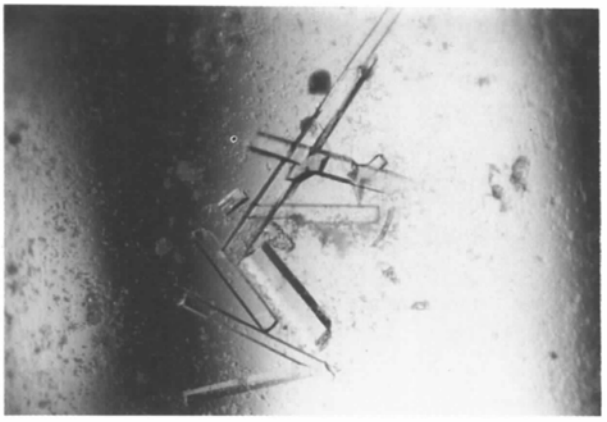
A new family of zinc-binding proteins has been identified in species as diverse as mammals, plants, mycoplasmas, eubacteria and cyanobacteria. All members of the family share a conserved, novel zinc-binding site *XXHXHXHXX* (where *X* is a hydrophobic residue, Robinson & Aitken, 1994; Robinson *et al.*, 1995). The maize member of this protein family has been named ZBP14, where ZBP stands for zinc-binding protein and 14 for its molecular mass of 14 kDa on sodium dodecyl sulfate plyacrylamide gel electrophoresis (SDS–PAGE) (Robinson *et al.*, 1995). The bovine brain member of this family was identified as an inhibitor of protein kinase C (PKCI-1) (McDonald, Groschel-Stewart & Walsh, 1987). The physiological role of maize ZBP14 has yet to be determined, although it shares high homology with bovine PKCI-1 (73% similarity and 53% identity, Pearson, *et al.*, 1990). ZBP14 at high concentration inhibited mammalian brain PKC to a maximum of 20% (Robinson *et al.*, 1995). This plant protein, expressed in *Escherichia coli* and purified on fast protein liquid chromatography (FPLC), has been crystallized and preliminary X-ray analysis carried out based on the data collected on synchrotron radiation sources.

Experimental

pMz2-12 (pU C13 plasmid containing the maize gene Mz2-12) was a kind gift of G. G. Simpson (Simpson, Clark & Brown, 1994). DNA manipulation and protein expression was carried out in *E. coli* TB1 with a pGEX-2T plasmid expression vector which produced a glutathione-S-transferase fusion protein. The protein was bound to a glutathione–Sepharose affinity matrix and treated overnight with thrombin (with continuous agitation). This cleaved the glutathione-S-transferase from maize ZBP14 and left the former still bound to the affinity support. The cleaved 14 kDa, ZBP14 was separated from the affinity matrix by centrifugation and further purified by anion-exchange chromatography on FPLC. SDS–PAGE indicated a purity greater than 99% (Robinson *et al.*, 1995).

The maize ZBP14 protein is a temperature-sensitive protein which necessitated all protein preparation and crystallization work was performed at 277 K. Despite these precautions the nucleation and crystal growth were affected by the small temperature fluctuations produced by the de-icing cycle in the cooling system. To alleviate this problem, crystallization trays were stored in polystyrene boxes at 277 K, which gave a sufficiently stable temperature. Crystals grew within a period of 2–30 d. Both sitting and hanging-drop methods of vapour diffusion were used (McPherson, 1989), and seeding techniques were tried (Stura & Wilson, 1992) but with no success.

Two crystals forms were grown from a mixture of ammonium sulfate, PEG 400, sodium chloride, pottasium phosphate and 1-O-octyl-β-D-glucopryranoside (β-OG) at pH 7.5. Variation in the concentration of the protein and the other components of the crystallization liquid, gave two crystal forms, as shown in Fig. 1. The details of the crystallization conditions are given in Table 1. A number of precipitants including PEG in the molecular weight range 200 to 20 000,



(*a*)

(*b*)

Fig. 1. Crystals of ZBP14. (*a*) Crystal form *A*, the longest dimension of the crystal is 1.5 mm, (*b*) crystal form *B*, the longest dimension of the crystal is 1 mm.

* Author for correspondence.

Table 1. *Crystallization conditions for ZBP*14

	Crystal form *A*		Crystal form *B*	
	Well	Drop	Well	Drop
Protein concentration (mg ml^{-1})	—	3	—	5
$(NH_4)_2SO_4$ (*M*)	1.8	0.7	1.4	0.75
PEG 400%(*w*/*v*)	1.8	0.7	1.5	0.75
NaCl (m*M*)	—	60	300	200
K_2HPO_4 (m*M*)	170	70	226	113
β-OG%(*w*/*v*)	1.11	0.46	1.9	0.95
$CdCl_2$ (m*M*)	—	—	0.04	0.02
Hepes (pH 7.5) (m*M*)	89	37	85	43

isopropanol, several salts were also screened along with various buffers (Tris, Hepes, acetate and phosphate) at pH 4.5–8.5, however, those proved to be unsuccessful. The addition of additives such as benzamidine hydrochloride, dioxane and 1, 2, 3-heptanetriol and detergents such as SB-12 (*n*-dodecyl-*N*, *N*-ammoniopropyl sulfonate), C_{12}DAO (*N*, *N*-dimethyl-dodecylamine-*n*-oxide) proved similarly unsuccessful apart from *β*-OG which will be discussed below.

Results and discussion

The addition of *β*-OG improved the crystallization significantly. For example, protein which previously failed to crystallize when equilibrated for two months with the solution in the well, crystallized when 1% *β*-OG was added after only two weeks.

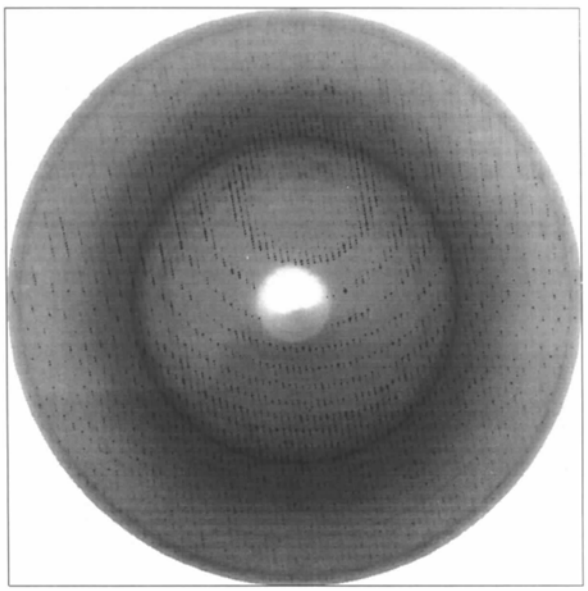


Fig. 2. X-ray diffaction pattern of native crystal in cryo buffer which was diffracted to 2.15 Å and collected at BL4 station, ESFR Grenoble, France.

It would seem that the addition of *β*-OG was an important factor in the growth of large diffraction-quality crystals.

Both the secondary and the quaternary structures of ZBP14 are altered upon zinc binding. Circular dichroism suggests that the ratio of *β*-sheet to *α*-helix is shifted towards *β*-sheet, and size-exclusion chromatography indicates a less extended structure with comparison with the apoprotein (Robinson *et al.*, 1995). Consequently, it is of great interest to determine the structure of both the zinc bound and unbound forms of ZBP14. To date, it has been possible to grow large crystals in the presence of cadmium but not with zinc (only small crystals). The morphology of the cadmium crystals (crystal form *B*) differs from that of the apoprotein (crystal form *A*). Circular dichroism experiments with cadmium show that it has similar effects on the structure of ZBP14 as zinc (Robinson *et al.*, 1995). We were able to grow crystal form *B* without Cd, but with higher concentrations of either sodium chloride, potassium phosphate or *β*-OG, compared with concentrations used in growing crystal form *A*. As yet we do not know if crystal form *B* is of the Cd-bound conformation of ZBP14, a question we aim to address using X-ray analysis.

Data were collected from crystal form *A* using synchrotron radiation sources at SRS Daresbury, UK, and at ESRF Grenoble France. The crystals were extremely sensitive to radiation damage. On the Grenoble source, an average lifetime of a single crystal at 277 K was less than 40 s of exposure. Incomplete native and heavy-atom derivatives data sets were collected at 277 K from several crystals. The native crystals diffracted to 2.1 Å while the two derivatives holmium(III) chloride and mercury acetate diffracted to 2.5 Å. Recently, we have managed to find a suitable cryo buffer and have collected, in the ESRF Grenoble, France, two complete data sets at 100 K: a native and a mercury derivative. The X-ray diffraction pattern of the native crystal is shown in Fig. 2. The crystals diffract to the same resolution as at 277 K. The crystals are orthorhombic, space group $P2_12_12_1$, with native unit cell of a = 92.64, b = 129.45 and c = 196.31 Å. Data processing and phase determination are in progress.

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