ESRF

Experiment title:

ZBP14 - Zinc binding protein

Experiment number:

LS42 / LS231

Beamline: ID2

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Report:

Recently a family of small proteins of about 12 to 16 Kd has been described [I]. The Zinc-binding site of PKCI-1 [2] consists of three histidines that are closely positioned. These histidines are perfectly conserved in all members of this family for which the name HIT, for HIstidine Triad, has been proposed [I]. Except for PKCI-1, the function of these proteins is not yet known. Their widespread evolutionary conservation suggests that they are important for some cellular process.

All members of the family share the conserved histidine triad Zinc binding sequence motif

Consensus pattern:
N-x(4) -[GA] -x-Q-x- [LIVM]-x-H-x-H-[LIVMF]-H

We have initiated structural studies on the *maize* member of this family, a 28kD homo-dimeric protein [3]. The *maize* protein does not inhibits *bovine* PKC. PKCI from *bovine* brain was shown to interact and to inhibit protein kinase C [4].

This protein shares high homology with the *bovine* member but its physiological role has not yet been determined. Both the secondary and the quaternary structure of PKCIH have been shown to be altered upon Zinc binding. Circular dichroism spectra have been used to suggest that the ratio of β–sheet to α–helix is shifted towards β–sheet after the Zinc is bound. Size exclusion chromatography indicates that the Zinc complex adopts a less extended structure when compared to the apo protein. The change in structure can be reversed by removal of the Zinc with EDTA. The inhibitory properties of the *maize* PKCIH towards *bovine* PKCI are different between the Zinc bound and the Zinc free species.

We have crystallised the Zinc free *maize* protein [5], and collected both native and heavy atom derivative data sets for this form. Orthorhombic crytals of the ape-protein, suitable for Xray diffraction studies, have been grown. On the LS42 visit to the ESRF, Unfrozen the crystals suffered from radiation damage, however we were able to collect sufficient data on both native and derivative crystals to determine the crystal characteristics and to establish that the mercury soaked material was a derivative. On the LS231 visit to the ESRF' we collected with frozen crystals, which were much more stable, and we collected both native and derivative data sets.

station	data	cell	reso	Rmerge	Rnat/der
esrf	apo	92.64129.45196.31	2.20	4.0	
esrf	Hg	92.29128.36196.60	2.45	8.6	23.0

The structure is proving exceptionably difficult to solve as there are at least 16 monomers in the asymmetric unit. These molecules do not appear to have a proper non-crystallographic symmetry arrangement involving rotation axes. Both patterson functions and self rot at ion functions have not been solved. Mass spectrometry has revealed that the apo crystals contain at least three different species of the protein, the expected PKCIH molecule, together with a di-sulphide dimer and a truncated version of the protein.

We have found that samples of the concentrated protein cannot be crystallised in the presence of Zinc ion, however diluting the protein a 1000 fold then titrating with Zinc acetate and monitoring with CD spectroscopy we have been able to get the Zinc complex in a form that can then be concentrated. Crystals have now been grown from this material.

We now have samples of the *human* PKCI and have produced crystals of this protein. Crystals have been grown which appear more robust than those produced from the *maize* protein. These crystals have orthorhombic cells (plates form:- 46.6 63.9 81.5; and rods form:- 45.9 63.3 74.8) that are about 10 times smaller than the maize crystals and appear to have just one dimer per asymmetric unit. Work is now in progress to determine the structure of the *human* Zinc bound protein, and our aim is to return to the *maize* system once we have solved the *human* protein's structure. The *human* protein also does not show a variation in its CD spectra with zinc ions.

Very recently the Xray structure of a Zinc free form of the human protein has been solved [6]. We are now concentrating our efforts on a Cys–Ser maize mutant to determine the Xray crystal structure on the Zinc bound form.

References

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