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Recombinant chemosensory protein (CSP2) from the moth *Mamestra brassicae*: crystallization and preliminary crystallographic study

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Chemosensory proteins (CSPs) are small proteins (13 kDa on average) present in several sensory organs from a wide range of insect species. They are believed to be involved in chemoperception (olfaction or taste) and to play a role in chemical transport from air or water to chemosensitive receptors. Here, the first crystals of a CSP originating from the moth *Mamestra brassicae* (Mbra) proboscis and expressed as recombinant protein in *Escherichia coli* periplasm are reported. Crystals of MbraCSP2 were obtained by the hanging-drop vapour-diffusion method under the following conditions: 1 µl of a 46 mg ml^{-1} protein solution in 50 m*M* Tris pH 8.0 containing cetyl alcohol as ligand (1:5 molar ratio) was mixed with 1 µl of well solution containing 30% PEG 4000, 0.2 *M* sodium acetate in 100 m*M* Tris at pH 8.4. The protein–cetyl alcohol complex crystallizes in space group $P2_1$, with unit-cell parameters $a = 47.9$, $b = 49.7$, $c = 50.3$ Å, $\beta = 110.1^\circ$. With two molecules in the asymmetric unit, the V_M is 2.15 Å^3 Da^{-1} and the solvent content is 42%. A complete data set has been collected at 1.6 Å resolution on beamline ID14-2 (ESRF, Grenoble). Se-Met expression has been performed with a view to solving the CSP2 structure with MAD data collection using the Se absorption edge.

1. Introduction

In the animal kingdom, chemical communication, vision and hearing allow individuals to recognize and respond to their environment and other individuals. Chemical communication is of primary importance in less evolved species such as insects. Proteins binding odorants and pheromones (GOBPs, general odorant-binding proteins; PBPs, pheromone-binding proteins) are transport proteins with an average length of 150 amino-acids and contain six conserved cysteines involved in three disulfide bridges (Breer *et al.*, 1990; Maida *et al.*, 1993; Maïbèche-Coisne *et al.*, 1998; Scaloni *et al.*, 1999). These proteins occur in the antennal lymph of insects and are very soluble. The pheromones and odorants perceived by insects are small hydrophobic organic molecules of 10–20 non-H atoms and are bound by the GOBPs/PBPs, making it possible for such chemical messengers to travel from the antenna lumen to the receptors through the aqueous compartment surrounding the olfactory neurones (Vogt *et al.*, 1988; Pelosi, 1996). The PBPs have been postulated to ferry specific pheromones, while GOBPs would perform the same task with odorant molecules, possibly related to the insect alimentary habits. These two classes of transport proteins have been extensively studied and ultimately the three-dimensional structure of a *Bombyx mori* PBP, a structural paradigm for all PBPs and GOBPs, has been solved in complex with bombykol (Sandler *et al.*, 2000). This crystallographic structure revealed a fold containing six α-helices which delineate a buried cavity filled with the alkyl alcohol. We have obtained crystals of PBP1 from *M. brassicae* (MbraPBP1) alone and the structure determination is under way (Campanacci *et al.*, 1999).

Another class of small proteins (chemosensory proteins; CSPs) has been identified in several sensory organs from a wide range of species of the insect phylum (Nomura *et al.*, 1992; Mameli *et al.*, 1996; Maleszka & Stange, 1997; Bohbot *et al.*, 1998; Angeli *et al.*, 1999; Adams *et al.*, 2000; Picimbon *et al.*, 2000). Owing to their localization in antennae, tarsi, labrum and proboscis, it has been postulated that these proteins might be involved in chemoperception, be it olfaction or taste (Nagnan-Le Meillour *et al.*, 1996; Angeli *et al.*, 1999). In this respect, they may also play a role in transport of hydrophobic chemicals (volatile or not) from air or water to olfactory or taste receptors. They differ, however, from the two former classes of chemosensory insect proteins, since they are smaller (∼110 residues), contain four conserved cysteines and are more widespread in the insect body and phylum. CSPs

Table 1
Crystal parameters and data-reduction statistics of the CSP2–cetyl alcohol complex.

Space group	$P2_1$			
Unit-cell parameters (Å,°)	a = 47.7, b = 49.7, c = 50.3, β = 110.1			
Beamline	ID14-EH2 (λ = 0.933 Å)			
Cutoff	None		$I/\sigma(I) > 3$	
Resolution (Å)	24.0–1.6	1.69–1.6	24.0–1.65	1.74–1.65
R_{sym} (%)	4.5	27.7	4.3	22.6
$I/\sigma(I)$	11.2	2.5	12.5	3.1
Completeness	97.5	97.5	97.6	97.6
Multiplicity	2.0	2.0	2.0	2.0

have not been extensively characterized until now, although their large distribution makes them of a functional interest wider than that of GOBPs and PBPs. Furthermore, they seem to share a carrier function quite similar to that of the larger OBPs (Nagnan-Le Meillour *et al.*, 2000). We have expressed CSP2 originating from the proboscis of the cabbage moth *M. brassicae* (MbraCSP2) as a recombinant protein in the periplasm of *E. coli*. Here, we report the first crystals of MbraCSP2 in complex with cetyl alcohol. These crystals diffract to at least 1.6 Å and are amenable for solution with MAD methods.

2. Material and methods

Cloning of *M. brassicae* CSP2 is described elsewhere (Jacquin-Joly *et al.*, 2001). Expression was carried out in *E. coli* Bl21(DE3) expression hosts. The cultures were grown at 310 K without induction. The periplasmic proteins were released by osmotic shock as described in the pET system manual. Large-scale preparations of periplasmic proteins were obtained from non-induced cultures of BL21(DE3) (pET22b/CSP2) and were purified by anion exchange on a ResourceQ column (Äkta FPLC, Pharmacia), followed by ammonium sulfate precipitation and FPLC gel filtration (Superdex 200 Column, Pharmacia). Fractions containing CSP2 were incubated overnight in the presence of a fivefold molar excess of cetyl alcohol (hexadecanol) and at a final ethanol concentration of 0.3%. The CSP2–cetyl alcohol complex was washed with 50 m*M* Tris–HCl pH 8.0 and concentrated (Nanosep-3, Filtron) to 46 mg ml^{-1} as determined by the UV absorbance using the theoretical ε_{280} of 19 300 M^{-1} cm^{-1}.

Expression of selenomethionine-substituted CSP2 was performed using the method described by Hendrickson *et al.* (1990) using a methionine auxotroph strain, *E. coli* B834(DE3). This strain was transformed by the calcium method with pET22b/CSP2. Cultures were grown at 310 K in LeMaster medium containing 40 mg l^{-1} of selenomethionine (France Biochem) and 50 µg ml^{-1} of carbenicillin. When the A_{600} reached the value of 0.5, the cultures were induced with 50 µ*M* IPTG and transferred at 301 K for 16–20 h. Purification of the selenomethionine protein was performed as described above; however, all buffers contained 1 m*M* EDTA and 0.2 m*M* DTT to prevent oxidation of the selenium.

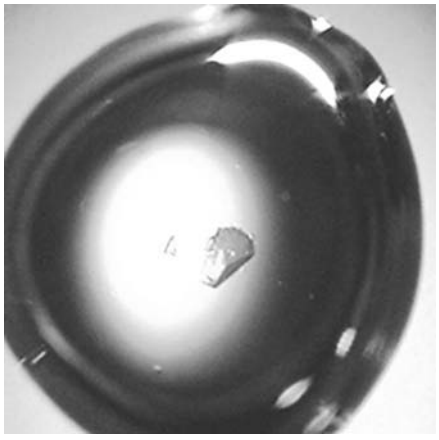

Figure 1
Single crystal of the recombinant chemosensory protein 2 from the moth *M. brassicae*. The size of this crystal is 0.2 × 0.2 × 0.3 mm.

3. Results and discussion

3.1. Crystallization of CSP2

Single crystals of MbraCSP2 were obtained at 293 K by the hanging-drop vapour-diffusion method. The initial droplet contained 1 µl of a 46 mg ml^{-1} protein solution in 50 m*M* Tris pH 8.0 containing five equivalents of cetyl alcohol as ligand mixed with 1 µl of well solution containing 36% PEG 5000 MME, 0.2 *M* sodium acetate at pH 5.5. Type I crystals generally appeared after 4 d. One such crystal, with dimensions 0.1 × 0.1 × 0.05 mm, was used for diffraction studies (see Table 1).

Larger crystals (0.2 × 0.2 × 0.3 mm, crystal type 2) have been obtained at 293 K under conditions close to those used for obtaining type 1 crystals, but in the presence of 30% PEG 4000, 0.2 *M* sodium acetate in 100 m*M* Tris pH 8.4. These crystals generally appeared after three weeks (Fig. 1).

3.2. Diffraction and data collection

Diffraction data were obtained exposing crystal 1 at a synchrotron-radiation source, beamline ID14-2 at the ESRF (Grenoble) tuned at 0.933 Å in the 16-bunch mode. Intensity data were collected using a ADSC-Q4 detector with a crystal-to-detector distance of 139 mm. The crystal used was cryocooled at 100 K without addition of cryoprotectant. Diffraction data were collected at 1.6 Å resolution: 100 1° oscillation images with 20 s exposure time were obtained as determined using *STRATEGY* (Ravelli *et al.*, 1997). Diffraction images were indexed and integrated with *DENZO* (Otwinowski, 1993) and scaled with *SCALA* (Collaborative Computational Project, Number 4, 1994). The CSP2–cetyl alcohol complex crystallizes in space group $P2_1$, with unit-cell parameters a = 47.9, b = 49.7, c = 50.3 Å, β = 110.1°. With two molecules in the asymmetric unit, the V_M is 2.15 Å^3 Da^{-1}, corresponding to a solvent content of 42% (Matthews, 1968). The diffraction data have an R_{merge} of 4.5% and a completeness of 97.5%. Data-collection statistics are summarized in Table 1.

We have postulated that two molecules are present in the asymmetric unit, leading to a V_M of 2.15 Å^3 Da^{-1}, since it is not very likely that 1.6 Å resolution could be obtained with a V_M of 4.3 Å^3 Da^{-1} from such a small crystal. Analysis with *GLRF* (Tong & Rossmann, 1997) did not give evidence of any twofold axis, however (data not shown). This is consistent with the TOCSY NMR data collected on CSP2, which indicate the presence of two spin systems per residue and hence a non-equivalent position for each monomer (data not shown).

Crystals of type 2 belong to another crystal form, space group $P4_x2_x2$, with unit-cell parameters $a = b$ = 71.6, c = 80.5 Å. These crystals diffract to 2.3–2.5 Å at best and we have not collected a complete data set from them.

We have expressed Se-Met labelled CSP2 in order to perform MAD experiments at the Se edge, although one Se per 13 kDa is higher than the average 15 e per 5 kDa (Hendrickson *et al.*, 1985). We have also obtained small crystals of CSP2 in complex with 12-bromo-1-dodecanol, which may give useful phasing information using the Br edge. Combination of both strategies should produce useful phases.

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