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Crystallization and preliminary crystallographic study of a pheromone-binding protein from the cockroach *Leucophaea maderae*

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Pheromone-binding proteins (PBPs) are small helical proteins (13–18 kDa) present in various sensory organs of moths and other insect species. An antennal protein from the cockroach *Leucophaea maderae* (LmaPBP) has been found to share all the hallmarks of the PBP family and is expressed specifically in the female adult antennae, the gender that perceives the sex pheromone. Here, the crystallization of LmaPBP expressed as a recombinant protein in *Escherichia coli* periplasm is reported. Crystals of LmaPBP were obtained by the sitting-drop vapour-diffusion method using a nanodrop-dispensing robot. The protein crystallizes in two different crystal forms. Form 1 belongs to space group $P1$, with unit-cell parameters $a = 43.2$, $b = 45.1$, $c = 45.7$ Å, $\alpha = 118.6$, $\beta = 93.0$, $\gamma = 106.9°$. With two molecules in the asymmetric unit, V_M is 2.7 Å^3 Da^{-1} and the solvent content is 47%. A complete data set has been collected at 1.6 Å resolution on beamline ID14-2 (ESRF, Grenoble). Form 2 was obtained in the presence of the pheromone (3-hydroxy-butan-2-one) and belongs to space group $P2_1$, with unit-cell parameters $a = 38.2$, $b = 62.2$, $c = 45.1$ Å, $\beta = 93.0°$. With two molecules in the asymmetric unit, V_M is 2.0 Å^3 Da^{-1} and the solvent content is 39%. A complete data set has been collected at 1.7 Å resolution on beamline BM14 (ESRF, Grenoble). SeMet expression has been performed with a view to solving the structure by MAD data collection using the Se absorption edge.

1. Introduction

Insect sexual behaviour and mate choice are often based on different stimuli and are mainly mediated by the sex pheromones. In lepidoptera, the males perceive the sex-pheromone blend emitted by females and respond to it (Vogt & Riddiford, 1981). Proteins that bind pheromones (pheromone-binding proteins; PBPs) occur in the antennal lymph of insects and are on average 130–150 amino acids long, with six conserved cysteines in three disulfide bridges (Vogt & Riddiford, 1981; Vogt *et al.*, 1991; Krieger *et al.*, 1996). PBPs produced in male antennae and secreted in the antennal lymph accommodate pheromones and make it possible for these messengers to travel from the air–antenna interface to the pheromonal receptors. The three-dimensional structure of a PBP from *Bombyx mori* (BmorPBP), a structural paradigm for all PBPs, has been solved in the apo form and in complex with bombykol (Sandler *et al.*, 2000; Horst *et al.*, 2001; Lee *et al.*, 2002). This crystallographic structure revealed a fold containing six α-helices which delineate a buried cavity filled with the bombykol pheromone, an alkyl alcohol. In both the X-ray and NMR structures, the protein was found to be monomeric.

In the cockroach *Leucophaea maderae*, in contrast to moths, the females perceive the sex-pheromone blend emitted by males (Sirugue, 1992). A protein of ~13 kDa has been isolated from the antennae of the females. Based on the dimorphism observed in its sex expression and on its low but significant identity with moth PBPs (Rivière *et al.*, 2003), this protein has

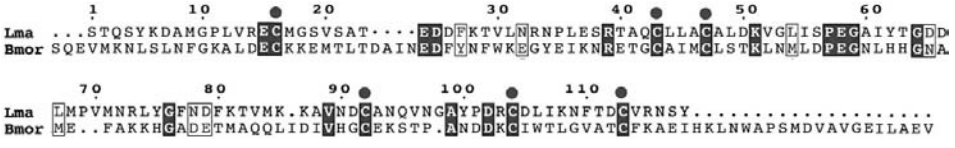

Figure 1
Alignment of the sequence of LmaPBP (Lma) with that of the PBP from *B. mori* (Bmor) of known three-dimensional structure.

been proposed to belong to the PBP family (LmaPBP). LmaPBP shares 17% sequence identity with BmorPBP, whose three-dimensional structure is known (Fig. 1).

Using a high-throughput crystallization technology using nanodrops, we have obtained crystals of LmaPBP diffracting to 1.6 Å resolution. Since the sequence identity between LmaPBP and BmorPBP was not sufficient for molecular replacement, the protein has been selenomethionine-labelled and structure determination is under way using MAD methods (Hendrickson, 1991).

2. Material and methods

LmaPBP was cloned from female adult antennae and subcloned in pET-22b+ as described elsewhere (Rivière *et al.*, 2003), with a subcloning scheme similar to that of other PBPs or CSPs (Wojtasek & Leal, 1999; Campanacci *et al.*, 2001). Briefly, expression was carried out in the periplasm of *Escherichia coli* Bl21(DE3) at 291 K after induction with 0.3 m*M* IPTG. The periplasmic proteins were released by osmotic shock and purified by anion exchange on a ResourceQ column (Amersham) and gel filtration (Superdex 200, Amersham). Expression of selenomethionine-substituted LmaPBP was performed using the methionine-biosynthesis pathway inhibition method (Doublié, 1997). Purification of the selenomethionine protein was performed as described above and crystal optimization is under way.

3. Results and discussion

3.1. Crystallization of LmaPBP

Single crystals of LmaPBP were obtained at 293 K by the sitting-drop vapour-diffusion method using nanodrops (Sulzenbacher *et al.*, 2002) dispensed by a nano-pipetting robot (Cartesian Technologies) in Greiner plates (Mueller *et al.*, 2001). Overall, 27 (out of 96) different crystallization conditions yielded crystals with the MDL kit (Molecular Dimensions Ltd; http://www.moleculardimensions.com/) and eight (out of 48) with the Stura kit (Stura *et al.*, 1992; Molecular Dimensions Ltd) (Fig. 2) leading to two different crystal forms (Table 1). For the first form, the initial droplet contained 200 nl of 40 mg ml^{-1} protein solution in 10 m*M* Tris pH 8.0, 10 m*M* NaCl mixed with 100 nl of well solution containing 0.2 *M* imidazole/malate pH 8.5, 22.5% PEG 10 000 (Fig. 2*b*). Crystals of dimensions 0.4 × 0.3 × 0.1 mm appeared after 24 h and were used without further optimization (Fig. 2). The crystals belong to space group *P*1, with unit-cell parameters a = 43.2, b = 45.1, c = 45.7 Å, α = 118.6, β = 93.0, γ = 106.9°. With two molecules in the asymmetric unit, V_M is 2.7 Å^3 Da^{-1} (Matthews, 1968) and the solvent content is 47%. A second crystal form was obtained only once, with 200 nl of 40 mg ml^{-1} protein solution in 10 m*M* Tris pH 8.0, 10 m*M* NaCl containing 5 equivalents of the pheromone 3-hydroxy-butan-2-one mixed with 100 nl of well solution containing 0.1 *M* sodium citrate pH 5.6, 0.2 *M* potassium tartrate and 2 *M* ammonium sulfate. Crystals of dimensions 0.2 × 0.3 × 0.1 mm appeared after 24 h and were used without further optimization (not shown). Form 2 belongs to space group $P2_1$, with unit-cell parameters a = 38.2, b = 62.2, c = 45.1 Å, β = 93.0°. With two molecules in the asymmetric unit, V_M is 2.0 Å^3 Da^{-1} (Matthews, 1968) and the solvent content is 39%.

3.2. Diffraction and data collection

Diffraction data were obtained using a synchrotron-radiation source at ESRF (Grenoble). Form 1 was collected on beamline ID14-4 tuned at 0.933 Å, in the weak 16-bunch mode. Intensity data were collected with an ADSC-Q4 detector, with a crystal-to-detector distance of 139 mm. The crystal used was cryocooled to 100 K in its

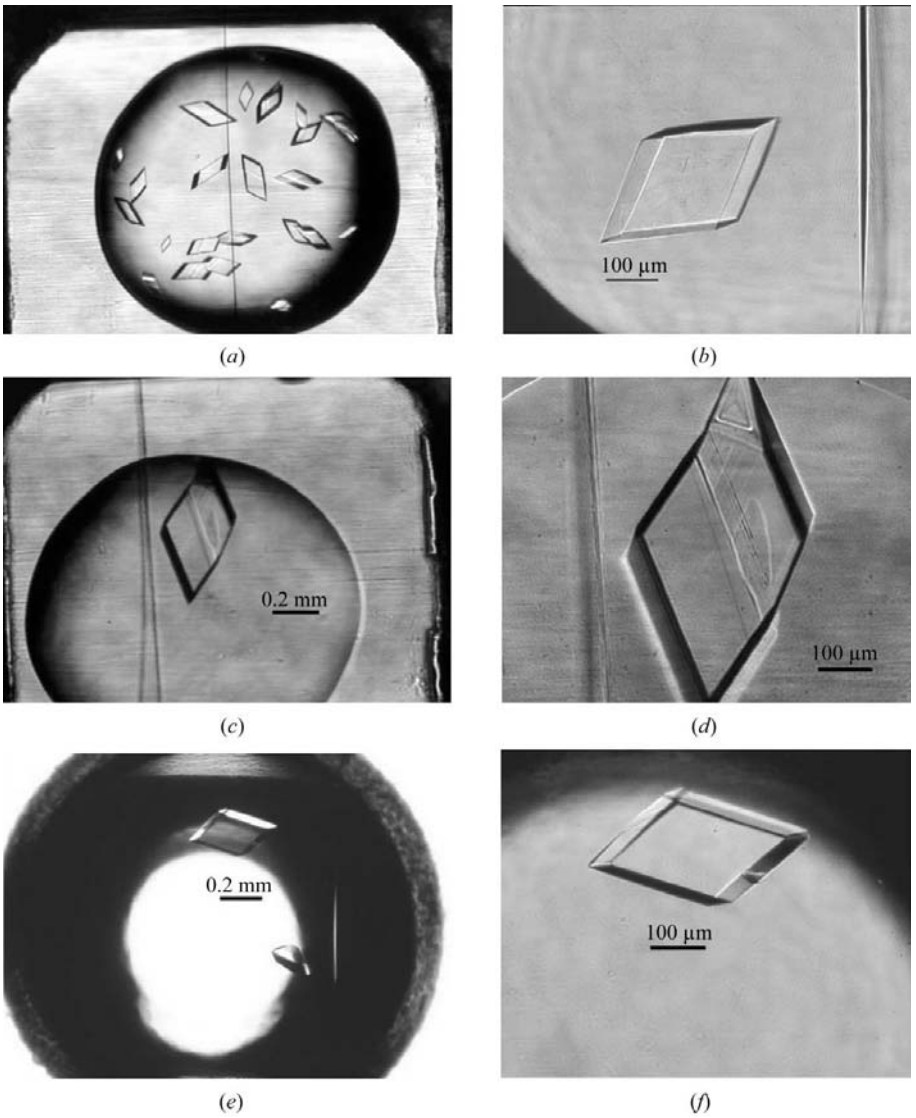

Figure 2
Crystals of recombinant PBP from the cockroach *L. maderae* obtained under various conditions using a nanodrop-dispensing robot. The initial volume of the drops was 200 nl protein + 100 nl reservoir. Drops (*a*)–(*d*) were obtained from Greiner 96 crystallization plates with a flat bottom and drops (*e*)–(*f*) from Greiner 96 crystallization plates with a round bottom. All crystals belong to crystal form 1 (Table 1). (*a*) Stura kit D2: 0.2 *M* imidazole/malate pH 7.0, 25% PEG 4000. (*b*) Stura kit D3: 0.2 *M* imidazole/malate pH 8.5, 22.5% PEG 10 000 (used for data collection). (*c*) MDL E10: 0.1 *M* Tris pH 8.5, 10 m*M* NiCl, 20% PEG 2000. (*d*) as (*c*), but zoomed four times. (*e*) Stura kit B2: 0.2 *M* imidazole/malate pH 7.0, 15% PEG 4000. (*f*) as (*e*), but zoomed four times.

Table 1
Crystal parameters and data-reduction statistics of LmaPBP crystals.

Values in parentheses are for the last resolution shell.

Crystal	Form 1 (Stura D3)	Form 2 (MDL G5)
Beamline	ID14-EH4	BM14
Wavelength (Å)	0.933	0.9192
Resolution (Å)	26.0–1.6 (1.6–1.69)	30–1.7 (1.7–1.79)
Space group	*P*1 (Fig. 2*b*)	$P2_1$
Unit-cell parameters (Å, °)	a = 43.2, b = 45.1, c = 45.7, α = 118.6, β = 93.0, γ = 106.9	a = 38.2, b = 62.2, c = 45.1, β = 92.5
R_{sym} (%)	4.3 (12.3)	3.9 (15.7)
$I/\sigma(I)$	7.6 (4.8)	10.1 (3.7)
No. unique reflections	31333	22683
Oscillation	300 × 1°	186 × 1°
Completeness (%)	84.4	97.0
Multiplicity	1.9	3.5

crystallization mother liquor with 25% glycerol. A complete data set was collected to 1.6 Å resolution from the native protein using 300 1° oscillation images with 20 s exposure time. Diffraction images were indexed and integrated with *DENZO* (Otwinowski & Minor, 1997) and scaled with *SCALA* (Collaborative Computational Project, Number 4, 1994). The diffraction data have an R_{merge} of 4.6% and a completeness of 85% (Table 1). This completeness will be improved by the choice of a better setting when collecting the SAD/MAD data. A data set from the second crystal form obtained in the presence of the pheromone was collected at BM14 using a procedure similar to that of form 1. It is likely that the crystal contains the pheromone, as it has been shown to bind tightly by fluorescence studies (Rivière *et al.*, 2003). In the 30–1.7 Å resolution range, R_{merge} was 3.9% and the completeness was 97.0% (Table 1).

4. Conclusions

Using nanodrop technology and commercial crystallization kits, LmaPBP readily yielded at least two crystal forms from many different conditions and without optimization. Both crystal forms proved to be suitable for structural studies. Molecular replacement using *AMoRe* (Navaza, 1994) failed, as expected, probably because of the level of identity between LmaPBP and BmorPBP. We have therefore expressed SeMet-labelled LmaPBP in order to perform MAD/SAD experiments at the Se edge.

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