Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray study of haem-binding protein from the bloodsucking insect *Rhodnius prolixus*

R. A. P. Nagem,[a,b] J. R. Brandão Neto,[a] V. P. Forrer,[a] M. H. Sorgine,[c] G. O. Paiva-Silva,[c] H. Masuda,[c] R. Meneghini,[a] P. L. Oliveira[c] and I. Polikarpov[a]*

[a]National Synchrotron Light Laboratory, LNLS, Caixa Postal 6192, CEP 13083-970, Campinas, SP, Brazil, [b]Gleb Wataghin Physics Institute, State University of Campinas, UNICAMP, Caixa Postal 6165, CEP 13083-970, Campinas, SP, Brazil, and [c]UFRJ, ICB, Departamento Bioquímica Médica, Rio de Janeiro, Brazil

Correspondence e-mail: igor@lnls.br

Received 11 December 2000
Accepted 12 March 2001

Rhodnius haem-binding protein (RHBP) from the bloodsucking insect *Rhodnius prolixus*, a 15 kDa protein, has been crystallized using polyethylene glycol as a precipitant. X-ray diffraction data have been collected at a synchrotron source. The crystals belong to the space group $P4_{1(3)}2_12$, with unit-cell parameters $a = b = 64.98$, $c = 210.68$ Å, and diffract beyond 2.6 Å resolution.

1. Introduction

Haem, iron protoporphyrin-IX, participates in several fundamental biochemical reactions such as respiration, oxygen transport in extracellular fluids and photosynthesis. On the other hand, haem may be a catalyst of the formation of reactive oxygen species and thus lead to oxidation of lipids, proteins and DNA (Aft & Mueller, 1983; Tappel, 1955; Vincent, 1989). Owing to its potential toxicity, haem is normally found associated with proteins such as haemopexin and albumin, free haem frequently being related to pathological conditions (Rytter & Tyrrel, 2000).

Haematophagous arthropods ingest enormous amounts of blood. The bloodsucking insect *R. prolixus* takes between five to ten times its body weight each single meal (Lehane, 1991). As vertebrate blood has about 10 m*M* of haem bound to haemoglobin, the digestion of blood in the midgut of this insect generates potentially toxic amounts of haem. Furthermore, after a blood meal water is massively excreted, leading to even higher haem concentrations in the gut lumen. Therefore, in order to use blood as the sole food source, haematophagous arthropods must develop efficient ways to counteract haem toxicity and a whole array of antioxidant defences designed to prevent radical formation or to scavenge oxygen reactive species. Among these defences, a haem-binding protein (RHBP; *Rhodnius* haem-binding protein) has been described in the haemolymph and oocytes of *R. prolixus*.

RHBP has a single 15 kDa polypeptide chain and in the haemolymph can be found free (apoRHBP) or associated with one haem molecule (holoRHBP). The binding of haem to circulating apoRHBP protects this insect from haem-induced lipid peroxidation (Dansa-Petretski *et al.*, 1995).

In this paper, we describe the purification, crystallization and results of preliminary X-ray diffraction study of holoRHBP as a first step toward its crystal structure solution. The knowledge of the three-dimensional structure of the protein will help in the understanding of the structural basis of its function.

2. Materials and methods

HoloRHBP was purified to homogeneity from *R. prolixus* oocytes as described (Oliveira *et al.*, 1995). Initial crystallization conditions were screened by the sparse-matrix method (Jancarik & Kim, 1991) using the macromolecular crystallization reagent kits I and II (Hampton Research). In each trial, a hanging drop of 1 µl of protein solution (10 mg ml^{-1} in water) was mixed with 1 µl of precipitant solution and then equilibrated against a reservoir containing 500 µl of precipitant solution. Small plane-like dark red crystals grew at 291 K in precipitant solution No. 9 [30%(*w*/*v*) polyethylene glycol 4000, 0.2 *M* ammonium acetate, 0.1 *M* trisodium citrate dihydrate pH 5.6] from Hampton reagent kit number I in three weeks. Further optimization at 291 K, including pH refinement (McPherson, 1995), leads to new values of precipitant concentration (25% PEG 4000) and pH (6.0). Well formed bipyramidal crystals of dimensions $0.05 \times 0.04 \times 0.03$ mm grew in three to six weeks.

All data sets were collected using a 345 mm MAR Research image-plate detector at the LNLS Protein Crystallography beamline (Polikarpov, Oliva *et al.*, 1998; Polikarpov, Perles *et al.*, 1998) by the oscillation method at 100 ± 1 K. The exposure time was approximately 5 min per frame. For the native data set the crystal-to-detector distance was set to 190 mm and two oscillation ranges were used: 0.8 and 0.6°. The crystal-to-detector distance for the derivative data set was 155 mm and an oscillation range of 0.7° was used. The incident radiation wavelength for native and derivative data sets was 1.55 Å. The wavelength was chosen in order to maximize the flux in the spectrum available at the beamline (Polikarpov *et al.*, 1997; Rossmann & Blow, 1962).

The data were processed using *DENZO* and *SCALEPACK* packages (Otwinowski & Minor, 1997). Results of data processing for the native and iodine derivative are summarized in Table 1.

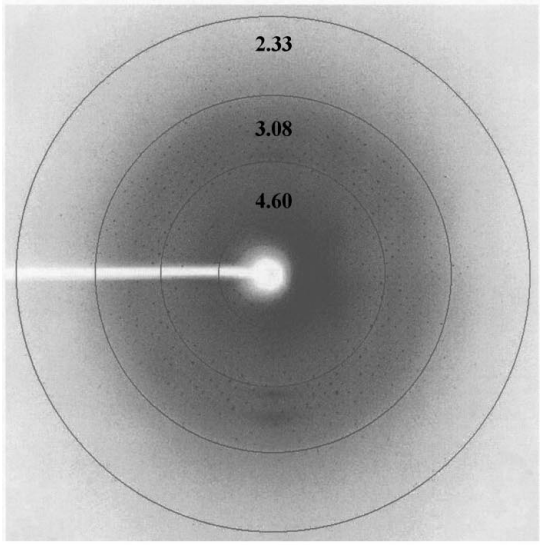
3. Results and discussion

The first crystals obtained diffracted to 3.2 Å resolution and presented considerable radiation decay when exposed to the X-ray beam at room temperature, resisting for only a few images. Cryocrystallographic techniques (Garman & Schneider, 1997) were employed to overcome radiation damage. Flash-freezing of the crystal in a gaseous nitrogen stream (Oxford Cryosystems) was performed after dipping the crystal in a mixture of the mother liquor with 20% ethylene glycol.

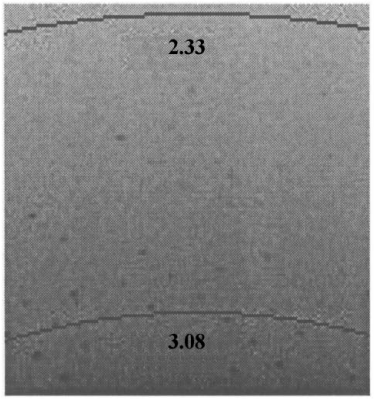
Three native data sets were collected with crystals obtained in the first refined crystallization round. The best native diffraction data set, extending to 3.2 Å resolution, is presented in Table 1. Slightly larger crystals were grown in subsequent crystallization trials, as evidenced by the first iodine-derivative data set recently collected to 2.6 Å resolution (Fig. 1). The iodine derivative was prepared by a rapid cryosoaking procedure (Dauter *et al.*, 2000) using a cryoprotectant solution containing in addition 1.0 *M* sodium iodide. Flash-freezing significantly improved the crystal resistance to X-rays, allowing a higher X-ray dose per image. An improvement in the $I/\sigma(I)$ ratio along with a high-redundancy data set led to better statistics and to a higher resolution cutoff.

The calculated unit-cell volume is 8.82×105 Å^3. Assuming three monomers per asymmetric unit, the calculated Matthews coefficient (V_M; Matthews, 1968) is 2.45 Å^3 Da^{-1}, which corresponds to 47.8% solvent content. On the other hand, if we presume a tetramer in the asymmetric unit, V_M is 1.84 Å^3 Da^{-1} and the solvent content is 30.5%. A self-rotation function calculation using the *POLARRFN* program from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) using different integration radii and resolution ranges did not give conclusive information about the asymmetric unit content. No consistent evidence for a threefold or fourfold non-crystallographic axis was observed, which might indicate that the non-crystallographic axis is approximately parallel to one of the cell axes. To elucidate this question, a complete structure determination will be required.

Sequence alignment has shown no significant homology with other proteins of known three-dimensional structure; therefore, a heavy-atom multiple isomorphous replacement method for the structure solution will have to be used. An extensive search for additional heavy-atom derivatives is currently under way.



(*a*)



(*b*)

Figure 1
(*a*) A typical diffraction pattern from the iodine-derivative data set. The crystal-to-detector distance was set to give a resolution limit of 2.33 Å at the outer edge of the image. (*b*) Enhanced contrast detail image from the highest resolution shell.

Table 1
Native and iodine-derivative crystal data and data-collection statistics.

Values for the highest resolution shell are shown in parentheses.

	Native	Iodine derivative
Space group	$P4_{1(3)}2_12$	$P4_{1(3)}2_12$
Unit-cell parameters (Å)	$a = b = 65.24$, $c = 207.14$	$a = b = 64.98$, $c = 210.68$
Resolution (Å)	35.0–3.20 (3.35–3.20)	22.0–2.60 (2.72–2.60)
No. of observations	24684 (3062)	48064 (5386)
No. of unique reflections	7642 (940)	19721 (2400)
$\langle I/\sigma(I)\rangle$	8.3 (3.9)	10.4 (2.8)
Multiplicity	3.2 (3.3)	2.4 (2.2)
Completeness (%)	96.0 (99.1)	74.7 (73.1)
R_{merge}† (%)	12.9 (31.3)	8.7 (40.8)
R_{fac}‡ (%)		36.9

† $R_{merge} = \sum(I - \langle I\rangle)/\sum I$. ‡ $R_{fac} = \sum |F_{PH} - F_P|/\sum F_P$.

JRBN, VPF, RM and IP gratefully acknowledge financial support from FAPESP and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

References

Aft, R. L. & Mueller, G. C. (1983). *J. Biol. Chem.* **258**, 12069–12072.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Dansa-Petretski, M., Ribeiro, J. M., Atella, G. C., Masuda, H. & Oliveira, P. L. (1995). *J. Biol. Chem.* **270**, 10893–10896.
Dauter, Z., Dauter, M. & Rajashankar, K. R. (2000). *Acta Cryst.* D**56**, 232–237.
Garman, E. F. & Schneider, T. R. (1997). *J. Appl. Cryst.* **30**, 211–237.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Lehane, M. J. (1991). *Biology of Bloodsucking Insects*, pp. 79–110. London: Harper Collins Academic.
McPherson, A. (1995). *J. Appl. Cryst.* **28**, 362–365.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Oliveira, P. L., Kawooya, J. K., Ribeiro, J. M. C., Meyer, T., Poorman, R., Alves, E. W., Walker, F. A., Machado, E. A., Nussenzveig, R. H., Padovan, G. J. & Masuda, H. (1995). *J. Biol. Chem.* **270**, 10897–10901.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Polikarpov, I., Oliva, G., Castellano, E. E., Garratt, R. C., Arruda, P., Leite, A. & Craievich, A. (1998). *Nucl. Instrum. Methods A*, **405**, 159–164.
Polikarpov, I., Perles, L. A., de Oliveira, R. T., Oliva, G., Castellano, E. E., Garratt, R. C. & Craievich, A. (1998). *J. Synchrotron Rad.* **5**(2), 72–76.
Polikarpov, I., Teplyakov, A. & Oliva, G. (1997). *Acta Cryst.* D**53**, 734–737.
Rossmann, M. G. & Blow, D. M. (1962). *Acta Cryst.* **15**, 24–31.
Rytter, S. W. & Tyrrel, R. M. (2000). *Free Radic. Biol. Med.* **28**, 289–309.
Tappel, A. L. (1955). *J. Biol. Chem.* **217**, 721–733.
Vincent, S. H. (1989). *Semin. Hematol.* **26**, 105–113.