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Crystallization and preliminary X-ray diffraction analysis of HeLp, a heme lipoprotein from the hemolymph of the cattle tick *Boophilus microplus*

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The main protein present in the hemelymph of the cattle tick *Boophilus microplus* is a lipoprotein able to bind heme (HeLp). It has an apparent molecular weight of 354 000 Da and is composed of two polypeptide chains found in stoichiometric amounts. It contains 33% lipids. The protein was crystallized using the hanging-drop vapour-diffusion method in the presence of 1,6-hexanediol as a precipitant. X-ray diffraction data were collected to 2.1 Å resolution using a synchrotron-radiation source. The crystal belongs to the triclinic space group $P1$, with unit-cell parameters a = 90.58, b = 105.50, c = 116.14 Å, α = 112.40, β = 111.64, γ = 91.35°. Owing to the lack of information about the amino-acid sequence, the structure of HeLp will be solved by the use of heavy atoms. Several possible derivatives have been collected and analysis is under way.

1. Introduction

Ticks are blood-sucking arthropods that infest a wide array of species (Sauer *et al.*, 1995), including humans and some animals of economic importance, and cause important losses in livestock production (Bowman *et al.*, 1996).

The southern cattle tick, *Boophilus microplus*, is one of the most important pests of cattle in tropical and subtropical countries. This ectoparasite causes severe production losses by its feeding activity and is the transmission vector of the protozoan parasites *Babesia* and *Anaplasma*, the causative agents of babesiosis and anaplasmosis, respectively (Jongejan & Uilenberg, 1994). Current control methods depend heavily on the use of acaricides. There are two major drawbacks to this approach: (i) the increasing resistance to the major classes of chemicals that has been reported in many parts of the world (Patarroyo & Costa, 1980; Nolan *et al.*, 1989) and (ii) the contamination of ecosystems and food by chemical residues.

Haem is a molecule that takes part in many fundamental biochemical reactions such as respiration, oxygen transport, photosynthesis and lipid desaturation (Ponka, 1999). In contrast to all these beneficial features, heme is a powerful catalyst of the formation of reactive oxygen species (Sadrzadeh *et al.*, 1984; Vincent *et al.*, 1988). A broad range of biomolecules can suffer heme-induced oxidative damage, especially lipoproteins, which are very susceptible to lipid peroxidation (Sadrzadeh *et al.*, 1987; Vincent, 1989; Miller *et al.*, 1995). Owing to its potential toxicity, heme is found associated with proteins capable of inhibiting heme-mediated generation of radical species, such as hemopexin, human albumin and *Rhodnius* heme-binding protein (Gutteridge & Smith, 1988; Vincent *et al.*, 1988; Dansa-Petretski *et al.*, 1995). Recently, it has been shown that binding to HeLp can reduce the pro-oxidant activity of heme (Maya-Monteiro *et al.*, 2004).

B. microplus obtains its own heme from the host hemoglobin present in ingested blood (Braz *et al.*, 1999), being the first multicellular organism that has been shown to be unable to synthesize the protoporphyrin ring. An important outcome of this finding, in the context of the tick's reproduction, is that the egg must provide all the heme necessary to build up a new organism. This dependence of the tick on heme from its diet necessitates the existence of mechanisms for heme absorption, transport and recycling, which have not been described for any other multicellular organisms.

The main protein of the hemolymph of the cattle tick *B. microplus* has been shown to be a heme lipoprotein (HeLp). It has an apparent molecular weight of 354 000 Da and contains two apoproteins (103 and 92 kDa) found in stoichiometric amounts (Maya-Monteiro *et al.*, 2000). HeLp contains 33% lipids, both neutral lipids and phospholipids, and 3% sugars. HeLp contains two heme molecules and is capable of binding six additional molecules of heme. HeLp is suggested to derived from an essential adaptation to the loss of the heme-synthesis pathway.

2. Crystallization

Protein was isolated from the cattle tick *B. microplus* as previously described (Maya-Monteiro *et al.*, 2000). Initial crystallization

experiments were carried out at a constant temperature of 293 K by the hanging-drop vapour-diffusion method (McPherson, 1982) using the sparse-matrix crystallization screening protocols described by Jancarik & Kim (1991). All crystallization trials were performed in VDX multi-well plates with 300 µl reservoir solution using Crystal Screen and Crystal Screen II from Hampton Research. Drops consisting of 2 µl protein solution (10 mg ml^{-1} in water) and 2 µl reservoir solution were prepared on siliconized cover slides. Small crystals were observed in 0.1 *M* Tris–HCl buffer pH 8.5 with 0.2 *M* $MgCl_2$ and 3.4 *M* 1,6-hexanediol as precipitant. New screenings varying the pH of the buffer and the concentration of the precipitant were performed and we obtained crystals suitable for X-ray data collection (Fig. 1) in 0.1 *M* Tris–HCl buffer at pH 7.7 with 0.2 *M* $MgCl_2$ and 3.0 *M* 1,6-hexanediol.

3. Data collection

Crystals were mounted in nylon loops and flash-frozen in a nitrogen stream at 100 K in the mother liquor, which acted as a cryoprotectant and did not show the formation of ice. X-ray diffraction intensities were collected at the D03B-CPR beamline, Laboratório Nacional de Luz Síncrotron (Campinas, Brazil) using a wavelength of 1.43 Å and a MAR CCD 165 detector (MAR Research) with 40 s exposures. Diffraction data were collected from 360 images using the oscillation method; individual frames consisted of 1° oscillation steps over a range of 360°. The data set was processed with the program *MOSFLM* (Leslie, 1992) and X-ray intensities were scaled with the program *SCALA* (Collaborative Computational Project, Number 4, 1994).

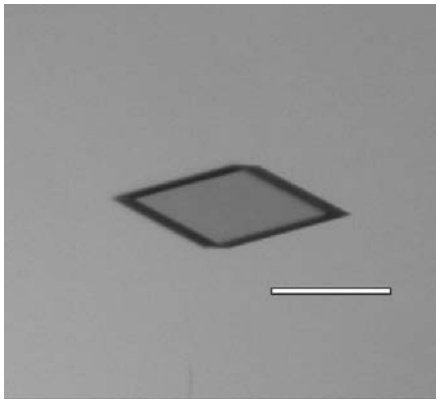

Figure 1
Crystal of HeLp from *B. microplus*. The bar represents 0.2 mm.

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Resolution range (Å)	50.0–2.1 (2.21–2.10)
No. total reflections	767133
No. unique reflections	201877
$R_{\rm merge}$† (%)	6.9 (43.9)
Completeness (%)	94.8 (91.1)
$\langle I/\sigma(I)\rangle$	9.0 (1.5)
Multiplicity	3.8 (3.3)
Solvent content (%)	51
Subunits per AU	1

† $R_{\rm merge} = \sum_h \sum_j |I(h)_j - \langle I(h)\rangle| / \sum_h \sum_j I(h)_j$; $I(h)_j$ is the observed intensity of the reflection and $\langle I(h)\rangle$ is the mean intensity of reflection h calculated after loading and scaling.

4. Results

The crystals of HeLp belong to the triclinic space group *P*1, with unit-cell parameters $a = 90.58$, $b = 105.50$, $c = 116.14$ Å, $\alpha = 112.40$, $\beta = 111.64$, $\gamma = 91.35°$. A nearly complete data set was collected to 2.1 Å resolution. The calculated packing parameter, based on a molecular weight of 354 kDa, indicates the presence of a monomer in the asymmetric unit. This corresponds to a typical Matthews coefficient ($V_{\rm M}$) of 2.63 Å^3 Da^{-1}, which is within the expected range (Matthews, 1968). This $V_{\rm M}$ value corresponds to a solvent content of approximately 51%, assuming a protein density of 1.29 g cm^{-3} (Maya-Monteiro *et al.*, 2000). The data-collection statistics are shown in Table 1.

Owing to the lack of amino-acid sequence data, the structure will be solved using heavy-atom derivatives. An effort to clone and sequence the gene (or genes) is currently under way in order to obtain the sequence of the protein. A search for suitable heavy-atom derivatives is in progress. In order to solve the structure we are planning to measure MAD data using the iron constitutively present in the protein. Derivatives with the iron substituted by palladium and tin will also be prepared in order to obtain more derivatives.

In summary, we have obtained well diffracting crystals of the main protein of the hemolymph of the cattle tick *B. microplus*. With the three-dimensional structure of HeLp, we expect to understand better how the protein binds and transport heme. To our knowledge, this is the first report of lipoprotein crystals that are capable of diffracting to high resolution. High-resolution crystals only were obtained for the apo proteins of a truncated human apoA-I of HDL in the absence of lipids (Borhani *et al.*, 1997). For human LDL, only low-resolution diffraction patterns (27 Å) have been reported (Lunin *et al.*, 2001). Therefore, resolution of the HeLp structure will provide unique high-resolution information on the lipid–protein interface in a eukaryotic lipoprotein.

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