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Crystallization and preliminary diffraction studies of a recombinant major urinary protein

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A monoclinic crystal form of a recombinant major urinary protein, (MUP) from mouse, has been obtained. MUP is a member of the lipocalin family whose biological function relates to olfaction and sexual communication. Crystals were grown by the vapour-diffusion technique against a mother liquor containing $CdCl_2$. Crystals belong to the monoclinic space group $P2_1$ with the cell parameters $a = 37.14$, $b = 55.79$, $c = 37.67$ Å, and $\beta = 93.24°$. The crystals diffract to beyond 1.4 Å resolution at a synchrotron beamline.

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1. Introduction

The major urinary protein (MUP) is an important component of the urine from the sexually mature male mouse, and a member of the lipocalin superfamily (Cavaggioni *et al.*, 1987; Adams & Sawyer, 1990; Böcskei *et al.*, 1992). Lipocalins have a variety of functions, which include odor-binding proteins found in the nasal mucosa or excreted in urine. Studies show that MUP has an important role in the transmission and reception of odor for sexual communication (Le Magnen, 1952). Many members of the lipocalin family bind hydrophobic ligands with high affinity and selectivity. MUP also has high homology with a pirazine-binding protein from nasal mucosa. The MUP binding process takes place in the blood plasma; subsequently, MUP is concentrated in the urine and, once excreted it slowly releases into the air volatile molecules with hormonal and behavioral activities.

Although MUP is present in large amounts in the urine of mature mice, it is expressed in several isoforms which are difficult to separate. A homogeneous and functional recombinant MUP (rMUP) was expressed from methylotropic yeast *Pichia pastoris* (Ferrari *et al.*, 1997). Binding active indicates that rMUP has a behavior similar to the native one, suggesting that the protein, and in particular its hydrophobic binding pocket, is folded properly. This was reported to be the first protein of the lipocalin family being expressed in quantitative yields, with correct folding and activity. Owing to its role in pherormonal communication there is large interest in studying in detail the binding site of MUP, and the interpretation of the relationship between structural and biological function. Tetragonal crystals of MUP have previously been obtained and its X-ray structure at 2.4 Å resolution was reported by Böcskei *et al.* (1992). However, recent NMR studies of rMUP (Dr Alberto Spisni, personal communication) reveal that the secondary structure of the molecule in solution differs from the one obtained on the basis of X-ray diffraction studies.

In order to investigate further the three-dimensional structure and the function of this urinary protein, to study the role of crystallographic contacts, and to compare it with the NMR structure, we initiated rMUP crystallization experiments. Crystals of the rMUP, diffracting to 1.39 Å resolution, were obtained in the monoclinic crystal form.

2. Methods and results

Recombinant MUP, kindly supplied by Dr A. Spisni (Università di Parma, Itália), was expressed and purified as previously described (Ferrari *et al.*, 1997). The crystals used for data collection were grown by hanging-drop vapour diffusion at 291 K in sodium phosphate buffer at pH values near 6.5, and cadmium chloride (60 m*M*) as the precipitant. Drops consisted of equal volumes of protein at 10 mg ml^{-1} concentration and well solution (well volume = 1.0 ml). Crystals of size (0.4 × 0.15 × 0.1 mm) grew in four weeks.

Diffraction data were collected on a MAR Research 345 imaging plate at the Protein Crystallographic (PCr) beamline (Polikarpov *et al.*, 1998) at the Laboratório Nacional de Luz Síncrotron (LNLS) located in Campinas, Brazil. The image plate was operated in the 300 mm scanning mode and the crystal-to-detector distance was set to 90 mm. During data collection the crystal was chilled to 277 K. Initially the resolution limit of diffraction was 1.39 Å, but then gradually decreased during data collection. Data were auto-indexed, and integrated with the program *DENZO* on a Silicon Graphics INDY system. Scaling and merging of data were achieved with the

Table 1
Data collection and processing statistics.

Statistical values for the highest resolution shell are shown in parentheses corresponding to 1.76–1.70 Å.

Space group	$P2_1$
Cell dimensions (Å, °)	
a	37.14
b	55.79
c	37.67
β	93.24
Wavelength (Å)	1.376
Crystal-to-detector-distance (mm)	90
Resolution range (Å)	14–1.7
Total No. of reflections	38851
No. of unique reflections	14693
$\langle I/\sigma(I)\rangle$	10.9 (2.46)
Multiplicity	2.64 (2.03)
Completeness (all data) (%)	87.0 (84.5)
$R_{\rm sym}$ (%)	0.079 (0.233)

program *SCALEPACK* (Otwinowski, 1993). A data set comprising 80 oscillation photographs with an oscillation range of 1.0° was collected from a single crystal to 1.7 Å. The resulting statistics for the data set are given in Table 1.

According to the molecular weight of the protein (approximately 18 kDa) and space group of the crystal, it can be inferred that one protein molecule is present in the asymmetric unit. The V_m value of the crystal is 2.36 $Å^3\,Da^{-1}$ and the solvent content is about 48% (Matthews, 1968).

A molecular-replacement solution was calculated with the program *AMoRe* (Collaborative Computational Project, Number 4, 1994; Navaza, 1994) using the previously reported crystal structure of MUP determined at 2.4 Å resolution as a search model (Protein Data Bank code: 1mup). The top solution for the rotation function has a correlation coefficient of 32.5%, whereas the next highest peak was below 17%. A translational position was found which yielded an R factor of 44%, and a correlation coefficient of 68.7%. The packing arrangement of the molecules in the unit cell for this solution yielded no unfavorable intermolecular contacts.

In the previously reported structure of MUP some spurious electron density was found in the ligand-binding pocket, however the ligand could not be unambiguously identified. Seven other side chains in the middle of the molecule were also not found, in addition to the first four residues at the N-terminus and five residues at the C-terminus. Refinement at 1.7 Å resolution is in progress and these problems will be addressed in the higher resolution structure of recombinant MUP, creating a better basis to study the structure–function relationship of this protein.

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