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Crystallization and preliminary X-ray diffraction studies of two different crystal forms of the lipase 2 isoform from the yeast *Candida rugosa*

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The yeast *Candida rugosa* produces several closely related lipases which show a high degree of sequence identity (between 77 and 88% for pairs of proteins). Despite this high sequence identity, they exhibit markedly different substrate specificities, indicating that subtle structural differences may produce significant functional changes. Isoform 2 (lip2) has been crystallized using the hanging-drop vapour-diffusion method at 291 K. Diffraction-quality crystals have been obtained from two different experimental conditions (designated *A* and *B*, respectively). Type *A* crystals belong to space group *P*1 and have unit-cell parameters $a = 62.15$, $b = 91.14$, $c = 108.46$ Å, $\alpha = 90.78$, $\beta = 106.31$, $\gamma = 86.91^\circ$; type *B* crystals are monoclinic with a nearly hexagonal topology, with unit-cell parameters $a = 116.11$, $b = 225.55$, $c = 116.06$ Å, $\beta = 119.89^\circ$, and belong to space group $P2_1$. Diffraction data were collected to a resolution of 1.97 Å at a synchrotron facility from type *A* crystals and to 2.65 Å on an in-house rotating-anode generator from type *B* crystals. Whereas the triclinic crystal reveals monomeric lip2, the monoclinic crystal contains dimeric lip2.

1. Introduction

Lipases are triacylglycerol ester hydrolases (EC 3.1.1.3) which catalyze the hydrolysis of long-chain acylglycerols. The hydrolytic activity of lipases, but not of esterases, is drastically enhanced upon contact with a lipid–water interface (Brockman *et al.*, 1973; Macrae, 1963), a phenomenon known as interfacial activation (Desnuelle *et al.*, 1960; Verger, 1980). A partial explanation of this property has been provided from the structural analysis of lipases from a wide variety of sources (Schrag & Cygler, 1997). These studies reveal the existence of an amphiphilic movable flap, which in the so-called closed or inactive state covers the active site of the enzyme, but in the open or active state makes it accessible to the solvent. All the lipases with known three-dimensional structure are serine hydrolases belonging to the α/β-hydrolase superfamily (Ollis *et al.*, 1992). Their catalytic machinery consists of a highly conserved triad and an oxyanion hole (Nardini & Dijsktra, 1999; Heikinheimo *et al.*, 1999).

The yeast *Candida rugosa* (ATCC 14830) produces several closely related extracellular lipases, which are the products of different genes (Longhi *et al.*, 1992; Lotti *et al.*, 1993). Each one codes for a 534 amino-acid residue polypeptide chain. Currently, the crystal structures of *C. rugosa* lipase (CRL) 1 (lip1), both in the open (Grochulski *et al.*, 1993) and closed (Grochulski *et al.*, 1994) conformations, and that of CRL 3 (lip3, or cholesterol esterase) complexed with cholesteryl linoleate (Ghosh *et al.*, 1995) have been elucidated. Structural comparisons revealed important differences affecting the flap and the substrate-binding pocket (Ghosh *et al.*, 1995), which presumably may explain their different specificity. Interestingly, whereas lip3 (Ghosh *et al.*, 1995) and lip2 (Lee *et al.*, 2002) exhibit a pronounced esterase activity, lip1 essentially behaves as lipase. The structural basis for their esterase/lipase preferences is not known. Recently, it has been proposed that small amino-acid changes between lip1 and lip3, affecting their flaps and binding pockets, may influence their oligomerization (Pernas *et al.*, 2001).

In this work, we have crystallized the CRL isoform 2 under two different conditions. Under conditions designated *A* a triclinic crystal was obtained, whereas conditions *B* yielded a monoclinic crystal. Molecular-replacement (MR) solutions have been found with the closed form of lip1 as a starting model, which strongly suggests that lip2 is in a closed conformation in both crystals. Protein packing in the monoclinic crystal revealed the existence of lip2 dimers, whereas lip2 monomers were observed in the triclinic crystal.

2. Experimental

2.1. *C. rugosa* lipase 2 purification and crystallization

Lip2 was purified from UAB extracts as previously described (Pernas *et al.*, 2000). The initial crystallization conditions were estab-

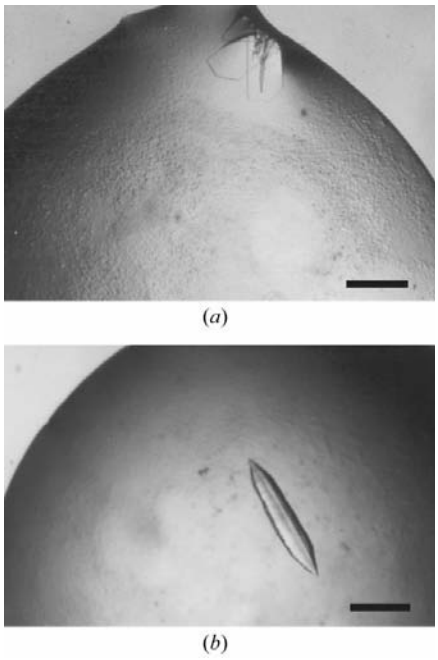

Figure 1
Crystal forms of the CRL 2 isoform. (*a*) Triclinic crystals of lip2 grown in 0.1 *M* sodium acetate trihydrate pH 5.0, 15%(*w*/*v*) PEG 4000 and (*b*) a monoclinic crystal of lip2 grown in 1.6 *M* magnesium sulfate heptahydrate, 0.1 *M* MES pH 6.5. Scale bars correspond to 0.2 mm.

lished using the sparse-matrix sampling technique (Jancarik & Kim, 1991) with the hanging-drop vapour-diffusion method at 291 K using Crystal Screens I and II (Hampton Research). Drops containing equal volumes (2 µl) of protein (7–8 mg ml^{-1}) and reservoir solution were equilibrated against 500 µl of reservoir solution. Crystals were obtained under different conditions that can be classified into two categories: samples containing polyethylene glycol 4000 (conditions 10, 40 and 41 from Crystal Screen I; type *A* crystals) and condition 20 from Crystal Screen II (1.6 *M* magnesium sulfate heptahydrate, 0.1 *M* MES pH 6.5; type *B* crystals). Initial type *A* crystals were aggregated thin plates that were suitable for diffraction after careful separation with Hampton Micro-Tools. Further optimization of conditions to 0.1 *M* sodium acetate trihydrate pH 5.0, 15%(*w*/*v*) PEG 4000 (2 µl of protein solution plus 2 µl reservoir solution) resulted in good-quality plate-like crystals (Fig. 1*a*). On the other hand, type *B* crystals were rod-shaped and were further improved in size using 1.6 *M* magnesium sulfate heptahydrate, 0.1 *M* MES pH 6.5 (4 µl of protein solution plus 4 µl reservoir solution) (Fig. 1*b*). The employment of Additive Screens I and II or Detergent Screens (Hampton Research) did not result in further improvement of the crystals.

2.2. X-ray diffraction experiments

Preliminary diffraction data were collected on an in-house MAR Research MAR345 image-plate detector with Cu $K\alpha$ X-rays generated by an Enraf–Nonius rotating-anode generator equipped with a double-mirror focusing system, operated at 40 kV and 90 mA. Prior to flash-freezing at 120 K, all crystals were soaked for 10 s in a cryoprotectant solution consisting of the crystallization solution plus 25% glycerol. A native data set from a type *A* crystal was finally collected using the synchrotron-radiation source at ESRF (Grenoble) on beamline BM14 using a MAR CCD detector; a data set from a type *B* crystal was collected in-house. In the first case, the wavelength used was 1.004 Å. The crystal-to-detector distances were set to 100 and 175 mm, respectively. All data were processed and scaled using the programs *MOSFLM* (Leslie, 1994) and *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

A systematic search for crystallization conditions resulted in two crystal forms of the CRL 2 isoform. Type *A* crystals diffract to 1.97 Å resolution (Fig. 2*a*) and belong to the *P*1 triclinic space group. The unit-cell parameters are a = 62.15, b = 91.14, c = 108.46 Å, α = 90.78, β = 106.31, γ = 86.91°. Specific volume calculations yielded four molecules of lipase 2 in the unit cell, with a solvent content of 50.9% (V_M = 2.46 Å^3 Da^{-1}). Data-collection and processing statistics are summarized in Table 1. Type *B* crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 116.11, b = 225.55, c = 116.06 Å, β = 119.89° and diffract to 2.65 Å resolution (Fig. 2*b*). These unit-cell parameters show nearly hexagonal topology; in fact, the diffraction images were easily indexed with *MOSFLM* (Leslie, 1994) in an hexagonal lattice, with unit-cell parameters $a = b$ = 115.83, c = 225.22 Å, but all efforts to merge the data with *SCALA* (Collaborative Computational Project, Number 4, 1994) were unsuccessful (R_{sym} > 20%). Finally, the data were successfully indexed in the less symmetric monoclinic space group $P2_1$. The asymmetric unit contains six lip2 molecules and has 67.0% solvent content (V_M = 3.74 Å^3 Da^{-1}). The statistics for this data set are shown in Table 1.

Phasing of the diffraction data is being attempted with the molecular-replacement method using the program *AMoRe* (Navaza & Vernoslova, 1995). The structure of lip1

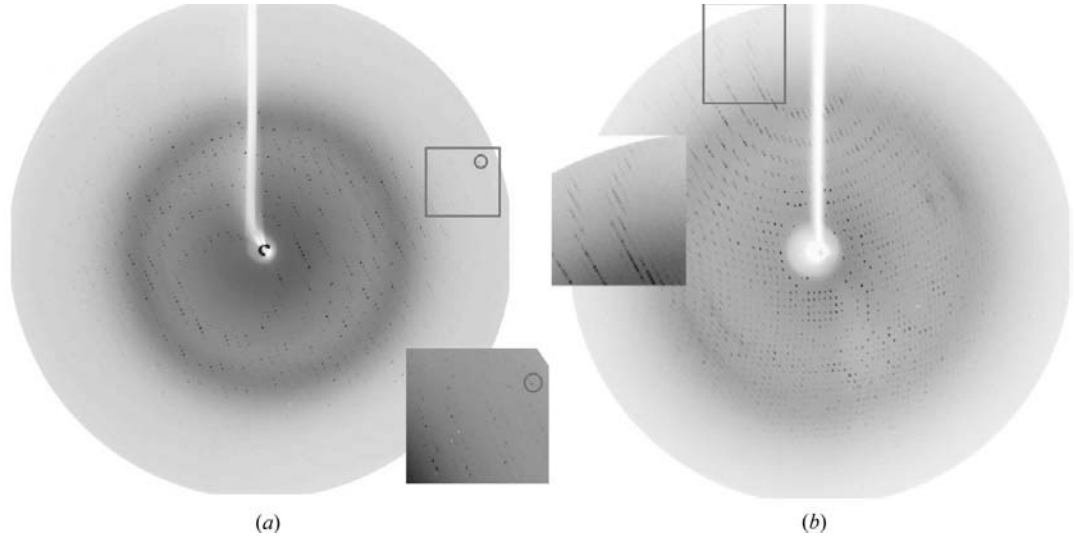

Figure 2
Diffraction patterns of the two crystal forms of the CRL 2 isoform. (*a*) Pattern from the data set corresponding to the triclinic crystal of lip2 (collected using synchrotron radiation on beamline BM14 at the ESRF) and (*b*) pattern from the data set of the monoclinic crystal (collected in-house). The circle in (*a*) indicates a 2.10 Å resolution reflection; in (*b*), reflections reach the edge of the plate, which corresponds to 2.20 Å resolution.

Table 1
Data-collection and processing statistics of the triclinic and monoclinic crystals of lip2.

Values in parentheses are for the highest resolution shell.

Space group	$P1$	$P2_1$
Unit-cell parameters		
a (Å)	62.15	116.11
b (Å)	91.14	225.55
c (Å)	108.46	116.06
α (°)	90.78	
β (°)	106.31	119.89
γ (°)	86.91	
Resolution limit (Å)	1.97	2.65
Observations	299637	327802
Unique reflections	152503	139328
Completeness (%)	94.6 (76.5)	93.3 (93.3)
Multiplicity	2.0 (1.1)	2.4 (2.5)
$R_{\rm merge}$† (%)	9.5 (29.9)	15.4 (35.8)
$I/\sigma(I)$	4.6 (2.2)	4.4 (2.0)
Solvent content (%)	50.9	67.0

† $R_{\rm merge} = \sum|I(h) - \langle I(h)\rangle|/\sum I(h)$, where $I(h)$ is the observed intensity and $\langle I(h)\rangle$ is the mean intensity of reflection h over all measurements of $I(h)$.

(79.4% sequence identity with lip2) has been used as search model for a rotational and translational search in the 15–3.5 Å resolution range. MR solutions have been found with the closed form of lip1 as a starting model for both diffraction data sets. The final correlation coefficient and R factor after rigid-body refinement are 50.8 and 36.5%, respectively, for the monoclinic crystal and 60.5 and 35.4%, respectively, for the triclinic crystal. The use of the open form of lipase 1 as a starting model produced solutions with significantly lower correlation coefficients and higher R factors. Whereas the protein packing in the triclinic crystal reveals monomeric lip2 (Fig. 3*a*), the rather loose packing in the monoclinic crystal (Fig. 3*b*) shows lip2 dimers formed by non-crystallographic symmetry-related monomers, similar to those described for lip1 (Grochulski *et al.*, 1993) and lip3 (Ghosh *et al.*, 1995). These dimers orientate themselves such that the substrate-binding pockets face each other. Determination of the three-dimensional structure of lip2 in both crystal forms, now in progress, will provide reliable information about the oligomerization process of lip2 and also about the structural determinants of the different substrate specificities of the CRL isoforms. As the MR results indicate, lip2 is in an inactive state, which is interesting as it would be the second CRL closed state to be described and the first time in which an oligomeric *and* inactive form of a CRL has been described, which may give new insights into the mechanism of lipase activation.

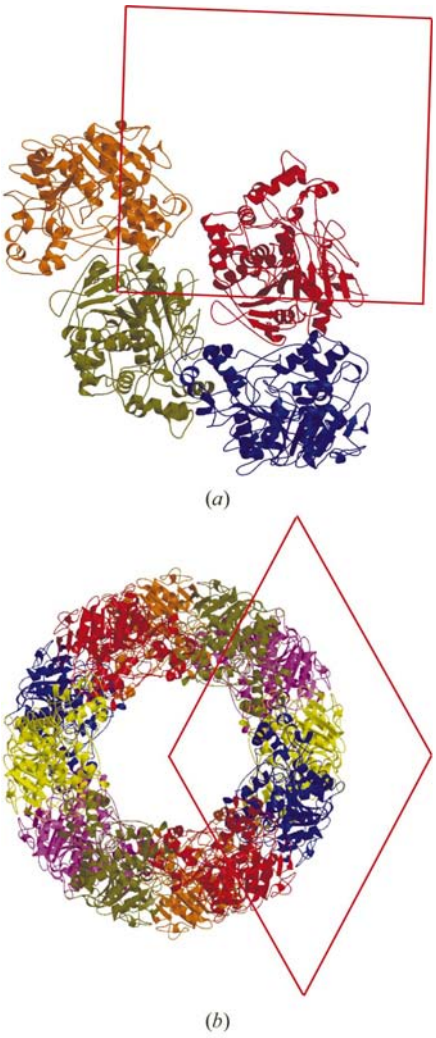

Figure 3
Crystal packing of the CRL 2 isoform. (*a*) Protein packing in the triclinic crystal. The four protein molecules of the unit cell are shown in different colours. (*b*) Lip2 packing in the monoclinic crystal as viewed normal to the *b* axis; the two asymmetric units making up the crystal unit cell are shown to illustrate the pseudo-hexagonal symmetry. In both cases outlines of the unit cell are included. The figures were prepared with *MOLSCRIPT* (Kraulis, 1991).

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