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Crystallization of a proteolyzed form of the horse pancreatic lipase-related protein 2: structural basis for the specific detergent requirement

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Horse pancreatic lipase-related proteins PLRP1 and PLRP2 are produced by the pancreas together with pancreatic lipase (PL). Sequence-comparison analyses reveal that the three proteins possess the same two-domain organization: an N-terminal catalytic domain and a C-terminal domain, which in PL is involved in colipase binding. Nevertheless, despite the high level of sequence identity found, they exhibit distinct enzymatic properties. The intrinsic sensitivity of the peptide bond between Ser245 and Thr246 within the flap region of PLRP2 to proteolytic cleavage probably complicates PLRP2 crystallization since, as shown here, this proteolyzed form of PLRP2 is only crystallized after specific detergent stabilization of this region. This has been performed by the hanging-drop vapour-diffusion method at 291 K and exclusively in the presence of *N*,*N*-dimethyldecylamine-β-oxide (DDAO). However, most crystals (>95%) are highly twinned and diffract poorly (to ∼7–5 Å resolution). Diffraction-quality trigonal crystals have unit-cell parameters $a = b = 128.4$, $c = 85.8$ Å and belong to space group $P3_221$. A 2.9 Å native data set was collected at ESRF on beamline ID14-2 with an $R_{\rm merge}$ of 12.7%. Preliminary structural analysis provides a structural basis for the specific roles of DDAO.

1. Introduction

Together with pancreatic lipase (PL), the key enzyme in intestinal fat digestion, the pancreas produces pancreatic lipase-related proteins PLRP1 and PLRP2. Despite the significant sequence identity (65–70%) and structural similarity (Winkler *et al.*, 1990; Roussel *et al.*, 1998) between PL and PLRP2, they exhibit clearly distinct enzymatic properties and are classified into different subfamilies. Both enzymes have a two-domain organization: an N-terminal catalytic domain and a C-terminal domain, which is involved in colipase binding. In addition, the catalytic machinery is buried owing to the presence of a loop (flap) which is displaced upon PL activation (Van Tilbeurgh *et al.*, 1993). Even so, PLRP2 shows specific functional properties in addition to lipase activity: it hydrolyzes phospholipids (Thirstrup *et al.*, 1994; Jayne, Kerfelec, Foglizzo, Chapus *et al.*, 2002) and galactolipids (Andersson *et al.*, 1996) and its behaviour towards colipase is clearly different to that of PL. In this regard, molecular-sieve experiments have not detected the formation of either a binary complex PLRP2–colipase or a ternary complex PLRP2–colipase–micelle (Jayne, Kerfelec, Foglizzo, Granon *et al.*, 2002) equivalent to that formed in the mechanism of PL activation (Hermoso *et al.*, 1996, 1997).

Recent studies have revealed a high sensitivity of the peptide bond Ser245–Thr246 within the flap region of PLRP2 to proteolytic cleavage, which does not affect the enzymatic activity (Jayne, Kerfelec, Foglizzo, Granon *et al.*, 2002). This susceptibility to peptide-bond cleavage may help in understanding the inhibition of PLRP2 activity by E600 (diethyl *p*-nitrophenyl phosphate). These studies reveal the accessibility of the catalytic serine residue to E600 in the presence of bile salts either in the monomeric or in micellar states.

In this work, we have crystallized horse PLRP2 in the presence of the detergent DDAO, which is shown to play a dual role: it stabilizes the flap region and mediates protein–protein contacts.

2. Experimental

2.1. PLRP2 purification

PLRP2 was purified from an acetonic pancreatic powder as described previously (Jayne, Kerfelec, Foglizzo, Chapus *et al.*, 2002; Jayne, Kerfelec, Foglizzo, Granon *et al.*, 2002). Briefly, the pancreatic powder was dissolved for 1 h at 277 K in Tris buffer (20 m*M* Tris–HCl pH 8.0) containing protease inhibitors. After centrifugation at 8000 rev min^{-1} for 1 h, the supernatant was precipitated with 50%(*w*/*v*) ammonium sulfate. The proteins were subsequently resuspended in Tris buffer containing 0.2 *M* NaCl, 1 m*M* benzamidine and 0.2 m*M* PMSF and dialyzed overnight at 277 K against

the same buffer. After centrifugation, the supernatant was loaded onto an Ultrogel AcA 54 column equilibrated in the same buffer. Fractions containing lipase activity were pooled and dialyzed against Tris buffer containing 1 m*M* benzamidine. After centrifugation, the supernatant was loaded onto a Q-Sepharose Fast Flow column (17 × 2.5 cm; Pharmacia) equilibrated with the same buffer. Elution with a linear salt gradient (from 0 to 200 m*M* NaCl) yielded two peaks with lipase activity corresponding to PLRP2 and colipase-dependent lipase, respectively. The fractions corresponding to PLRP2 were pooled and dialyzed against 10 m*M* sodium acetate buffer pH 5.8 and loaded onto a SP-Sepharose Fast Flow column (17 × 2.5 cm Pharmacia). Elution was performed with a linear salt gradient (from 0 to 100 m*M* NaCl). Fractions corresponding to PLRP2 were pooled and lyophilized.

2.2. Crystallization

Protein concentration was determined from absorbance measurements employing an extinction coefficient at 280 nm (ε) of $6.65 \times 10^4\ M^{-1}\ \mathrm{cm}^{-1}$ on a Cintra 5 UV–Vis spectrophotometer (GBC Scientific Equipment) with 1 cm quartz cells. The protein powder was systematically diluted in 10 m*M* Tris–Gly pH 8.5 to a final concentration of $\sim$10 mg ml^{-1}. Extensive screening for crystallization conditions employing the sparse-matrix sampling technique (Jancarik & Kim, 1991) with the hanging-drop vapour-diffusion method at 291 K and commercial screens did not yield successful results in terms of protein crystals. Promising protein precipitates were obtained with 18–20%(*w*/*v*) polyethylene glycol (PEG) 6000 as precipitant in Tris–Gly buffer (0.1 *M* Tris–Gly pH 8.5). Drops contained equal volumes (2 µl) of protein and reservoir solution equilibrated against 500 µl reservoir solution. Small PLRP2 crystals were obtained in condition No. 15 of Detergent Screen 1 from Hampton Research containing *N*,*N*-dimethyldecylamine-β-oxide (DDAO) (2 µl protein + 2 µl detergent + 2 µl precipitant). Further optimization of conditions to volume ratios of 2 + 1 + 2 µl, respectively, yielded crystals that rapidly ($\sim$8 h) grew to dimensions of 0.6 × 0.3 × 0.2 mm (Fig. 1).

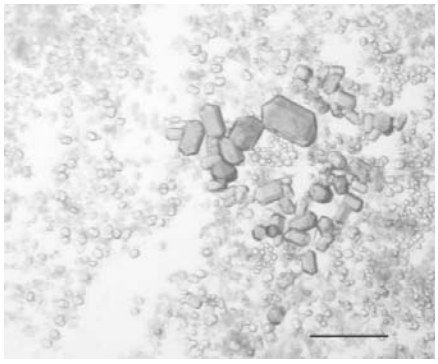

Figure 1
Crystals of PLRP2 grown at 291 K in 28%(*w*/*v*) polyethylene glycol 6000, 0.1 *M* Tris–Gly pH 8.5 containing 20.8 m*M* *N*,*N*-dimethyldecylamine-β-oxide (DDAO). The bar indicates 0.3 mm.

2.3. X-ray diffraction experiments

Diffraction data were collected at ESRF (Grenoble, France) on beamline ID14-2 (λ = 0.934 Å) using an ADSC Q4 CCD detector. Crystals were cryoprotected by a quick soak (10 s) in reservoir solution containing 30%(*v*/*v*) glycerol. After exhaustive crystal screening, a diffraction data set from a suitable crystal was collected at 100 K. It consisted of 240 images with 1° oscillation and extended to 2.99 Å resolution (Fig. 2). The crystal-to-detector distance was set to 165 mm. 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

PLRP2 crystals diffract to 2.99 Å resolution (Fig. 2) and belong to the trigonal space group $P3_221$ (unit-cell parameters $a = b = 128.4$, $c = 85.8$ Å). Data-collection and processing statistics are summarized in Table 1. A total of 229 946 measured reflections were merged into 16 857 unique reflections with an $R_{\rm merge}$ of 12.7% and an overall $I/\sigma(I)$ of 5.4. The merged data set was 100% complete to 2.99 Å resolution. The asymmetric unit contains one PLRP2 molecule, giving a crystal volume per protein weight ($V_{\rm M}$) of 4.08 Å^3 Da^{-1}, which corresponds to a solvent content of 70% (Matthews, 1968). This high solvent content is not unreasonable in view of the weak diffraction.

The structure of horse PLRP2 was solved by molecular replacement with *MOLREP* from the *CCP*4 package using the structure of rat PLRP2 (PDB code 1bu8; Roussel *et al.*, 1998). The cross-rotation function using data in the 26–3.5 Å resolution range gave one clear solution with Rf/σ = 11.41 (the next highest solution was 4.87). The translation function gave a unique solution (TF/σ = 61.21) with an *R* factor of 0.508 and a correlation coefficient of 0.32. The next solution was TF/σ = 35.39, with an *R* factor and correlation coefficient of 0.548 and 0.197, respectively. The structure was remodelled using the program *O* (Jones *et al.*, 1991). Subsequent model refinement and calculation of electron-density maps was performed with *REFMAC* (Murshudov *et al.*, 1997).

Table 1
Data-collection and processing statistics.

Values for the outermost shell (3.15–2.99 Å) are given in parentheses.

Space group	$P3_221$
Unit-cell parameters (Å)	$a = b = 128.4$, $c = 85.8$
Resolution range (Å)	46.6–2.9
No. measured reflections	229946
No. unique reflections	16857
$R_{\rm merge}$ (%)	12.7 (47.0)
Completeness (%)	100 (100)
Multiplicity	13.6 (12.2)
Average $I/\sigma(I)$	5.4 (1.6)

The final model refined using data to 2.9 Å resolution provides the structural basis for the specific requirement of DDAO for PLRP2 crystallization. Each PLRP2 molecule binds two detergent molecules (Fig. 3) in such a way that they sandwich the flap region. Whilst one DDAO molecule perfectly fits within an internal hydrophobic cavity, where it makes numerous van der Waals contacts and a salt bridge with Asp81, the other one interacts hydrophobically with the external side of the flap and with the adjacent symmetry-related PLRP2 molecule. Furthermore, the high quality of the electron-density map in the flap region allows unambiguous identification of proteolysis, which is in agreement with previous results indicating cleavage of the peptide bond between Ser245 and Thr246 (Jayne, Kerfelec, Foglizzo, Granon *et al.*, 2002). Thus, it can be concluded that the

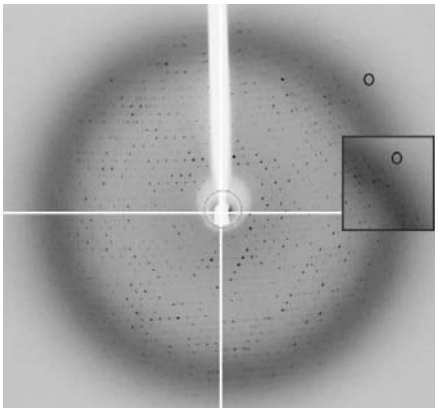

Figure 2
X-ray diffraction pattern of a trigonal PLRP2 crystal collected at the ESRF (beamline ID14-2) using an ADSC Q4 CCD detector. The exposure time was 7 s, the crystal-to-detector distance 165 mm and the oscillation angle 1.0°. The circle indicates a 3.00 Å reflection.

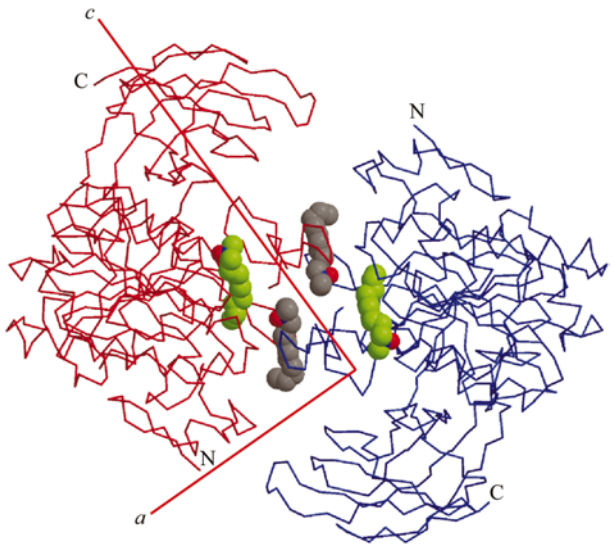

Figure 3
Packing of PLRP2 trigonal crystals in the *ac* plane showing the location of the detergent DDAO (as CPK models). Colour code for DDAO molecules: green, molecules stabilizing the inner part of the flap; grey, molecules mediating contacts between PLRP2 molecules.

detergent DDAO plays a dual role in the crystallization of PLRP2: firstly, it stabilizes the proteolyzed flap region, which is presumably a highly flexible region (no electron density is observed for residues 243 to 246), and secondly, it mediates contacts with the immediately adjacent protein molecule. These effects are critical in the crystallization process, considering that the most important contacts between PLRP2 molecules are between the flap regions.

The model is currently being analyzed to provide information about the structural basis of the distinct enzymatic behaviour of PLRP2 when compared with that of PL.

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References

Andersson, L., Carrière, F., Lowe, M. E., Nilson, A. & Verger, R. (1996). *Biochim. Biophys. Acta*, **1302**, 236–240.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Hermoso, J., Pignol, D., Kerfelec, B., Crenon, I., Chapus, C. & Fontecilla-Camps, J. C. (1996). *J. Biol. Chem.* **271**, 18007–18016.
Hermoso, J., Pignol, D., Penel, S., Roth, M., Chapus, C. & Fontecilla-Camps, J. C. (1997). *EMBO J.* **16**, 5531–5536.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jayne, S., Kerfelec, B., Foglizzo, E., Chapus, C. & Crenon, I. (2002). *Biochim. Biophys. Acta*, **1594**, 255–265.
Jayne, S., Kerfelec, B., Foglizzo, E., Granon, S., Hermoso, J., Chapus, C. & Crenon, I. (2002). *Biochemistry*, **41**, 8422–8428.
Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Leslie, A. (1994). *MOSFLM User Guide. MOSFLM* v.5.2. MRC Laboratory of Molecular Biology, Cambridge.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst.* D**53**, 240–255.
Roussel, A., Yang, Y., Ferrato, F., Verger, R., Cambillau, C. & Lowe, M. (1998). *J. Biol. Chem.* **273**, 32121–32128.
Thirstrup, K., Verger, R. & Carrière, F. (1994). *Biochemistry*, **33**, 2748–2756.
Van Tilbeurgh, H., Egloff, M. P., Martínez, C., Rugani, N., Verger, R. & Cambillau, C. (1993). *Nature (London)*, **362**, 814–820.
Winkler, F. K., D'Arcy, A. & Hunziker, W. (1990). *Nature (London)*, **343**, 771–774.