

# Crystallization and Preliminary Diffraction Studies of Porcine Pancreatic Elastase in Complex with a Novel Inhibitor

Tânia F. Oliveira<sup>a</sup>, Jalmira Mulchande<sup>b</sup>, Rui Moreira<sup>b</sup>, Jim Iley<sup>c</sup> and Margarida Archer<sup>a,\*</sup>

<sup>a</sup>Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, ITQB-UNL, Av. República, Apt. 127, 2781-901 Oeiras, Portugal; <sup>b</sup>CEFC, Faculdade de Farmácia, Universidade de Lisboa, Av. Forças Armadas, 1600-083 Lisboa, Portugal; <sup>c</sup>Chemistry Department, The Open University, Milton Keynes, MK7 6AA, UK

**Abstract:** Porcine pancreatic elastase (PPE) was crystallized in complex with a novel inhibitor at pH 5 and X-ray diffraction data were collected at a synchrotron source to 1.66 Å. Crystals belong to the orthorhombic space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with unit cell parameters a = 50.25 Å, b = 57.94 Å and c = 74.69 Å. PPE is often used as model for drug target, due to its structural homology with the important therapeutic target human leukocyte elastase (HLE). Elastase is a serine protease that belongs to the chymotrypsin family, which has the ability to degrade elastin, an important component in connective tissues. Excessive elastin proteolysis leads to a number of pathological diseases.

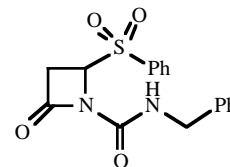
**Keywords:** Human leukocyte elastase, Porcine pancreatic elastase, β-Lactams inhibitors, Crystallization.

## INTRODUCTION

Human leukocyte elastase (HLE, EC 3.4.21.37) is a serine protease released from polymorphonuclear leukocytes (neutrophils) in response to inflammatory stimuli and mediators [1]. HLE degrades very efficiently various tissue matrix proteins such as elastin and in healthy individuals its proteolytic activity is regulated by potent endogenous anti-proteases such as α<sub>1</sub>-antitrypsin and secretory leukocyte proteinase inhibitor [2]. Imbalance between HLE and its endogenous inhibitors leads to excessive elastin proteolysis and destruction of connective tissues in a number of inflammatory diseases such as pulmonary emphysema, adult respiratory distress syndrome (ARDS), chronic bronchitis, chronic obstructive pulmonary disease (COPD), pulmonary hypertension and rheumatoid arthritis [3-6], as well as in other pulmonary pathophysiological states, e.g. cystic fibrosis [7].

The design of low molecular-weight inhibitors of elastases has received considerable attention due to their potential therapeutic usefulness [8, 9]. Understanding the nature of inhibition and structure of the ligand-enzyme complex is a fundamental step in any structure-based lead optimization project. This is particularly true for mechanism-based inhibitors that can lead to a double-hit inactivation process [10]. β-Lactams (azetidin-2-ones) are potent inhibitors of a wide range of enzymes that contain serine as the catalytic residue [11-13]. Many structural and inhibition studies with β-lactams have been conducted with the readily available porcine pancreatic elastase (PPE) [14, 15]. Indeed, the sequence identity between PPE and HLE is ca. 40% [16], and despite small differences in the primary specificity pockets [1], PPE is considered to be a good model for HLE to be used in preliminary inhibition studies.

Herein we report the crystallization and preliminary X-ray diffraction analysis of PPE inactivated with the β-lactam (Fig. 1), which contains a potential leaving group at C-4 position of the four-membered ring. Compound JM54 (Fig. 1) is a novel potent irreversible inhibitor of PPE ( $k_{\text{obs}}/[I]$  of 290 M<sup>-1</sup>s<sup>-1</sup>) that requires only ca. 1 mol. equivalent to inactivate the enzyme [17]. We aim towards a detailed structural characterization of the molecular interactions of PPE with this potential mechanism-based inhibitor.



**Figure 1.** Molecular structure of the synthesized inhibitor JM54.

## MATERIALS AND METHODS

### Crystallization

Elastase from porcine pancreas (E.C. 3.4.21.36) was purchased from Serva (Cat No 20929) and was used without any further purification. The protein (MW of 25.9 kDa) was dissolved in bidistilled water to a concentration of 40 mg/ml. A concentrated solution (~150 mM) of the synthesized inhibitor JM54, was freshly prepared in DMSO. JM54 was incubated with PPE for 30 minutes at room temperature (inhibitor in a 10 molar excess, final concentration of 10% DMSO). The crystallization trials were set up at 293 K using the sitting drop vapour-diffusion method, and were based on previously known crystallization conditions [18, 19]. PPE co-crystallized with the inhibitor JM54 in 200 mM sodium sulfate and 100 mM sodium acetate at pH 5.1 yielded good quality crystals. Native crystals were also grown under the same experimental conditions to test soaking procedures with the inhibitor.

\*Address correspondence to this author at the Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, ITQB-UNL, Av. República, Apt. 127, 2781-901 Oeiras, Portugal; E-mail: archer@itqb.unl.pt



HLE = Human Leukocyte Elastase

DMSO = Dimethyl Sulfoxide

## REFERENCES

- [1] Bode, W., Meyer, E., Jr. and Powers, J.C., (1989) *Biochem.*, 28, 1951-1963.
- [2] Barnes, P.J., (2000) *N. Engl. J. Med.*, 343, 269-280.
- [3] Cowan, K.N., Heilbut, A., Humpl, T., Lam, C., Ito, S. and Rabinovitch, M., (2000) *Nat. Med.*, 6, 698-702.
- [4] Ohbayashi, H., (2005) *Expert. Opinion on Therapeutic Patents*, 15, 759-771.
- [5] Donnelly, L.E. and Rogers, D.F., (2003) *Drugs*, 63, 1973-1998.
- [6] Fujita, J., Nelson, N.L., Daughton, D.M., Dobry, C.A., Spurzem, J.R., Irino, S. and Rennard, S.I., (1990) *Am Rev. Respir. Dis.*, 142, 57-62.
- [7] Griese, M., von Bredow, C., Birrer, P. and Schams, A., (2001) *Pulm. Pharmacol. Ther.*, 14, 461-467.
- [8] Malhotra, S., Man, S.F. and Sin, D.D., (2006) *Expert Opin. Emerg. Drugs*, 11, 275-291.
- [9] Bernstein, P.R., Edwards, P.D. and Williams, J.C., (1994) *Prog. Med. Chem.*, 31, 59-120.
- [10] Powers, J.C., Asgian, J.L., Ekici, O.D. and James, K.E., (2002) *Chem. Rev.*, 102, 4639-4750.
- [11] Konaklieva, M.I., (2002) *Curr. Med. Chem. Anti-Infective Agents*, 1, 215-238.
- [12] Konaklieva, M.I. and Plotkin, B.J., (2004) *Mini Rev. Med. Chem.*, 4, 721-739.
- [13] Leung, D., Abbenante, G. and Fairlie, D.P., (2000) *J. Med. Chem.*, 43, 305-341.
- [14] Wilmouth, R.C., Kassamally, S., Westwood, N.J., Sheppard, R.J., Claridge, T.D., Aplin, R.T., Wright, P.A., Pritchard, G.J. and Schofield, C.J., (1999) *Biochemistry*, 38, 7989-7998.
- [15] Wilmouth, R.C., Clifton, I.J. and Neutze, R., (2000) *Nat Prod Rep*, 17, 527-533.
- [16] Sinha, S., Watorek, W., Karr, S., Giles, J., Bode, W., Travis, J., (1987) *Proc. Natl. Acad. Sci., USA*, 84, 2229-2232.
- [17] Mulchande, J., Martins, L. and Moreira, R., (2006) *Drugs Fut.*, 31, 194.
- [18] Weiss, M.S., Panjikar, S., Nowak, E. and Tucker, P.A., (2002) *Acta Cryst. D*, 58, 1407-1412.
- [19] Wurtele, M., Hahn, M., Hilpert, K. and Hohne, W., (2000) *Acta Cryst. D*, 56, 520-523.
- [20] Leslie, A.G.W., (1992) Joint CCP4 + ESF-EAMCB Newsl. Protein Crystallogr., 26.
- [21] Collaborative Computational Project, N. 4, (1994) *Acta Cryst. D*, 50, 760-763.
- [22] Murshudov, G.N., Vagin, A.A. and Dodson, E.J., (1997) *Acta Cryst. D*, 53, 240-255.
- [23] Matthews, B.W., (1968) *J. Mol. Biol.*, 33, 491-497.
- [24] Ay, J., Hilpert, K., Krauss, N., Schneider-Mergener, J. and Hohne, W., (2003) *Acta Cryst. D*, 59, 247-254.
- [25] Kinoshita, T., Kitatani, T., Warizaya, M. and Tada, T., (2005) *Acta Cryst. F*, 61, 808-811.
- [26] Wilmouth, R.C., Clifton, I.J., Robinson, C.V., Roach, P.L., Aplin, R.T., Westwood, N.J., Hajdu, J. and Schofield, C.J., (1997) *Nat. Struct. Biol.*, 4, 456-462.