Conclusions and Future Perspectives

Granzyme B and Granzyme K

The immune response is a highly sophisticated defence system that needs a precise regulation to exert its functions. The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, and viral infections, as well as from the growth of tumour cells. Many of these cell types have specialised functions being this the case of the natural killer cells and the cytotoxic T lymphocytes, which use a set of mechanisms to induce cell death in the target cell.

The granzymes are mediators of one of these major mechanisms and the crystal structure elucidation of two members of this family has been crucial for a better understanding of the molecular processes underlying the immune response. The present work analyses the structural determinants of substrate specificity of two different proteinases from a common source, the cytotoxic granzyme B and pro-granzyme K. Both proteins belong to the same family of serine proteinases and exhibit high homology at the sequence level. However, they display distinct biochemical properties concerning their substrate specificities and inhibition profiles.

Granzyme B displays a unique substrate specificity for a serine proteinase as it cleaves peptide bonds after aspartic residues. The structural determinants responsible for this exceptional substrate specificity were unveiled in the three-dimensional structure of granzyme B. The primary specificity for Asp residues is basically determined by the presence of an Arg residue (Arg226) located at the back of the specificity pocket, which will establish a salt bridge with the entering aspartic acid of a substrate. Additional residues would also contact a GzmB substrate defining the extended substrate specificity profile of this enzyme. To date, GzmB has been the only granzyme to be clearly implicated in triggering apoptosis in target cells. Several substrates have been identified, some of which, i.e. caspase 3 and Bid, have shown to be physiological substrates. GzmB is thus an important cellular tool to combat threatening cells to our bodies. It is then of extreme importance to precisely regulate GzmB site and way of action. In normal defence responses GzmB is a very effective immune mediator but its activity can turn hazardous

once its action is not anymore under physiological and healthy circumstances, resulting for instance in the onset of rheumatoid arthritis (RA). RA is a chronic, systemic, inflammatory disease that chiefly affects the synovial membranes of multiple joints in the body. The prevalence of the disease is 1-2% of the general population and is found world-wide. Females with RA outnumber males by a 3:1 margin. Onset of the disease in adults is usually between 40 and 60 years, although it can occur at any age. The structural data here presented provides a solid ground for future developments for structure-based design of specific inhibitors for human GzmB. These future compounds would have a medical application for the treatment of this painful auto-immune disorder.

As for the structure of pro-GzmK, it is the first granzyme zymogen to be solved to date. Pro-GzmK crystal structure has revealed unexpected features for a serine proteinase zymogen. In contrast to the other serine proteinases zymogen structures, pro-GzmK most closely resembles an active enzyme. Whether this is a general feature of granzyme zymogens is still unclear, being necessary to have additional structures of this family of proteinases. In comparison to GzmB, studies on GzmK are less abundant and no physiological substrates have been discovered so far. It has been though implicated also in triggering apoptosis in target cell. Certainly, both granzymes may be expected to activate different substrates due to their strikingly distinct substrate binding pockets. It is most likely that both enzymes, and the other granzymes described, may act synergistically to induce the programmed cell death in target cells.

Procarboxypeptidase from *Helicoverpa armigera*

H. armigera (Hubner) is the world's worst agricultural pest. This voracious and polyphagous insect is a pest of 181 plant species, including important crops (i.e. cotton, maize, and tobacco) in Australia, China, India and Pakistan. All the methods developed so far to eradicate such a pest have been fruitless, due mainly to the build up of pesticide resistance.

The crystal structure of a digestive procarboxypeptidase from *H. armigera* provides new insight at the structural level that could be employed to develop new strategies to control this pest. Until recently, studies of the protein digestion in the insect gut have focus primarily on the initial phases (trypsin and chymotrypsin), being exopeptidases as the carboxypeptidases far less studied. This is the first crystal structure of an insect carboxypeptidase, and the second structure of an insect protease (the first being that of a fire ant chymotrypsin).

This *H. armigera* procarboxypeptidase structure reveals the preservation of the overall fold of exopeptidases throughout the animal kingdom. However, it has some structural features that distinguish it from the mammalian counterparts already elucidated. Furthermore, the modelling of the C-terminal tail of LCI has revealed which conformation should adopt the residues forming the substrate-binding subsites of the enzyme. There is a compelling need to develop new effective, safe and environmentally-friendly means to fight against such insect pest. The crystal structure here presented could be a useful target to design specific inhibitors with a practical agricultural application.

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Curriculum Vitae

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- First Degree in Psychology by the Universitat Autònoma de Barcelona, 1990-1995.
- First Degree in Biochemistry by the Universitat Autònoma de Barcelona, 1994-1998.
- PhD studies at the Max Planck Institut für Biochemie, Munich, 1998-present.

Seminars, Symposiums and Conferences Attended

- Artificial Insemination Laboratory Course. Organised by the Col.legi Oficial de Biòlegs and The Dexeus Institute (Prof. A. Veiga), Barcelona 1994.
- Immunology Conference organised by AECS, Faculty of Medicine, Barcelona, 1993.
- Thyroid Hormone International Conference. Fundación Ramón Areces and CSIC (Consejo Superior de Investigaciones Científicas, CSIC), Madrid, 1995.
- Postgraduate seminars on Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona (UAB), 1996 and 2000-2001.
- 24th Meeting of the Federation of European Biochemical Societies (FEBS´96), Barcelona, 1996.
- II Simposium de Neurobiologia Experimental, Barcelona, 14-15 December, 1998. Poster presented about the "Effects of dysthyroidism on the morphology of the hippocampal pyramidal neurons" (Sala, J., **Estébanez, E**., Darbra, S., Garau, A., Martí, M. A., Balada, F. Efecte del distiroidisme en la morfologia de cèl.lules piramidals de l'hipocamp).
- 17th Winter School on Proteinases and their Inhibitors, Recent Developments. Tiers (Italy), 1999. Human Blood Coagulation Factor XII (oral presentation).
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- Gordon Conference in Proteases and their Inhibitors in New London, New Hampshire, USA, Juny 2000. Poster: "Crystal structure of the novel midgut procarboxypeptidase from the cotton pest Helicoverpa armigera". **Estébanez-Perpiñá**, E., Bayes, A., Vendrell, J., Bode, W., Huber, R., Bown, D.P., Gatehouse, J.A., Avilés, F.X. and Reverter, D.

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