CRYSTAL STRUCTURE OF HUMAN BUTYRYLCHOLINESTERASE: A KEY STEP FOR ENGINEERING OF CATALYTIC SCAVENGERS AGAINST ORGANOPHOSPHATE POISONING

Patrick MASSON [a], Florian NACHON [a], Oksana LOCKRIDGE [b]
[a] Centre de Recherches du Service de Sante des Armees, Departement de Toxicologie, BP 87, 38702 La Tronche Cedex, France
[b] University of Nebraska Medical Center, Eppley Institute, NE 68198-6805, USA

Enzymes capable of degrading highly toxic organophosphate (OP) esters are emerging as safe and efficient means for destruction of nerve agent stockpiles, remediation of contaminated areas, decontamination of materials. Stoichiometric and catalytic biological scavengers are also promising alternative medical countermeasures against poisoning by nerve agents. Different OP-degrading enzymes are potential candidates for pretreatment, decontamination of skin, mucosa and open wounds or for treatment as supplement to current therapy. Here we will focus on human butyrylcholinesterase (BuChE, EC.3.1.1.8). Our goal is to engineer cholinesterase-based enzymes, having high OP-hydrolyzing activity, stable on storage and, stable in the bloodstream and immunologically compatible to be injected in humans.

Starting from the x-ray structure of *Torpedo californica* acetylcholinesterase, the structure of human BuChE was modeled and used to design mutants of BuChE. Several mutants, in which a nucleophile pole was created, have been produced [1]. As predicted, these mutants were found to be capable of hydrolyzing OP. However, their activity was too low to be of operational interest and their catalytic mechanism was not clearly established. Attempts to make new muteins with improved OP-hydrolase activity failed. Because understanding the molecular mechanisms underlying the phosphotriesterase activity of BuChE mutants requires detailed knowledge of the structure and conformational dynamics of the enzyme, we endeavoured to determine the three-dimensional structure of human BuChE.

A truncated and partially glycosylated monomer of BuChE was produced in CHO cells. Crystals suitable for x-ray analysis were obtained and the enzyme structure was solved at 2 Å resolution at the European Synchrotron Research Facility [2]. Knowledge of the BuChE structure is essential for the rational redesign of binding and active site pockets and for understanding the effects of active-site remote mutations to be made by directed evolution.

1. C. Millard, O. Lockridge and C. Broomfield, Biochemistry, 37 (1998) 237-247.
2. F. Nachon, Y. Nicolet, N. Viguie, P. Masson, J-C. Fontecilla and O. Lockridge, submitted.

Correspondence: Patrick Masson
Centre de Recherches du Service de Sante des Armees, Departement de Toxicologie
BP 87, 38702 La Tronche Cedex
France

Received: 11. 10. 2001