Elsa Reiner, Vera Simeon-Rudolf, Bhupendra P. Doctor, Clement E. Furlong, Martin K. Johnson, Marcello Lotti, Israel Silman, and Palmer Taylor (Guest Editors)
Esterases Reacting with Organophosphorus Compounds

Special Issue of *Chemico-Biological Interactions,* a journal of molecular and biochemical toxicology, Vols. **119–120** (1999) 1–620, Elsevier Science, Amsterdam, 1999, 619 pp.
ISSN 0009–2797, CBINA (119–120) 1–620 (paperback).

A collection of papers presented at the Third International Meeting on Esterases Reacting with Organophosphorus Compounds, Dubrovnik, Croatia, April 15–18, 1998.

The sixty-seven papers in this book are grouped under the following chapters: Esterase Structures, Catalytic Properties of Cholinesterases, Catalytic Properties of Paraoxonases and Related Esterases, Biology of Cholinesterases, Biology of Paraoxonases and Related Esterases, Toxicity and Degradation of Organophosphorus Compounds, Neuropathy Target Esterase, and Miscellaneous. The chapters are preceded by a Preface and followed by the Author and Subject Indexes.

The book represents investigations of cholinesterases, esterases hydrolyzing organophosphorus compounds (OP) and neuropathy target esterases, carried out in the last six years, in succession of the results published in the collection of papers presented at the Second International Meeting on Esterases in 1992 (*Chemico-Biological Interactions*, Vol **87**, No. 1–3, 1993).

In order to hydrolyze acetylcholine in the extracellular space, cholinesterases need to be exposed on the outside layer of the cell membrane or exported from cells. In vertebrates, these molecules are generated from a single gene: the catalytic domain may be associated with a small C-terminal region, encoded by several exons, and their alternative splicing defines the types of catalytic subunits ($AChE_S$, $AChE_H$, $AChE_T$) and determines their post-translational maturation. $AChE_S$ generates soluble monomers whereas $AChE_H$ generates GPI-anchored dimers in *Torpedo* muscles and on mammalian blood cells. In all vertebrate cholinesterases, $AChE_T$ is the only type of catalytic subunit, appearing as monomer, dimer or collagen-tailed and hydrophobic-tailed tetramer. In the collagen-tailed forms, $AChE_T$ subunits are associated with a specific collagen, Col Q, which through its hydrophobic tail anchors AChE in mammalian brain membranes.

The three-dimensional structure of AChE from *Torpedo californica,* together with kinetic, spectroscopic and side-directed mutagenesis studies, has permitted identification of the amino acid residues that interact with the natural substrate. The structure revealed that the active side is located near the bottom of a deep and narrow gorge lined with 14 conserved aromatic amino acids. It was found that the AChE active site possesses an oxyanion hole, an acyl binding pocket, and a choline binding site. The aromatic side chains constituting the acyl pocket and the residues in the oxyanion hole participate in acylation and deacylation of AChE. The intermediate complex is accommodated at the bottom of the gorge.

There are two sites of ligand interaction in AChE: an acylation site at the base of the gorge, and a peripheral site at its mouth. Ligands can bind selectively to either the acylation or the peripheral sites. It was found that peripheral site inhibitors affect the rate of phosphorylation of AChE with organophosphates. A series of chiral methylphosphonates was studied, in which the leaving group and the retained alkoxy group were systematically modified in order to analyze inactivation and oxime reactivation of wild-type and mutant cholinesterases. The efficacy of oxime reactivation is dependent on both oxime and phosphonate structures.

Besides AChE, mammalian organisms also possess butyrylcholinesterase (BChE), another acethylcholine-hydrolyzing enzyme. Administration of butyrylcholinesterase has been proposed as a treatment for organophosphate poisoning in mice, rats and non-human primates. This protective effect results from the covalent binding of BChE to organophosphates (OP), thus precluding OP binding to and inactivation of AChE at nerve synapses of the central and peripheral nervous system. Human BChE is composed predominantly of tetramers. The tetramerization domain of BChE is at the carboxy-terminus. Stabilization of the tetramer is mediated through the interaction of seven conserved aromatic residues and the proline-rich attachment domain of collagen. Differences in active-site gorge dimensions of cholinesterases were revealed by binding inhibitors to human BChE. It was found that the volume of the BChE active-site gorge is $\approx A^3$ larger than that of the AChE gorge. The results obtained suggest that the dimensions and the micro-environment of the gorge play a significant role in determining the selectivity of the substrate and inhibitors for cholinesterases.

Human serum paraoxonase/arylesterase (PON) is a calcium-dependent enzyme that hydrolyzes a wide variety of organophosphates, including paraoxone, diisopropyl-fluoro-phosphate, sarin and soman. By group selective labelling and site-directed mutagenesis, the essential amino acid residues were identified in the enzyme. In human populations, serum paraoxonase exhibits a substrate dependent polymorphism. It was shown that PON plays a major role in the detoxication of organophosphates processed through the P450/PON1 pathway. PON is a key component of the HDL complex, and

PON is able to inactivate toxic products resulting from the oxidation of lipid components of the blood LDL. Mature PON retains its N-terminal hydrophobic signal sequence that may be needed for binding to the Apo A-1 protein of HDL. Thus, the anti-atherogenic qualities of HDL may derive in part from its content of PON. Protection of endothelial and other cells against oxidative damage due to oxidized LDL lipoproteins, and the *in vivo* protection by PON against endotoxic damage by bacterial lipopolysaccharides suggest that PON has a stabilizing property for cellular membranes that undergo either acute or chronic exposure to oxidative agents and free radicals.

Organophosphorus compounds are a broad class of neurotoxic chemicals, which may be used as pesticides or chemical warfare agents. The toxicity of OP compounds is dramatically reduced by chemical or enzymatic hydrolysis. Enzymes hydrolyzing OP compounds, like human paraoxonase, organophosphorus hydrolase (OPH) and organophosphorus anhydrolase (OPAA), accomplish this hydrolysis by cleaving P–C, P–O, P–F, P–S and P–CN bonds in specific OP compounds. Recently, and for the first time, an OPAA has been reported in a plant source: in duckweed, germinated mung bean and in a slime mold (*Dictyostelium discoideum)*.

The interest in OPH and OPAA enzymes received a boost with the purification of the enzymes from *Pseudomonas diminuta* MG and *Flavobacterium* ATCC 27551. The gene (*opd*) encoding OPH was found to be associated with the large plasmid of dissimilar size in *P. diminuta* and *F.* sp. The DNA sequences for *opd* genes have been determined and the gene has been expressed in a variety of vectors using host-specific promoters. Recently, the genes encoding OPAA from two species of *Alteromonas* were cloned and sequenced. Efforts to overproduce an OPAA from *A.* sp. ID 6.5 with the goal of developing strategies for long term storage and decontamination have been successful. Inclusion of OPAA enzyme into spray or foam provides a basis for the development of an enzyme-based decontaminant system.

In order to protect OP-contaminated persons, a sponge product composed of a ChE, oxime, and polyurethane foam was prepared for the removal and decontamination of OP compounds from biological surfaces such as skin. These polyurethane ChE-sponges exhibit remarkable long-term stability and increased resistance to elevated temperature.

It was established long ago that OP-esters exert their primary toxic effect on cholinergic synapses. The main disadvantage of the use of OP compounds as pesticides is the associated toxicity. The toxic effect of certain OP compounds, quite distinct from the inhibition of AChE, is their ability to cause progressive degeneration of long axons, leading to ataxia. Degeneration can occur over a period of several weeks after a single insult with a neuropathic OP compound. This delayed neuropathy is mediated through reaction of the OP compound with neuropathy target esterase (NTE). The mechanism of OP-induced delayed polyneuropathy and the function of NTE

are still unknown. Simple inhibition of NTE esterase activity does not initiate neuropathy; the latter requires aging of the OP bound to the catalytic serine residue. This may indicate that a non-esterase function of NTE is important for axonal maintenance. Recently, NTE was cloned and it was found that it contains a novel C-terminal domain which is conserved from bacteria to man. The catalytic serine is located within this domain. NTE is defined as phenyl valerate esterase, resistant to non-neuropathic paraoxon and susceptible to neuropathic mipafox.

Only one paper concerning the nomenclature system for the paraoxonases was published. A consensus was reached as to a preferred system for numbering the paraoxonase amino acid residues that could be recommended for general use by all workers in this field.

In conclusion, this book represents a balanced and comprehensive account of the achievements in the last six years. Scientists from all prominent laboratories in this field have contributed to this book. Out of the published papers, I have described those which represent a particular challenge to further investigations. Readers who have access to previous volumes will be able to follow the history of the field and the ebb and tide of » hot topics« at each Meeting, while those who pick up this new volume might welcome some papers appearing in each chapter. This book will be of interest to biochemists, toxicologists and pharmacologists.

Pavao Mildner