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Special Topic

Carboxylesterases – from function to the field: an overview of carboxylesterase biochemistry, structure–activity relationship, and use in environmental field monitoring

This issue of the *Journal of Pesticide Science* highlights carboxylesterases (CEs; EC 3.1.1.1) and their role in the agrochemical sciences. Carboxylesterases are members of the α/β hydrolase family, a superfamily of enzymes in which the core of each enzyme is similar: an α/β sheet consisting of eight β-sheets connected by α-helices. These enzymes have diverse function and include cholinesterases, epoxide hydrolases, lipases and phosphotriesterases (such as paraoxonase) as well as multiple other enzymes. Carboxylesterases, also known as B-esterases based upon their inhibition by organophosphates, are serine hydrolases that hydrolyze ester, amide, and carbamate bonds. These ubiquitous enzymes are traditionally regarded as xenobiotic metabolizing enzymes, which hydrolyze esterified xenobiotics to the corresponding alcohol and carboxylic acid. Carboxylesterases have been extensively studied for their role in agrochemical metabolism based upon their interactions with three major classes of agrochemicals: organophosphates, carbamates and pyrethroids. In addition, CE levels and activity are involved in insecticide resistance mechanisms. It is well known that variability in CE levels as well as relative isozyme abundance contribute to the selective toxicity of ester-containing insecticides in both mammals and insects and reports of insecticide resistance associated with CE activity are widespread. Accordingly, CE biochemistry is clearly of importance to the agrochemical research community. However, what is often less recognized by agricultural chemists and biochemists, is the role of CE activity in the metabolism of numerous ester-containing therapeutics. Carboxylesterases are of clinical importance because of their high abundance and location in a diverse array of tissues, including liver, blood, lung, small intestine, brain, stomach, spleen, heart, testis, prostate, pancreas, colon, macrophages, and monocytes. For example, human carboxylesterase 1 (hCE1, CES1A1) is the 10^{th} most abundant protein in the human liver and is therefore almost certainly significantly involved in the metabolism of multiple endogenous and exogenous compounds. In addition, studies are beginning to indicate that CE activity may have a role in fundamental biochemical processes involving lipid trafficking and the onset of cardiovascular disease. Given the diverse functions and roles of this enzyme family, this special issue of the journal seeks to provide an overview of these different activities towards the goal of increasing the general knowledge and more importantly to synergize interactions between efforts from the agrochemical, environmental and pharmaceutical fields in understanding this fascinating and ubiquitous enzyme family.

This issue opens with a review by Satoh and Hosokawa, examining current developments in molecular-based studies of the structure and function of CEs. One of the current obstacles in the field of CE research is confusion surrounding the nomenclature. Accordingly, Satoh and coworkers have proposed a system based upon molecular properties similar to that used for cytochrome P450s. Satoh and Hoskokawa then go on to describe mechanisms of regulation of gene expression of CEs by xenobiotics, and the involvement of CEs in drug metabolism. Of particular relevance to the agrochemical community, they present data on the continued effort to develop CE activity as a biomarker for organophosphate exposure instead of the more commonly employed endpoint involving inhibition of cholinesterase activity. This paper is followed up by Imai *et al.* who discuss the use of CE activity in the design and activation of prodrugs. Carboxylesterases hydrolyze a wide array of common therapeutics, including the anti-thrombogenic agents aspirin and clopidogrel, the anti-influenza prodrug oseltamivir (Tamiflu), the cholesterol reduction drug lovastatin, and the chemotherapeutic agent CPT-11 (irinotecan-7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin), as well as the β-blockers flestolol and esmolol, the Ca^{2+}-activated K^+ channel blocker cetiedil, cocaine, and heroin. Imai *et al.* discuss some of the more challenging issues in clinical studies with CEs including inter-individual differences in CE expression levels and the subsequent potential effects in pharmacological studies. In addition, they examine species differences in CE-mediated tissue hydrolase activity, which is paramount when extrapolating CE-based preclinical studies from animals to humans. This work is followed-up by Wadkins and coworkers, who in collaboration with Philip Potter's group have devoted extensive effort to effecting controlled CE-mediated activation of the chemotherapeutic agents CPT-11. Towards this end, Wadkins and coworkers discuss the major classes of known mammalian CE inhibitors and describe their impressive computational efforts to design new scaffolds for the development of novel and selective inhibitors. In particular, the review focuses on their efforts to create CE isozyme-specific inhibitors, which for example significantly inhibit human intestinal CE

(hiCE, CES2A1) involved in the activation of CPT-11 in the intestine, but do not affect hCE1 activity. They then discuss several strategies for *in silico* drug development methods, including structure docking, database searching, multidimensional quantitative structure–activity relationship (QSAR) analysis, and a new approach that uses QSAR combined with *de novo* design. Taken together, these efforts provide insight into strategies for designing the next generation of selective CE inhibitors.

It is well known that variability in CE levels and relative isozyme abundance contribute to the selective toxicity of ester-containing insecticides in a range of organisms from fish to insects and mammals. However, stereochemistry is also important in esterase-associated metabolism. Many agrochemicals, including pyrethroids and some organophosphates, contain chiral centers that greatly affect their subsequent metabolism. Since varying stereoisomers of both organophosphates and pyrethroids exhibit differential toxic effects, stereospecific hydrolysis is essential in the determination of their toxic properties. Redinbo and coworkers discuss results from their extensive experience in solving more than a dozen CE X-ray crystal structures. In particular, their work on examining the stereospecific interactions of human carboxylesterase 1 (hCE1 or CES1A1) with organophosphates and pyrethoids has provided valuable insight into agrochemical binding and metabolism by CEs. For example, they have examined the substrate selectivity of stereoisomers of organophosphates to illustrate selectivity based upon selective orientation in the two binding pockets. Pyrethroid isomers, in contrast, likely impact the positioning of the oxyanion hole required to stabilize the negatively-charged transition-state oxygen. These structure-based studies are vital to increasing our understanding of CE biochemistry as well as for designing isozyme-selective inhibitors as discussed by Wadkins and coworkers.

One of the interesting areas of current CE research involves identifying endogenous substrates. The next paper by Ross *et al.* discusses some of their pioneering efforts in this area, with emphasis on examining the potential role of CE activity in lipid metabolism. In particular, they have shown that endogenous lipids including cholesteryl esters, triacylglycerols, and 2-arachidonoylglycerol are substrates for CEs. Based upon these results and further functional studies, their data suggest that CEs may be a novel target for the treatment of multiple diseases including atherosclerosis. In this review, they further discuss the known physiological functions of CEs, the interactions between xenobiotics (primarily pesticides) and lipids, and the possible implications of these findings in terms of health and disease. They raise the intriguing possibility of drug–agrochemical interactions, in which exposure to agrochemicals such as organophosphates or carbamates can inhibit CE activity, which may have a subsequent effect upon the phamacokinetics of the metabolism of ester-containing therapeutics. In addition, the work of Ross and coworkers attempts to draw a link between organophosphate exposure and effects upon lipid trafficking and potentially onset of cardiovascular disease, an intriguing hypothesis that warrants further investigation. In terms of endogenous activity, one of the few CEs whose endogenous substrate is understood is juvenile hormone esterase (JHE), which has a clearly defined physiological substrate. The next paper by Kamita and Hammock examines the biochemistry of JHE in detail. Normal insect development requires a precisely timed, precipitous drop in the hemolymph titer of juvenile hormone (JH), which has a diverse range of functionality in the insect life cycle including roles in development, metamorphosis, reproduction, diapause, migration, polyphenism, and metabolism. Two pathways for the degradation of JH have been intensively studied in insects: 1) the methyl ester moiety at one end of the JH molecule is hydrolyzed by JHE resulting in the conversion of the methyl ester into a carboxylic acid and 2) the epoxide moiety at the other end of the JH molecule is hydrolyzed via a microsomal epoxide hydrolase to the corresponding diol. Kamita and Hammock review the biochemistry and structure of authentic and recombinant JHEs from six insect orders, and present updated diagnostic criteria that help to distinguish JHEs from other CEs. In addition, the authors discuss the use of a JHE-encoding gene to improve the insecticidal efficacy of biopesticides.

The theme of insect CE activity is presented in further detail by Oakeshott and coworkers, who examine the nature of CE-based resistance to agrochemicals. Elevated esterase activities and increased staining intensities of multiple esterase isozymes are commonly associated with resistance to organophosphate, pyrethroid and carbamate insecticides in various heliothine and spodopteran pests. The involvement of CE activity in pest resistance to agrochemicals has been extensively studied over the years. Oakeshott and coworkers examine the body of literature relating to heliothine and spodopteran pests in detail. They present three possible explanations for the multiplicity of bands involved in CE-mediated resistance: multiple isozymes encoded by individual loci, coordinated regulation of multiple esterase loci, and/or genetic hitchhiking among multiple, closely linked esterase loci. They propose the intriguing hypothesis that these observations can be explained by a “master regulator” mutation in a more general chemical stress response. These observations are exceedingly important for understanding the involvement of CE activity in agrochemical resistance mechanisms.

The next few papers move into discussions of the environmental applications of CE activity. An organism’s sensitivity to pyrethroid, organophosphate, or carbamate exposure may be influenced by its endogenous CE activity. Therefore, measurement of CE activity may be useful in predicting the effects of agrochemical exposure upon ecosystem health. However, there are currently insufficient data available in the literature to fully examine this issue. The use of acetylcholinesterase (AChE) activity as a biomarker of organism exposure to agrochemicals (organophosphates and/or carbamates) is well doc-

umented in the literature. However, recent studies have suggested that AChE activity alone is not an appropriate biomarker because some organophosphates have increased affinity for CE over AChE. The preferential inhibition of CE over AChE following exposure to organophosphates and potentially carbamates suggests that CE activity will provide a more sensitive endpoint. Towards this end, Sanchez-Hernandez and coworkers have proposed the use of earthworm CE activity as a biomarker of exposure to organophosphates. This paper describes the use of earthworm esterases as biomarkers to be included in a broad-based field toxicity test. The potential role of gut CEs in the modulation of pesticide toxicity is also discussed in view of its contribution to the natural tolerance of earthworms to pesticides, and the appropriate selection of earthworm species for regulatory toxicity testing. Finally, Sanchez-Hernandez presents the intriguing concept of using earthworm gut CE activity in the enzymatic bioremediation of pesticide-contaminated soils. The use of CE activity in environmental monitoring is further examined by Phillips *et al*., who perform Toxicity Identification Evaluation (TIE) studies for the identification of toxicity in receiving waters and sediment samples. The extreme toxicity of pyrethroids to many aquatic organisms, combined with their hydrophobicity, has resulted in concern regarding their potential environmental effects. This concern is exacerbated by the fact that current TIE protocols devised for the identification of insecticides (and other environmental contaminants) in aqueous and sediment samples do not identify pyrethroid-associated toxicity with complete certainty. To address this shortfall, the use of CE activity to hydrolyze pyrethroids in aquatic toxicity testing has been proposed as a simple, mechanistically based method to selectively identify pyrethroid-associated toxicity. This approach has been employed by Phillips and coworkers, who have successfully applied it in real-world scenarios. In this research paper, they present data showing the use of CEs in TIEs as part of a statewide assessment of pyrethroid pesticides and sediment toxicity in urban creeks in California. Sediment samples from four sites containing varied concentrations of pyrethroids were investigated using TIEs to determine causes of toxicity. Treatments were conducted on both whole sediment and interstitial water to determine the role of pyrethroids in the observed toxicity to the amphipod *Hyalella azteca*, and to evaluate TIE method performance. Results from their studies demonstrated the real-world application of CEs in a weight-of-evidence approach for identifying pyrethroids in environmental samples, which is an exciting advance in applications of CE activity.

This issue wraps up with two commentaries, one by Shiotsuki and coworkers that reviews the recent genomic and phylogenetic analysis of insect Carboxyl/cholinesterases (CCEs). In the CCE phylogenetic tree, a species or order specific CCE cluster is evident, but for some CCEs 1 : 1 orthologous relationships are observed. This information could be useful for further functional analysis of CCEs or for the development of species-specific pesticides and/or synergists. Lastly, Chatonnet and coworkers discuss the ESTHER database, which gathers and annotates all the published information related to gene and protein sequences of the α/β hydrolase superfamily, including CEs. In their commentaries, they provide an example of how the ESTHER resource can be used to examine the role of specific mutations in the development of resistance of arthropods to organophosphates and carbamates.

Our knowledge of CEs and CE-mediated metabolism has significantly increased; however, it is still very much an evolving field. Accordingly, this is an exciting time in the field of CE research. For decades, CEs have been referred to as having “unknown function”, with the majority of studies describing xenobiotic-based metabolism. This trend is now shifting with multiple groups beginning to investigate/elucidate the endogenous function of these enzymes. As discussed above, CEs metabolize a diverse array of substrates from chemotherapeutic agents and anti-virals, to pesticides, and insect hormones involved in metamorphosis as well potentially playing a role in lipid trafficking and disease. Questions of particular interest center around further establishing the endogenous physiological function of CEs, their potential role in disease processes, and the synthesis of selective, water-soluble inhibitors for use in elucidating function as well as effecting controlled activation/metabolism of CE-mediating therapeutics such as CPT-11. In addition, the potential application of CE activity in environmental monitoring as both a biomarker of agrochemical exposure as well as an active tool in TIE protocols requires further development. Initial work has been promising, but further refinement of protocols will be necessary for wide-scale use and acceptance. Another promising area of research will be to examine other CEs. Studies in humans have primarily focused on hCE1 and hiCE (CES1A1 and CES2A1, respectively), but there are other CEs including a brain-specific hCE3. Very little work has been performed to characterize CEs in organisms besides target insect pests and humans. For example, some studies have suggested that CE levels and isozyme distribution in fish associate with sensitivity to agrochemical exposure, warranting further efforts to study CE activity in multiple species. Taken together, the information presented in the papers in this special issue highlight the diverse array of functions and biological activities of CEs, while demonstrating the challenges as well as fascination in studying these enzymes. Given the importance of the functions described in this wide range of biological activities, this field represents a rich future for further investigation into CE biochemistry from agricultural chemists to clinicians examining the etiology of cardiovascular disease.

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