

MEETING ABSTRACTS

EVOLUTION OF THE FIRST DISULFIDE BOND IN THE CHOLINESTERASE-CARBOXYLESTERASE (COESTERASE) FAMILY: POSSIBLE CONSEQUENCES FOR CHOLINESTERASE EXPRESSION IN PROKARYOTES

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Within the alpha/beta hydrolase fold superfamily of proteins, the COesterase group (carboxylesterase type B, block C, cholinesterases...) diverged from the other groups through addition of an N-terminal disulfide bond and simultaneous increase in the mean size of the protein (1). This disulfide bond creates a large loop, which is essential for the high catalytic activity of cholinesterases through formation of the upper part of the active center gorge. In some non-catalytic members of the family, the loop may be necessary for heterologous partner recognition. The shuffling of this portion of protein occurred at the time of emergence of the fungi/metazoan lineage. Homologous proteins with this N-terminal disulfide bond are absent in plants but they are found in a limited number of bacterial genomes. In prokaryotes, the genes coding for such homologous enzymes may have been acquired by horizontal transfer. However the cysteines of the first disulfide bond are often lost in bacteria. Natural expression in bacteria of CO-esterases comprising this disulfide bond may have required compensatory mutations or expression of new chaperones. This disulfide bond may also challenge expression of the eukaryote-specific cholinesterases in *E. coli*. Recently, catalytically active human acetylcholinesterase and butyrylcholinesterase were successfully expressed in *E. coli*. The key was the use of a peptidic sequence optimized through the Protein Repair One Stop Shop process, an automated structure- and sequence-based algorithm toward expression of properly folded, soluble eukaryotic proteins with an enhanced stability (2,3). Surprisingly however, the crystal structure of the optimized butyrylcholinesterase variant expressed from bacteria revealed co-existing 'close' and 'open' states of the first disulfide bond. Whether the 'open bond' involves two cysteines (i.e., the bond never formed) or two half-cystines (i.e., the bond properly formed, then broke during the production/analysis process) cannot be inferred from the structural data. Yet, this observation suggests that this first bond is difficult to maintain in *E. coli*-expressed cholinesterases.

References

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