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Human butyrylcholinesterase (BChE) is a homodimer of two identical polypeptide chains of ~390 kDa which fold to give a very compact globular structure. It is a unique enzyme containing long-chain fatty acids, primarily palmitate, as well as long hydrocarbon tails covalently attached at its active center. The functions of the covalently modified fatty acids and the length of the hydrocarbon tails remain unknown. Progress in this area has been hampered by the lack of a BChE structure in a lipid environment. We believe that a detailed structural understanding of the human BChE active site, coupled with the elucidation of its lipid modified structure, will be a major step toward an understanding of the role of the covalently linked fatty acids and the hydrocarbon tails in its function. This grant proposes the first structure of human BChE in a lipid environment. We have solved the structure of the BChE active site in the absence of bound fatty acids or lipid, in spite of strong evidence that they are present. We propose that the human BChE active site resembles that of the squid BChE, which has an acetylcholine analog as the binding site. Our solution structure of human BChE reveals that fatty acids are tightly bound at the active center, forming a distinctly novel binding site which occupies nearly 30% of the total volume. Based on our proposed structure, we hypothesize that the fatty acid binding site of the human BChE accommodates the entire acyl-chain of the palmitate in the E2 conformation, and that the hydrocarbon tails of long-chain fatty acids are embedded in the interfacial region between the protein and the lipid. Here we propose to elucidate the functions of these fatty acids and the lipid tails. We plan to generate monoclonal antibodies directed at specific fatty acid binding sites on the human BChE active site. We will determine the binding affinities of these antibodies for human BChE, and also investigate the possibilities that the fatty acids and lipid hydrocarbon tails are essential for the normal function and regulation of the enzyme. We will also investigate the possibility that the fatty acids and the lipid tails may be required for the long-term viability of the enzyme, as humans with a knockout for this gene are developmentally and neurologically impaired.

Indigo solenopsis Indigo solenopsis is a genus of solenopid trilobite from Upper Cambrian (Ordovician) shale deposits of eastern Canada, it is also found in the United States.



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