

HUMAN CHOLINESTERASE GENES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 087,724, filed Aug. 21, 198, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 875,737, filed Jun. 18, 1986, now abandoned the contents of which are fully incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to genetically engineered proteins having human cholinesterase activity, comprising either acetylcholinesterase activity or pseudocholinesterase activity. This invention is also directed to the cloning and production of these proteins. This invention further relates to using these proteins to produce antibodies interacting with human cholinesterase. The proteins of this invention can also be used in medical treatment of organophosphorus poisoning.

BACKGROUND OF THE INVENTION

Structural and Physiological Properties of Cholinesterases

At the cholinergic synapse, the enzyme acetylcholinesterase terminates the electrophysiological response to the neurotransmitter acetylcholine (ACh) by degrading it very rapidly. (For a review, see Silver, A., *The Biology of Cholinesterases*. North-Holland Pub. Co., Amsterdam (1974)). Mammalian cholinesterases (ChEs) exhibit extensive polymorphism at several levels. They can be distinguished by substrate specificity into acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7, AChE) and butylcholinesterase or pseudocholinesterase (acylcholine acylhydrolase, EC 3.1.1.8, ψ ChE). These cholinesterases differ in their susceptibility to various inhibitors. Both are composed of subunits of about 600 amino acid residues each and are glycosylated (10–20%).

ChEs occur in multiple molecular forms, which exhibit different sedimentation coefficients on sucrose gradients, display different hydrodynamic interactions with non-ionic detergents and are composed of different numbers of subunits. (For a comprehensive review see Massoulie, J., and Bon, S., *Ann. Rev. Neurosci.* 5:57–106 (1982)). There are secreted, cytoplasmic, and membrane-associated pools of ChEs in the mammalian nervous tissue, but all forms of ChEs possess similar catalytic properties, suggesting that they share common acetylcholine binding sites (Chubb, I. W., In: *Cholinesterases—Fundamental and Applied Aspects*. M. Brzin, E. A. Barnard, and D. Sket, Eds., pp. 345–359, (1984)). Various ChEs may also contain distinct polypeptide regions, responsible for the subcellular segregation of various AChE forms, for their different amphipathic properties and for their different modes of assembly into multisubunit protein molecules. This is supported by the reports of antibodies which display homologies (Fambrough et al., *Proc. Natl. Acad. Sci. USA* 79:1078–1082 (1982)), as well as differences (Doctor et al., *Proc. Natl. Acad. Sci. USA* 80:5767–5771 (1983)) between different forms of AChE from various organisms.

Interaction of Cholinesterases with Organophosphorus Insecticides and Nerve Gases

Most of the commonly used insecticides are organophosphorus (OP) compounds, acting by blocking the

insect's AChE. In many cases where such insects develop insensitivity to the insecticides, this occurs because of the enhanced expression of AChE forms which do not interact with the organophosphate. To deal with such phenomenae successfully, it is necessary to know the mechanisms by which the appearance of such AChE forms is regulated.

Complete inhibition of mammalian AChE (i.e., by administration of OP-poisons) is lethal, due to the formation of a stable stoichiometric (1:1) covalent conjugate with the active site serine. Treatment of OP poisoning has involved the use of reversible ChE inhibitors, and reactivation of the inhibited enzyme with active-site directed nucleophiles (e.g., quaternary oximes) which detach the phosphoryl moiety from the hydroxyl group of the active site serine. A parallel competing reaction, termed "aging," transforms the inhibited ChE into a form that cannot be regenerated by the commonly used reactivators by dealkylation of the covalently bound OP group, and renders therapy of intoxication by certain organophosphates, such as Sarin, DFP, and Soman, exceedingly difficult (National Academy of Sciences Report, 1982).

Frequent Mutations in Cholinesterase Genes

Pseudocholinesterase (ψ ChE) is particularly enriched in the serum. Subjects with "null" ψ ChE activity (i.e., samples of their serum cannot hydrolyze acetylcholine) do not exhibit any health problem under natural conditions but suffer from prolonged apnea when they are given succinylcholine (a drug commonly used as a short-acting muscle relaxant) in surgical operation. However, there are no rapid, simple, routine methods to detect and characterize the atypic forms of the enzyme prior to surgery.

The amino acid sequence of human AChE is not known. It has recently been found that the same six amino acids are included in the organophosphorus binding site of both Torpedo AChE tetramers and human pseudoChE tetramers. (McPheeQuigley, K., *J. Biol. Chem.* 206:12185–12189 (1985); Schumacher et al., *Nature* 319:407–409 (1986)).

The amino acid sequence in this hexapeptide from the organophosphate-binding site of ChEs has been determined recently in several laboratories, by partial proteolytic digestion of the purified enzymes, labeled with [3 H]-diisopropylfluorophosphate, and was found to be Gly-Glu-Ser-Ala-Gly-Ala, with the serine residue bound to organophosphates. It has been suggested that the "null" forms of ψ ChE are modified in this peptide.

Common Alterations in the Level and Properties of Cholinesterases

Modifications in both the level (Spokes, E.G.S., *Brain* 103:179–183 (1980)) and the composition of molecular forms (Atack, J. R., et al., *Neurosci. Lett.* 40:199–204 (1983)) of human brain AChE have been reported in several neurological or genetic disorders, such as Senile Dementia of the Alzheimer's type (SDAT; about 5% of the population above 65), or Down's syndrome (trisomy of chromosome 21). The levels of AChE in cholinergic brain areas drops by about 50%, and the tetrameric form of the enzyme disappears completely in SDAT. Individuals with Down's syndrome invariably develop SDAT manifestations before the age of 40. In addition, open neural tube defects in human embryos are clinically characterized by secretion of AChE tetra-