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Old and new questions about cholinesterases

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ABSTRACT

Cholinesterases have been intensively studied for a long time, but still offer many fascinating and fundamental questions regarding their evolution, activity, biosynthesis, folding, post-translational modifications, association with structural proteins (ColQ, PRiMA and maybe others), export or degradation. They constitute an excellent model to study these processes, particularly because of the sensitivity and specificity of enzymic assays. In addition, a number of provocative ideas concerning their proposed non-conventional, or non-catalytic functions deserve to be further documented.

The existence of enzymes which hydrolyze the neurotransmitter acetylcholine has been established since the 1930s [1,2]. The two cholinesterases of vertebrates, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) have been very rapidly distinguished [3–5] and these enzymes have been the subject of considerable research [6]. In particular, specific and potent inhibitors have been synthesized and the catalytic activity of cholinesterases has been explored in great detail. The presence of a reactive serine was demonstrated and its peptidic environment was determined as early as 1959 [7].

The electric organs of the electric ray (*Torpedo marmorata* and *Torpedo californica*) and of the Amazonian electric eel (*Electrophorus electricus*) provided an abundant source of AChE [8], and of other cholinergic synaptic components such as the nicotinic receptor [9]. Biochemical analyses of AChE from electric organs and from other tissues allowed the characterization of multiple molecular forms of AChE, in which the enzyme was shown to be associated with the specific collagen ColQ, with the transmembrane protein PRiMA, or anchored in cell membranes by a post-translationally added glycophosphatidylinositol (GPI) [10,11].

Quite remarkably, the complete sequence of human BChE has been determined by direct chemical sequencing by Lockridge in 1987 [12]. The sequences of *Torpedo californica* and *marmorata* AChE were determined from the coding sequences [13,14], and were followed by many others. This revealed that, in some species, alternatively spliced transcripts generate several variants of AChE, which differ in their post-translational modifications and oligomerization properties, thus explaining the origin of the molecular diversity of AChE forms [10,11]. The associated proteins ColQ and PRiMA have been cloned [15–17]. ColQ constitutes the collagenous tail which inserts the T or "tailed" variant of AChE or BChE in an extracellular basal lamina (e.g. at neuromuscular junctions), while the transmembrane protein PRiMA anchors these enzymes in cell membranes (e.g. in neurons). Although the association of AChE with its membrane anchor PRiMA has not yet been analyzed in as much detail as its association with ColQ [18–20], the origin of multiple molecular forms and their structure are reasonably well understood.

The crystallographic structure of the dimeric, GPI-anchored form of *Torpedo* AChE was determined by Sussman et al. in 1991 [21], and other structures were then obtained for natural and recombinant AChEs, both alone

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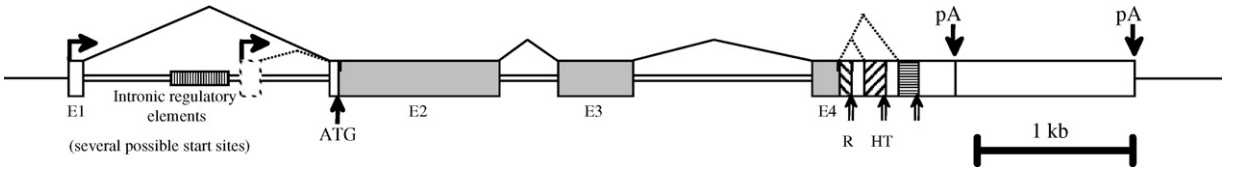

Fig. 1. Organization of the mammalian AChE gene. This scheme is based on the results obtained for the mouse gene by several groups [33,52,172,173] (see Genbank AF312033, AK036443, NT_039315). The scheme is approximately to scale, except that the non-coding sequences at the 5′ end of E2 and following the R region are expanded threefold; note that the mammalian AChE gene is quite compact, which facilitates its study [29]. Sequences that may be retained in mature transcripts (exons) are shown as boxes. Transcription initiation sites (arrows pointing to the right) and lines indicating splicing are shown above. The non-coding exons labelled E1 and shown as a dotted box correspond respectively to E1c and E1a/b of Ref. [52]. Atanasova et al. described a start site located about 500 bp upstream of E1, generating an alternative non-coding exon that would be spliced to E2 [33,52,172,173]. Meshorer et al. also reported a further upstream start site, generating an upstream exon containing a translation initiation ATG codon, in frame with the coding sequence; this would add an N-terminal peptide upstream of the secretion signal peptide [52]. Regulatory elements exist in the first intron (between E1 and E2), indicated by a narrow vertically hatched box. There are two alternative termination polyadenylation sites (pA) in the 3′ untranslated region (arrows pointing downwards). Common coding regions corresponding to the signal peptide and the catalytic domain are shown in grey; 3′ regions encoding alternative C-terminal peptides are hatched (R, H and T). Note that the R transcript contains regions H and T, and the H transcript contain region T. ATG and stop codons are indicated below, by single and double vertical lines, with arrows pointing upwards. In the case of dog AChE, the R region is predicted to run without frameshift into the H region.

and associated with inhibitors. The catalytic mechanism of AChE was explored by mutagenesis of various residues and by sophisticated physical methods using low temperature crystallography and substrate analogs, as reported in several chapters of this volume.

The transcriptional regulation of the genes encoding AChE, ColQ, and PRiMA has provided partial explanations for the observed distributions of AChE molecular forms in cholinergic tissues. For example, the relative expressions of AChE and ColQ in slow and fast muscles may explain their differences in the levels and proportions of collagen-tailed forms in the junctional and extra-junctional regions of fast and slow muscles [22–25]. Genetic modifications of the AChE, BChE, ColQ and PRiMA genes, in the mouse, make it possible to explore the physiological functions of AChE, BChE and their molecular forms. It was greatly surprising that AChE−/− mice, although very weak, can live at all, considering the acute toxicity of anti-cholinesterase poisons [26]. Suppression of ColQ or PRiMA have shed considerable light on the roles of the corresponding modes of enzyme localization, for example by showing that, under normal conditions, acetylcholine hydrolysis at neuromuscular junctions depends essentially on the presence of collagen-tailed AChE forms in the junctional basal lamina [27]. Current studies of genetically modified mice will clarify the respective physiological roles of the various splice variants of AChE [28,29].

All these findings, ranging from the atomic level with crystallography, to cell biology and physiology of a living animal, have been regularly reported at the International Meetings on Cholinesterases [30], and constitute an exceptional corpus of knowledge for a single enzyme. This makes it possible to consider a number of intriguing old and new unresolved questions. We would like to argue that, in addition to their physiological importance in cholinergic systems and perhaps in other contexts, cholinesterases constitute extremely valuable models for numerous biological processes, including protein folding and dynamics, enzymatic activity, trafficking in the secretory pathway, and even evolution of gene organization. Studies on AChE greatly benefit from the convenient, sensitive and specific assays of its catalytic activity, which allow a detailed analysis of even minor active components, such as precursors of the mature enzyme species. Activity assays provide quantitative data, in contrast with assays based on immunodetection of an inactive protein, e.g. in Western blots, which may be highly non-linear and are subject to threshold and non-specific background effects.

During 40 years of research focused on the molecular forms of cholinesterases, our group has made some intriguing observations, which we hope that others will explore further. We also wish to present our views on some questions concerning cholinesterases, which are currently under discussion in the scientific community.

1. Gene organization and evolution of cholinesterases

Vertebrates possess two cholinesterase genes, encoding AChE and BChE. These genes appear to result from a duplication in the lineage leading to vertebrates [31]. In mammals, AChE is encoded by a single gene, which seems to possess several transcription start sites, with alternative splicing patterns before the first coding exon, and an alternative choice of splice acceptor sites in the 3′ region of the transcripts (Fig. 1) [32,33]. It may thus generate proteins which possess the same catalytic domain of about 500 amino acids, but different C-terminal peptides (Fig. 2) [32,34]. Depending on the choice of splice acceptor sites following the constitutive exon that encodes the C-terminal part of the common catalytic domain, more or less extended parts of the pre-mRNA may be removed, thus placing different sequences in a coding position to determine distinct C-terminal peptides. These alternative coding regions, each ending with its own stop codon, are often called “exons”, but this terminology, although convenient, is incorrect since it introduces a confusion between RNA processing, which defines exons and introns, and protein synthesis, which depends on the coding sequence. In fact, each mature transcript contains its own alternative coding region, but also those located downstream in the genome, although they are preceded by stop codons and therefore in a non-coding position. For example, in mouse, the 3′ untranslated region of the R transcript contains the H and