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Photoregulation of Acetylcholinesterase Activity with Photolabile Cholinergic Ligands

Ling Peng, and Maurice Goeldner

Laboratoire de Chimie Bio-organique, URA 1386 CNRS - Faculté de Pharmacie, Université Louis Pasteur Strasbourg, B.P. 24, F-67401 Illkirch cedex, France
Tel: 03 88 67 69 60; Fax: 33 3 88 67 88 91; Email: peng@aspirine.u-strasbg.fr

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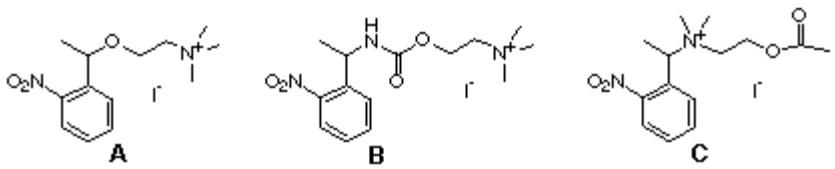
Abstract: We synthesized and characterized photolabile cholinergic ligands, and assessed their potential use in the exploration of the catalytical mechanism of acetylcholinesterase.

Keywords: Acetylcholinesterase, cholinergic ligands.

Photolabile precursors of biologically interesting molecules can provide control of temporal and spatial release of desired molecules by rapid photolysis, and are thus important tools in the study of fast biological processes. Acetylcholinesterase (AChE) is a particularly fast enzyme which hydrolyses the neurotransmitter acetylcholine with a turnover number approaching 20 000 s^{-1}. Knowledge of the 3-D structure of AChE [1] has permitted a better understanding of structure-function relationships of this enzyme, but has also raised cogent new questions concerning the traffic of substrate and products to and from the active site in view of the high turnover rate. An ideal approach to investigate this issue would be time-resolved crystallography which could afford, in real time scale, the 3D-structure of macromolecule in motion, provided that suitable probes, which could regulate the enzymatic activity by a temporally and spatially controled release of enzyme substrate or product at the active site, are available. For this purpose, we synthesized and characterized photolabile cholinergic ligands, and assessed their potential use in the exploration of the catalytical mechanism of AChE.

Scheme 1 shows the different types of photolabile cholinergic ligands. They are all 2-nitrobenzyl derivatives, and are precursors of choline (the product of the enzymatic reaction) [2], of carbamylcholine (enzyme substrate) [3] and of nor-acetylcholine (enzyme substrat, a close analogue of acetylcholine) [4, 5]. Upon photolysis, they generate the corresponding ligand rapidly and efficiently, and thus can photoregulate the activity of AChE. Time-resolved crystallographic studies with these compounds should allow visualization at atomic level of the clearance of choline from the enzyme active site (probe **A**), the carbamylation of AChE by carbamylcholine (probe **B**), and the acylation/deacylation process of AChE by nor-acetylcholine (probe **C**), respectively [6].



Scheme 1. Photolabile cholinergic ligands of AChE. **A.** Precursor of choline; **B.** Precursor of carbamylcholine; **C.** Precursor of nor-acetylcholine.

All three photolabile cholinergic ligands are reversible inhibitors of AChE, which bind at the enzyme active site with inhibition constants in the micromolar range (Table 1). Their photofragmentation processes occur

rapidly (in microsecond range) and with a high quantum yield (Table 1), without substantial photochemical damage to the enzyme.

Probes **A** and **B** photoregulate AChE activity in two different ways (Figure 1): while photolysis of AChE complexed with **A** resulted in regeneration of enzymic activity, a single laser falsh photolysis of AChE complexed with **B** led to time-dependent inactivation. Both sets of experiments demonstrated photoregulation of AChE activity. In the former case, choline (enzyme product) was produced photochemically at the active site and subsequently cleared from the active site, leading to the regeneration of enzymic activity. In the latter case, carbamylcholine (enzyme substrate) was photogenerated within the active site, with in situ carbamylation of enzyme, leading to a covalent inactivation of the enzyme, which could be reversed upon decarbamylation.

Table 1. Photofragmentation parameters and inhibition constants of **A, B** and **C**. $t_{1/2}$: Half-time of photofragmentation; F: Quantum yield; K_i: Inhibition constant.

	A	**B**	**C**
$t_{1/2}$ (usec)	10	24	25
[Phi]	0.27	0.25	0.10
K_i, AChE (uM)	13.0 +/- 0.3	44.0 +/- 0.9	0.70 +/- 0.05

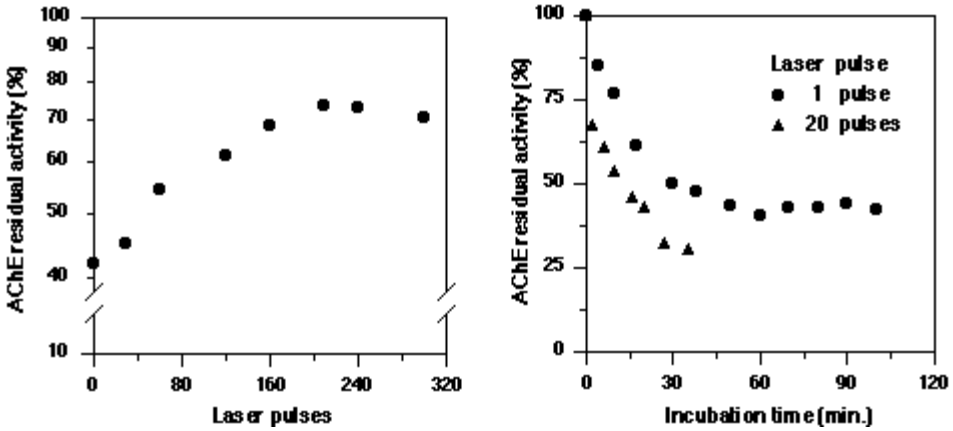


Figure 1. Photoregulation of AChE activity using probe **A** (1a) and probe **B** (1b).

In conclusion, **1a** and **1b** possess photochemical properties and a specificity for AChE which render them suitable as probes for time-resolved crystallography studies. Since the two probes photorelease either enzyme product or enzyme substrate in the active site, allowing photoregulation of AChE activity in complementary manners, they constitute complementary tools for the time-resolved crystallographic studies envisaged. Studies are presently in progress to establish the catalytic mechanism of AChE by time-resolved crystallography with these probes complexed in the AChE crystals.

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