**Introduction**

Acetylcholinesterase is an important enzyme found near the surface of the postsynaptic membrane in the synaptic cleft between the presynaptic terminal and the postsynaptic cell. (Wilson, 2002) This enzyme has the primary responsibility of hydrolyzing acetylcholine, which terminates the chemical message between the two nerves. (Goodsell, 2004) Acetylcholine and acetylcholinesterase are found in the between nerve endings in the central nervous system, the peripheral nervous system, and in the region between the nerve endings and a skeletal muscle cell of vertebrates. (Garrett, 2007) The chemical roles of acetylcholine and acetylcholinesterase are targets for many nerve gases, insecticides, and venomous agents. These agents can effectively block acetylcholinesterase, which prevents the hydrolysis of acetylcholine. The removal of acetylcholine molecules from the postsynaptic terminal is prohibited and further nerve impulses cannot be transmitted. In many situations this causes paralysis and death. (Wilson, 2002)

**Reaction**

![Reaction diagram](image)

(Garrett, 2007)

The chemical reaction induced by acetylcholinesterase is a hydrolysis reaction. Acetylcholinesterase breaks down acetylcholine to an acetate molecule and choline molecule through a nucleophilic addition reaction.

**Primary Sequence**

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<td></td>
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</tbody>
</table>

(PDB ID: 1acj)
Acetylcholinesterase consists of a sequence containing 537 amino acids that are arranged in fifteen alpha helixes and nine beta sheets. Two series of the $\beta$-sheets are anti-parallel to one another.

**Secondary Structure**

- Isolated $\alpha$-helix (with $\beta$-sheet in background) from functional pocket of acetylcholinesterase
  
  (viewed using KiNG from PDB ID: 1acf—Harel, 993)

- Isolated anti-parallel $\beta$-sheet (all other structures cropped) from acetylcholinesterase
  
  (viewed using KiNG from PDB ID: 1acf—Harel, 1993)
Wire model for Acetylcholinesterase + tarcrine (rotated 180 around Z axis)

(PDB ID: 1acj -- Harel, 1993)

(Hopp-Woods Scale)

Hydropathicity plots demonstrate the hydrophilicity (Hopp-Woods Scale) or hydrophobicity (Kyte-Doolittle scale) of an amino acid sequence. These diagrams can reveal important information about the polar and non-polar regions of the protein sequence of acetylcholinesterase. Hydrophilic regions are commonly exposed on the folded surface of proteins while the hydrophobic regions tend to show where the protein may interact with a lipophilic region. In the Hopp-Woods scale of acetylcholinesterase (PDB-1acj), there are thirteen strong (>1.0) hydrophilic regions, with the strongest ranging from amino acids 46-51, 90-92, 240-246, 266-272, 389-393, and 463-467. These amino acid sequences usually consist of arginine, aspartic acid, glutamic acid, and/or lysine. In the Kyte-Doolittle scale, there are 13 hydrophobic regions (>1.0) while five of the thirteen are considered significantly hydrophobic (>2.0). The regions that register >2.0 are found at amino acid 31, 142-145, 153-155, 294, and 448-451. These amino acid sequences usually consist of cysteine, isoleucine, leucine, methionine, phenylalanine and/or valine. (Bowen, 1998)

(Bowen, 1998)
**Tertiary Structure**

The combination of three disulfide bonds and numerous hydrogen bonding interactions gives this enzyme its functional, central groove to hydrolyze acetylcholine as well as its complex, convoluted structure.

**Interaction between acetylcholinesterase and acetylcholine**

**Note**—two acetylcholinesterase molecules can breakdown three acetylcholine molecules.

(PDB ID-2ha4; Bourne, 2006)
**Ligands**

In 1acj from the Protein Data Bank, acetylcholinesterase is shown bonded with tacrine. Tacrine is a cholinesterase-inhibiting molecule, which bound to the active site of acetylcholinesterase. (Bourne, 2006) Tacrine has a higher affinity to the active site of acetylcholinesterase than does acetylcholine. Acetylcholinesterase does not quickly degenerate tacrine, therefore ineffectively removing acetylcholine from the postsynaptic cleft. Tacrine was once used as a therapeutic agent for the treatment of Alzheimer's disease, but many patients could not tolerate the side effects. (Goddsell, 2004) Tacrine forms many bond interactions with acetylcholinesterase as shown in the following diagrams. (PDB ID: 1acj, Harel, 1993)

![Tacrine](image)

Acetylcholinesterase and Tacrine (ligand) interaction

![Hydrogen Bonding between Acetylcholinesterase and Tacrine](image)

(Moreland, 2005)

Hydrogen Bonding between Acetylcholinesterase and Tacrine
Hydrophobic interactions between Acetylcholinesterase and Tacrine

(Moreland, 2005)

Hydrophilic interactions between Acetylcholinesterase and Tacrine

(Moreland, 2005)

Other molecular interactions between Acetylcholinesterase and Tacrine

(Moreland, 2005)
**Reaction Mechanism (proposed)**

The hydrolysis of acetylcholine begins with the hydrolysis of the ester through nucleophilic attack. The imidazole ring of histidine480 forms a hydrogen bond with water, allowing the oxygen to attack the carboxyl carbon of the acetate group. This triggers the rearrangement of electrons to break the ester bond between acetate and choline. (Note: it is suggested that this could also occur with the indole ring of Tryptophan83.) (Sant’Anna, 2002)

![Chemical structures](image1)

(Sant’Anna, 2002)

The final mechanism involves the deacylation of acetate from histidine so it can be reabsorbed by the presynaptic region of the synaptic cleft for the transmission of the next nerve impulse. For this to occur, the acetate group undergoes a nucleophilic attack by water which is hydrogen bonded to histidine. This causes a rearrangement of electrons to form acetate. The imidazole ring is returned to its original state so acetylcholinesterase can be used again. (Sant’Anna, 2002)

![Chemical structures](image2)

(Sant’Anna, 2002)
When acetylcholinesterase breaks down acetylcholine the rate of reaction is extremely fast. This is necessary so if a second nerve impulse is triggered, the presynaptic region has enough acetate and the postsynaptic cleft is open to receive the impulse. Through experimentation it has been determined that deacylation is not the rate-limiting step of this reaction. $K_{\text{cat}}$, or turnover number, represent the measure of an enzymes maximum catalytic activity. It is the number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate. $K_m$, or Michaelis constant, describes the kinetics of an enzyme when the enzyme is completely saturated because it is at a significantly lower concentration than the substrate. (Froede, 1983)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$K_{\text{cat}}$ value (Jarv, 1976)</th>
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</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>140,000 s$^{-1}$</td>
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<table>
<thead>
<tr>
<th>pH</th>
<th>$(S)$</th>
<th>$K_m$</th>
<th>$E'/E^0$</th>
<th>$(E'/E^0)_{\text{max}}$</th>
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$K_m$ was determined by using acetylcholine as an inhibitor of acetylthiocholine hydrolysis; acetylthiocholine hydrolysis was determined by the spectrophotometric method.

## Table II

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$</th>
<th>$K_m$</th>
<th>$k_2$</th>
<th>$k_2$</th>
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<tbody>
<tr>
<td>Acetylcholine</td>
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<td>$5.9 \times 10^6$</td>
<td>$9.1 \times 10^6$</td>
<td>$1.7 \times 10^6$</td>
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<td>Acetylthiocholine</td>
<td>0.24</td>
<td>$5.3 \times 10^6$</td>
<td>$9.3 \times 10^6$</td>
<td>$1.23 \times 10^6$</td>
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</tbody>
</table>

Table 1—rate of acetylation of acetylcholinesterase (top), acetylcholine (middle), and constants (bottom) (Acquired from Froede & Wilson, 1984)
Enzyme regulation

Acetylcholinesterase has two regulatory mechanisms. One is found at the neuromuscular junction of skeletal muscles. Acetylcholinesterase is bound to the basal lamina of the postsynaptic cell by calcium ions. As a nerve impulse changes the membrane potential down a neuron, calcium ions move into the postsynaptic cell and acetylcholinesterase is released into the synaptic cleft to break down acetylcholine. As the neuron potential difference is restored through active transport processes, acetylcholinesterase reforms with calcium ions on the basal lamina. About 1/3 of the available acetylcholinesterase is bound more tightly at the synaptic cleft by a protein, collagen Q. A second regulatory function is under control by thyroid hormones. Thyroid hormones can have an enhancing effect on acetylcholinesterase levels in muscle tissue. Interestingly, thyroid hormones increase acetylcholinesterase levels in slow twitch muscle but not fast twitch muscle. (Skeltej, 2002)
References


Objective

- Students will be able to derive the amino acid sequence of acetylcholinesterase.
- Students will be able to describe how the amino acid sequence of acetylcholinesterase affects the enzyme's secondary and tertiary structure.
- Students will be able to evaluate the reaction mechanism between acetylcholinesterase and acetylcholine.
- Students will be able to describe the rate of reaction between acetylcholinesterase and acetylcholine.
- Students will be able to determine how acetylcholinesterase is regulated in skeletal muscle cells.
- Students will be able to describe the importance of acetylcholinesterase in the transmission of a nerve impulse.

Overview (Setting)

This lesson is designed for a college prep chemistry class with an extended lab period. (This lab period may coincide with the lecture period or it may follow at some other time.) Students are connecting knowledge learned in last year's Biology course and now beginning to develop a more comprehensive and complete understanding of the chemical nature of proteins, specifically enzymes.

Pre-Class Activity

Students will be broken into ten different groups. Each group will be given approximately 50 amino acids in sequence of acetylcholinesterase. Using a decoder table, students will determine the amino acids in the respective sequence.
Content—Enzyme Notes (Introduction)
Enzymes
- Proteins consisting of primary amino acid sequence
- secondary folding
- tertiary folding
- sometimes quaternary folding
- used to catalyze chemical reactions
  - speed up rate of reactions so chemical reactions can occur in feasible time for body/cells to use

Acetylcholinesterase
- important enzyme in nerve transmission
- breaks down neurotransmitter (acetylcholine)
  - allows next nerve impulse to be received
  - allows regeneration of acetate and choline molecules

Structured Practice (Classwork)
How does the enzymes primary, secondary, and tertiary structures allow for the catalyst of acetylcholinesterase?

- Students will diagrams, hydrophobicity plots, hydrophilicity plots, and reaction mechanism to propose possible solutions.

Independent Practice (Homework)
Students Research: Alzheimer's Disease
- students will research drugs that block neurotransmitters (neurotransmitter enzymes)
- students will produce a tri-fold pamphlet identifying at least 3 different drugs
- students will provide benefits of taking drug
- students will provide side-effects of taking drug
- students will provide chemical structure of drug
- students will provide short description of how drug interacts with protein to work effectively