

Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 17:

Irreversible Enzyme Inhibitors: Part II

5 March 2018

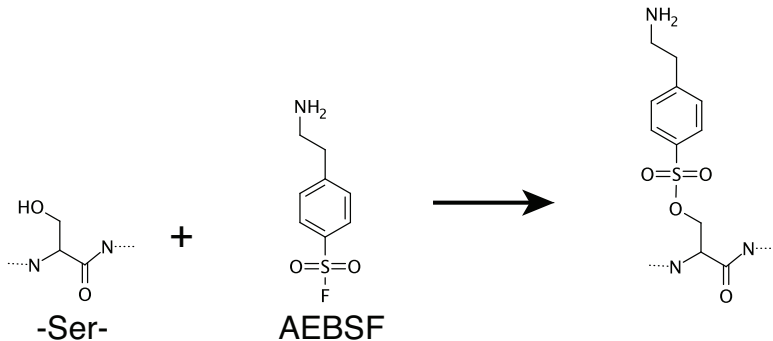
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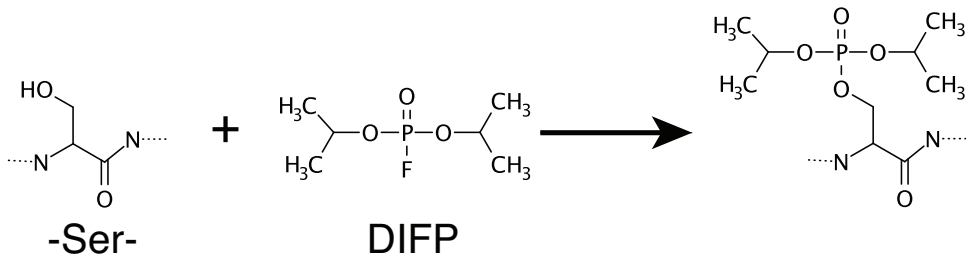
Irreversible Inhibition of Trypsin by AEBSF

4-(2-aminoethyl)-benzenesulfonyl fluoride



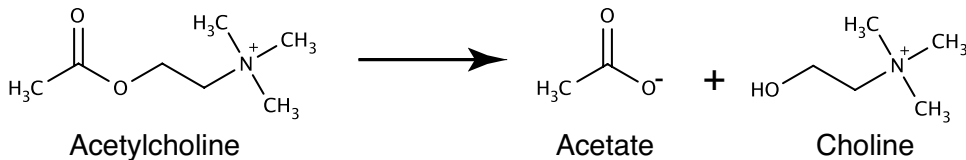
- Reaction is specific for the catalytic Ser residue.
- Reaction is irreversible.

An Earlier Irreversible Inhibitor of Serine Proteases: Diisopropyl Fluorophosphate



- First synthesized in the 1930's as a potential chemical weapon with neurotoxic effects.
- Found to inhibit esterases and proteases.
- Used as pesticide.
- Reported to be among chemical agents found in Syria in 2013.

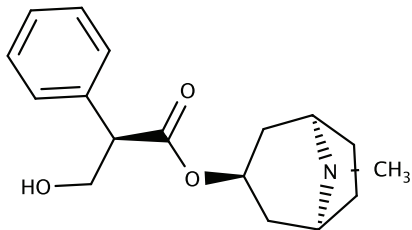
Acetylcholine Esterase



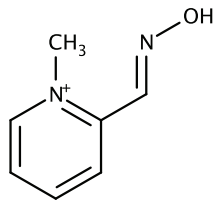
- Acetylcholine is a major neurotransmitter in vertebrates, insects and other animals.
- Esterase reaction is very similar to peptide hydrolysis.
- Enzyme uses a catalytic triad (Ser-His-Glu).
- Enzyme is inhibited by DIFP and other serine-reactive agents.

Two Antidotes for Organophosphate Poisoning

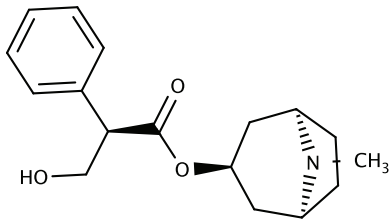
Atropine



Pralidoxime

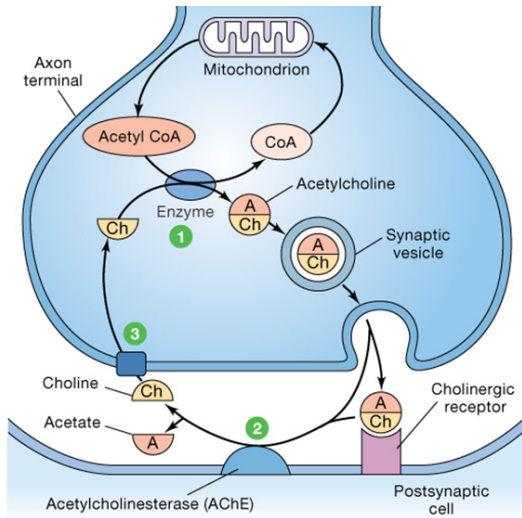


Atropine



- Isolated from *Atropa belladonna*, “deadly nightshade”.
- Inhibitor of acetylcholine receptors.
- Causes many symptoms, including dilation of pupils.
- “Bella dona” is Italian for “beautiful lady”. Plant extracts were used by women to dilate their eyes.
- Shorter acting drugs are used by ophthalmologists for dilation.

A Cholinergic Synapse



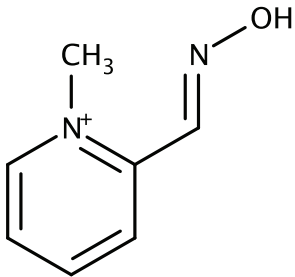
1 Acetylcholine (ACh) is made from choline and acetyl CoA.

2 In the synaptic cleft ACh is rapidly broken down by the enzyme **acetylcholinesterase**.

3 Choline is transported back into the axon terminal and is used to make more ACh.

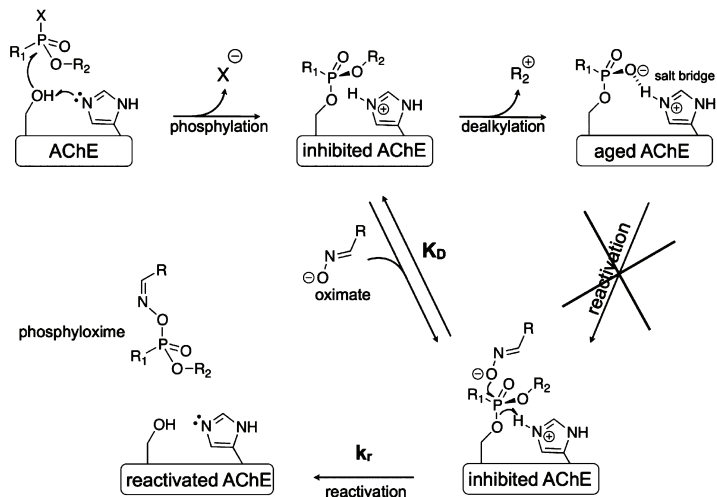
- Atropine competes with acetylcholine for binding to acetylcholine receptors.
- Atropine is an acetylcholine antagonist; binds to receptor without activating.
- Blocks synaptic transmission; better than continuous activation.

Pralidoxime



- Reactivates inhibited acetylcholine esterase.
- What makes this molecule special?
- Hydroxyl has unusually low pK_a , ≈ 8 .
- Oxygen is especially reactive.

Inactivation and Reactivation of Acetylcholine Esterase



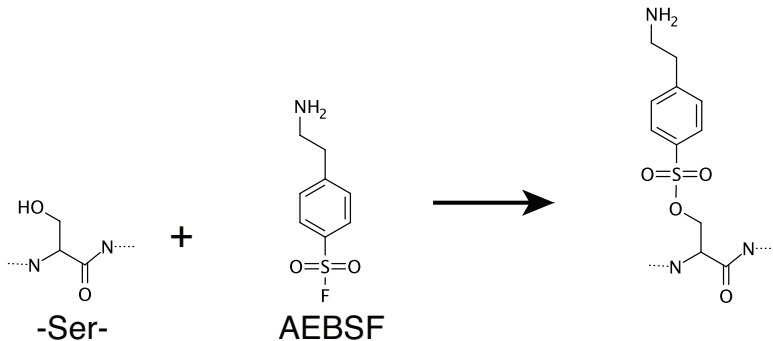
Mercey, G., Verdet, T., Renaou, J., Kiliachynai, M., Baatli, R., Nachon, F., Jean, L. & Renard, P.-Y. (2012). Reactivators of acetylcholinesterase inhibited by organophosphorous nerve agents. *Acc. Chem. Res.*, 45, 756–766. <http://dx.doi.org/10.1021/ar2002864>

Organophosphorous Poisoning and Treatment

- ~200,000 deaths per year by self-administered organophosphorous pesticides in rural Asia.
- Muscineric acetylcholine antagonists (*e.g.*, atropine) are generally the initial treatment.
- Oxime reactivators (*e.g.*, pralidoxime) are often used in conjunction with atropine.
 - Oximes are not very general. Different oximes are specific for different organophosphorous toxins.
 - Efficacy of oximes is debated.

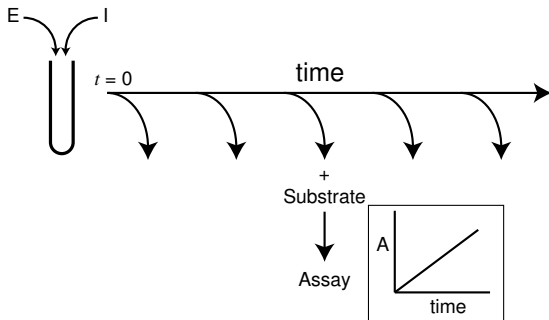
Back to Irreversible Inhibition of Trypsin by AEBSF

4-(2-aminoethyl)-benzenesulfonyl fluoride



Experimental Protocol for Studying Irreversible Inhibition

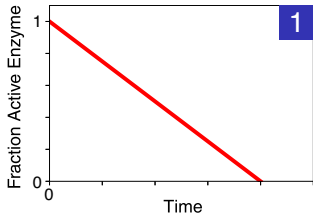
- Follow the reaction by measuring enzymatic activity at increasing times after mixing enzyme and inhibitor.



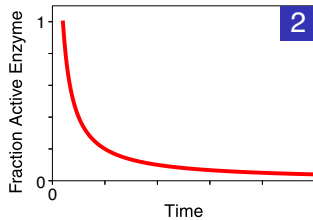
- For each sample withdrawn, measure reaction velocity.
- $V \propto$ concentration of uninhibited enzyme.
- Time for assay must be short relative to time of inactivation.

Clicker Question #1

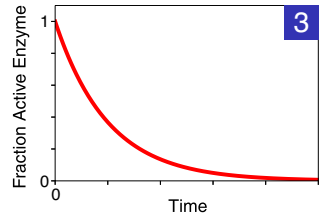
How does the concentration of active enzyme change with time?



$$\frac{[E]}{[E_0]} = 1 - kt$$



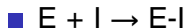
$$\frac{[E]}{[E_0]} = \frac{1}{kt}$$



$$\frac{[E]}{[E_0]} = e^{-kt}$$

All answers count for now.

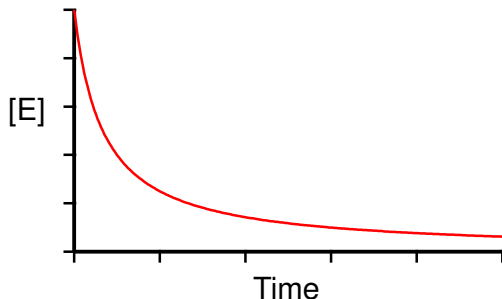
Kinetics of Irreversible Inactivation



- Second-order kinetics:

$$\frac{d[E]}{dt} = \frac{d[I]}{dt} = -k_2[I][E]$$

- If initial concentrations of enzyme and inhibitor are equal:



- This is *not* an exponential decay function!
Both [I] and [E] decrease with time, and both decreases contribute to reduced rate with time.

Pseudo First-Order Kinetics

- If $[I] \gg [E]$, $[I]$ will remain approximately constant during the reaction.

$$\frac{d[E]}{dt} = - \underbrace{k_2[I]}_{\text{constant}} \cdot [E]$$

- Define a pseudo first-order rate constant: $k_{app} = k_2[I]$

$$\frac{d[E]}{dt} = -k_{app}[E]$$

- Rearrange and integrate the rate expression:

$$\int_{[E]_0}^{[E]} \frac{d[E]}{[E]} = \int_{t=0}^t -k_{app} dt$$

$[E]_0$ = Initial enzyme concentration.

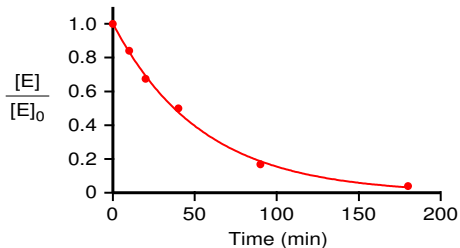
Pseudo First-Order Kinetics

- Integrated rate expression:

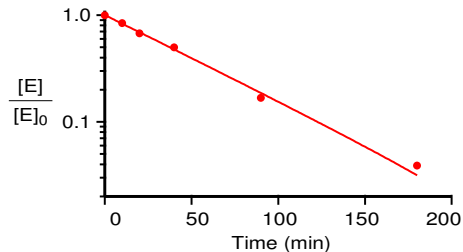
$$\ln \left(\frac{[E]}{[E]_0} \right) = -k_{app}t,$$

$$\frac{[E]}{[E]_0} = e^{-k_{app}t}$$

Standard plot:



Semi-logarithmic plot:



Why Do We See Exponentials and Logarithms Everywhere?

- The exponential function, $y = e^x$, is its own derivative:

$$\frac{de^x}{dx} = e^x$$

- More generally, if k is a constant:

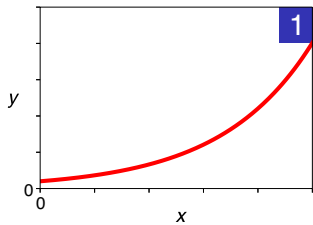
$$\frac{de^{kx}}{dx} = ke^{kx}$$

- Why is this important?
- There are many physical and biological processes for which the rate of change is proportional to the quantity that is changing!

Clicker Question #2

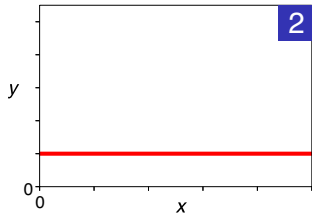
(Multiple answers possible!)

Which of these represents an exponential function: $y = e^{kx}$?



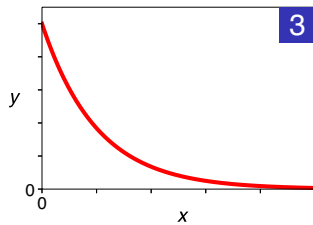
$$k > 0$$

$$\frac{dy}{dx} = ke^{kx} > 0$$



$$k = 0$$

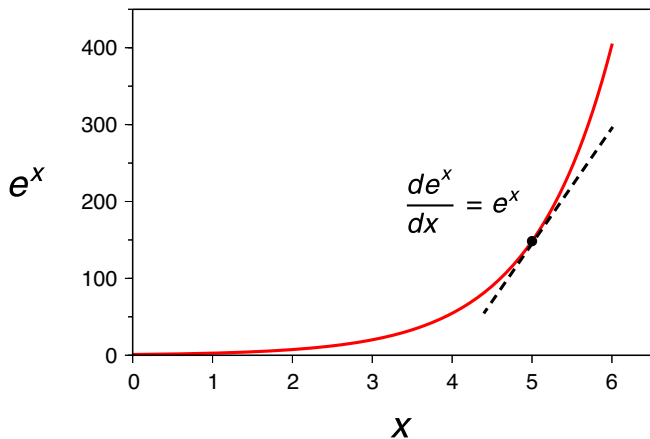
$$\frac{dy}{dx} = ke^{kx} = 0$$



$$k < 0$$

$$\frac{dy}{dx} = ke^{kx} < 0$$

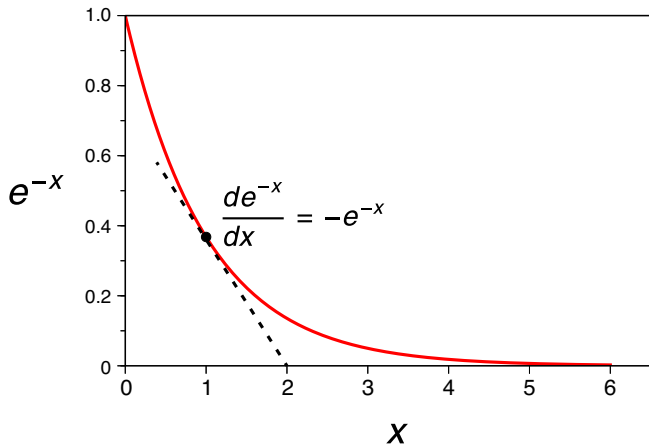
Exponential Growth



Example: Growth of bacteria:

- Each bacterium has an equal probability of dividing during a given time period.
- The number of new bacteria in a short time period is proportional to the number already present.

Exponential Decay

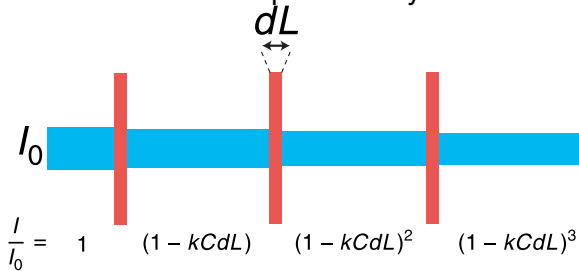


Example: Radioactive decay:

- Each nucleus has an equal probability of decaying during a given time period.
- The number of nuclei that decay in a short time period is proportional to the number of nuclei present.

Beer-Lambert Law Revisited

A cuvette divided up into many thin slices



- Equal probability of a single photon being absorbed in each slice.
- **But**, As more light is absorbed, the total number of photons absorbed in each slice decreases.
- Intensity of light decreases exponentially as it passes through the cuvette.

$$\frac{I}{I_0} = e^{-kCL}$$

Back to Pseudo First-Order Kinetics

- Second-order kinetics:

$$\frac{d[E]}{dt} = \frac{d[I]}{dt} = -k_2[I][E]$$

- If [I] does not change significantly, we can define an apparent rate constant:

$$k_{app} = k_2[I]$$

- Pseudo first-order kinetics

$$\frac{d[E]}{dt} = -k_{app}[E]$$

- Integrated rate expression:

$$\ln \left(\frac{[E]}{[E]_0} \right) = -k_{app}t, \quad \frac{[E]}{[E]_0} = e^{-k_{app}t}$$

Data Interpretation

- Estimate k_{app} from fit of $[E]/[E]_0$ versus time.
- Calculate second-order rate constant, k_2 from k_{app}

$$k_2 = k_{app}/[I]$$

- Can use estimate of k_2 to predict kinetics of inactivation at other inhibitor concentrations.