Lecture 9 - Co-factors Part II

PLP - Alanine Racemase

How to inhibit the conversion of L-Ala to D-Ala?

Another covalent inhibitor:

Designing a mechanism based PLP inhibitor?

-Product
-Reactant State

inactive covalent adduct

 inactive covalent adduct

\[
\frac{k_{\text{inact}}}{k_{\text{cat}}} \approx \text{Partitioning}
\]
Thiamine pyrophosphate (Vitamin B₁)
Like PLP, the electron-deficient heterocyclic ring of thiamine pyrophosphate (TPP) stabilizes the formation of carbanionic species.

Breslow (1961) was the first to propose and provide evidence for the C-2 carbanion, showing that this proton exchanges readily in D₂O.

Reactions involving thiamine pyrophosphate:
1. Decarboxylation of alpha-ketoacids: pyruvate decarboxylase

2. Oxidative decarboxylations: exemplified by pyruvate dehydrogenase

Lipoic acid - source of 2e⁻, 2H⁺
Redox Enzymes:
- NADPH/NADH \( \text{nicotinamide dependent (} 2e^- \text{, } H^+ \) \\
- FAD/FADPH \( \text{flavin dependent} \) \( 1e^- \rightarrow 1e^- \text{, } 2H^+ \)

Nicotinamide adenine dinucleotide (phosphate)

\[
\text{Nicotinamide adenine dinucleotide (phosphate)}
\]

\[
\begin{align*}
\text{Nicotinamide adenine dinucleotide (phosphate)} & = \text{nicotinamide} + \text{adenine} + 2 \text{phosphate groups} \\
\end{align*}
\]

Overall Reaction:
\[
\begin{align*}
\text{NAD}^+ + 2H^+ + 2e^- & \rightarrow \text{NADH} + \text{H}^+ \\
\text{E}'_0 & = -0.32 \text{V} \\
\text{E}^- & = -0.19 \text{V} \\
\text{E}^{+} & = 0.13 \text{V} \\
\text{G} & = -nF \text{E}_0 \\
\end{align*}
\]

Note compounds with lower redox potential thoroughly reduce those with higher redox potentials.

\[
\begin{align*}
\Delta G & = -nF \Delta E_0 \\
\Delta G & \approx 23 \text{ kcal/mol} \\
\end{align*}
\]

Reactions of NAD\(^+\)/NADPH\(^+\) Enzymes (\( 2e^-/H^+ \))

Note that these are all sterospecific.

To deduce whether it is pro R or pro S replace that H by D and determine R or S configuration.
Reactions of Flavin Enzymes (2e⁻/2H⁺)

Flavin

oxidized

1e⁻ reduced

2e⁻ reduced

Handle

R⁻ OH → R⁻ OH

R⁻ OH → R⁻ OH
Reaction of glutathione (glutathione reductase)

\[ \text{NADH} + \text{Enz.FI} + \text{GS-SG} \rightleftharpoons \text{2GSH} + \text{Enz.FI} + \text{NAD}^+ \]

\[ \text{FAD} \rightleftharpoons \text{FADH}_2 \rightleftharpoons \text{FAD} + \text{G-S-S}^+ \rightleftharpoons \text{FAD} + \text{G-S-S}^+ + \text{GSH} \rightleftharpoons \text{FAD} + 2\text{GSH} \]

Molecular oxygen

"peracid"

Source of $^\cdot\text{OH}$