The Chemistry of Cofactors: Thiamine, Biotin and Pyridoxal Phosphate

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Enzyme Catalysis

- Enzymes rely on amino acid functionalities for increasing catalytic activity

\[ E + S \xleftrightarrow{k_1, k_{-1}} E-S \xleftrightarrow{k_2, k_{-2}} E-P \xleftrightarrow{k_3, k_{-3}} E + P \]

\[
\begin{align*}
\text{Me} & \text{NH} & \text{NH} & \text{CO} & \text{OH} \\
\text{H}_{2}\text{N} & \text{N} & \text{N} & \text{OH} & \text{H}_{2}\text{N} & \text{N} & \text{OH}
\end{align*}
\]
\[
\xrightarrow{\text{H}_2\text{O}}
\]
\[
\begin{align*}
\text{Me} & \text{NH} & \text{NH} & \text{CO} & \text{OH} \\
\text{H}_{2}\text{N} & \text{N} & \text{N} & \text{OH} & \text{H}_{2}\text{N} & \text{N} & \text{OH}
\end{align*}
\]
rate: $1.8 \times 10^{-11}$ s$^{-1}$
$t_{1/2} = 1100$ years

\[
\begin{align*}
\text{Ph} & \text{NH} & \text{NH} & \text{CO} & \text{OH} \\
\text{H}_{2}\text{N} & \text{N} & \text{N} & \text{OH} & \text{H}_{2}\text{N} & \text{N} & \text{OH}
\end{align*}
\]
\[
\xrightarrow{\text{carboxypeptidase B}}
\]
\[
\begin{align*}
\text{Ph} & \text{NH} & \text{NH} & \text{CO} & \text{OH} \\
\text{H}_{2}\text{N} & \text{N} & \text{N} & \text{OH} & \text{H}_{2}\text{N} & \text{N} & \text{OH}
\end{align*}
\]
rate: $238$ s$^{-1}$

Enzyme Catalysis

- Enzymes rely on amino acid functionalities for increasing catalytic activity
Enzyme Catalysis

- Enzymes rely on amino acid functionalities for increasing catalytic activity

![Chemical structure](image1)

- What about more challenging transformations?

![Chemical structure](image2)

Side chain residues alone would not be able to stabilize the reaction pathway
What are Coenzymes?

- Coenzymes facilitate non-amino acid derived enzyme reactivity

Adenosine triphosphate, ATP (phosphorylation)

Flavin adenine dinucleotide, FAD (oxidation/reduction)

Nicotinamide adenine dinucleotide, NAD$^+$ (oxidation/reduction)

Coenzyme A (acyl transfer)

What are Coenzymes?

- Coenzymes facilitate non-amino acid derived enzyme reactivity

Lipoic acid (acyl transfer)

Tetrahydrofolate (transfer of C₅ units)

Thiamine diphosphate, TDP (decarboxylation)

Biotin (carboxylation)

Pyridoxal phosphate, PLP (amino acid metabolism)

Thiamine Diphosphate: Nature's Carbene Catalyst

- Thiamine diphosphate (TDP):

Deficiency in vitamin B₁ results in the neurological disorder Beriberi

Thiamine dependent enzymes are involved in the Krebs cycle through production of acetyl-CoA from pyruvate

Structure proven through synthesis in 1937

Thiamine Diphosphate: Nature's Carbene Catalyst

Thiamine diphosphate (TDP):

Thiamine dependent pyruvate decarboxylase initially thought to proceed through an amine catalyzed process.
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Thiamine diphosphate (TDP):

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Thiamine: Unraveling the Mystery

- Thiamine shown to incorporate $D_2O$: Breslow's first proposal

\[ \text{Structure} \]

- Correlation to Benzoin condensation

\[ \text{Reaction} \]


Thiamine: Unraveling the Mystery

- Thiamine shown to incorporate $D_2O$: Breslow's first proposal

- Westheimer analyzed for deuterium incorporation

Thiamine: Unraveling the Mystery

- Thiamine shown to incorporate D\textsubscript{2}O: Breslow's second proposal

- Breslow confirmed C-2 deuterium incorporation through IR and NMR

Thiamine: Nature's Umpolung Catalyst

- TPP catalyzes a range of chemical transformations
Thiamine: Nature's Umpolung Catalyst

- TPP catalyzes a range of chemical transformations

![Chemical reactions and structures]
Thiamine: Nature's Umpolung Catalyst

[Diagram of the thiamine catalytic cycle with chemical structures and arrows indicating the reaction pathway.]
Thiamine: Enzyme Specific Acceleration

- Mechanistic studies of pyruvate decarboxylase

\[
\text{MeCO}_2\text{OH} \xrightarrow{\text{pyruvate decarboxylase}} \text{MeCO}_2\text{H}
\]

Rate data at pH 6.2, 30 °C, 1 mM of pyruvate:

- Thiamine catalyzed process: \(2 \times 10^{-11} \text{ s}^{-1}\)
- Pyruvate Decarboxylase: \(63 \text{ s}^{-1}\)

\[\rightarrow 3 \times 10^{12} \text{ rate enhancement}\]

How does Pyruvate Decarboxylase Mediate the Decarboxylation?

- Rate acceleration: enzyme assisted deprotonation
How does Pyruvate Decarboxylase Mediate the Decarboxylation?

- Kinetics of nonenzymatic system highlight rate acceleration

PDC: 640 s\(^{-1}\)
Thiamine: 5 \times 10^{-5} \text{ s}^{-1}
Acceleration: 1 \times 10^7

PDC: 82 s\(^{-1}\)
Thiamine: 2 \times 10^{-11} \text{ s}^{-1}
Acceleration: 4 \times 10^{12}

Thiamine: Side Reactivity

- Separate model system indicates specific acid catalysis towards productive reactivity

Decarboxylation rate is highest in pH ranges that result in protonation of pyrimidine N1

At high pH, decarboxylation is not favored and α-keto acid is formed

Addition of most buffers has no effect on the rate of decarboxylation but altered the ratio of fragmentation vs. protonation

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Thiamine: Side Reactivity

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Key glutamic acid residue accelerates decarboxylation

Replacement of Glu$_{473}$ with a side chain of similar size (Gln$_{473}$) results in similar enzyme kinetics without drastic loss of reactivity. Other side chain mutants show reduced proficiency.

Enzyme Acceleration of Pyruvate Decarboxylase

- Hydrophobic residues create van der Waals interactions and stabilize the decarboxylation transition state

  I415V modification of enzyme results in 16-fold loss of activity

- Hydrogen bonding network surrounds the enzyme active site for rapid proton shuttling

  E473D modification of enzyme results in 3000-fold loss of activity

Active site structure of pyruvate decarboxylase from Zymomonas mobilis

Thiamine: Nature's Umpolung Catalyst
**Pyridoxal Phosphate**

- **Pyridoxal phosphate (PLP):**
  - [Chemical structures of vitamin B₆, pyridoxal phosphate, and pyridoxamine phosphate]

- **PLP is critical for the synthesis of amino acids through transamination**
  - [Chemical structures of amino acids and ketones with PLP-dependent transaminases]
Pyridoxal Phosphate

Mode of amine activation:

PLP activates through schiff base formation which weakens the α-amino bonds.

Resonance assisted hydrogen bond between the C-3 OH and imine provides stability.

How does the enzyme control selectivity for bond breaking and forming events?
Pyridoxal Phosphate

Dunathan model for enzyme specificity

Dunathan Hypothesis: Enzyme control through directional carboxylate binding

Deprotonation:

Decarboxylation:

Pyridoxal Phosphate Mediated Transformations

- Pyridoxal phosphate (PLP) as a versatile activation mode in biology

Pyridoxal Phosphate Mediated Transformations

- Pyridoxal phosphate (PLP) as a versatile activation mode in biology

![Pyridoxal Phosphate Structure and Reactions Image]

Pyridoxal Phosphate Mediated Transformations

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Pyridoxal Phosphate Mediated Transformations

- Pyridoxal phosphate (PLP) as a versatile activation mode in biology

- [Chemical structures and reactions shown]

**Pyridoxal Phosphate**

- Protein binding of PLP: similarities between separate protein classes

![Chemical structure of PLP and PLP coenzyme bound to enzyme](image)

PLP coenzyme is covalently bound to enzyme through backbone lysine residue which is removed during catalysis

Second highly conserved series of side chain residues create a phosphate binding "cup" to orient PLP

Substrate specificity is determined through binding pocket interactions with substrate side chain

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Pyridoxal Phosphate

PLP mediated transamination
Pyridoxal Phosphate

- PLP mediated racemization

How can an enzyme control such similar transformations?
Enzyme control over extent of pyridinium formation

From aspartate aminotransferase

From bacterial alanine racemase

Residue adjacent to pyridine has important implications for reaction type based on the stability of the quinoid intermediate

The active site lysine is thought to act as the direct base in both processes

Pyridoxal Phosphate Mediated Claisen Condensation

Pyridoxal Phosphate Mediated Tryptophan Synthesis

Pyridoxal Phosphate Mediated Tryptophan Synthesis

- Crystal structure of bacterial enzyme tryptophan synthase

**Biotin: Vitamin H**

- Biotin: highly specialized carboxylation catalyst

![Biotin Structure](image)

Covalently bound to enzyme through amide bond with lysine sidechain which is found in a highly conserved -Ala-Met-Lys-Met protein sequence

Critical CO₂ carrier for fatty acid biosynthesis as well as gluconeogenesis

Discovered through interactions with egg protein avidin

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Role of Biotin in Fatty Acid Biosynthesis

Biotin mediated acetyl-CoA carboxylase

Acetyl CoA

Malonyl CoA

MeSACP

MeSACP

MeSACP
Role of Biotin in Acetyl-CoA Carboxylase

- Biotin mediates the transfer of CO₂ from solution to acetyl-CoA
Biotin mediates the transfer of CO₂ from solution to acetyl-CoA
Biotin: Carboxylation Catalyst

- Biotin mediates the transfer of bicarbonate from solution to an acetate
Role of Biotin in Acetyl-CoA Carboxylase

- Biotin mediates the transfer of CO₂ from solution to acetyl-CoA
Biotin Carboxylation

- Early $^{18}$O labelling observations

\[
\begin{align*}
\text{H}^{18}\text{O}^{18}\text{O}^{-} & \quad \text{pyruvate carboxylase} \\
\text{HO}^{-}\text{ADP} & \quad \text{ADP}^{-}\text{S}^{17}\text{O}^{19}\text{O}^{-} \\
\end{align*}
\]

- Analysis of phosphate stereochemistry

\[
\begin{align*}
\text{ADP}^{-}\text{S}^{17}\text{O}^{19}\text{O}^{-} & \quad \text{pyruvate carboxylase} \\
\text{S}^{17}\text{O}^{16}\text{O}^{-} & \quad \text{ADP}^{-}\text{S}^{17}\text{O}^{16}\text{O}^{-} \\
\end{align*}
\]

Biotin Carboxylation

- Phosphate ester hydrolysis

\[
\begin{array}{c}
\text{HO}^- \\ \text{R'}\text{O}^- \text{P}^- \text{OR} \\
\text{R'O}^- \text{OR''}
\end{array}
\]

inversion at phosphorus

Phosphate hydrolysis is achieved through a trigonal bipyramidal intermediate.
Incoming nucleophiles and leaving groups must occupy axial positions.

- Pseudorotation can occur for cyclic phosphates

\[
\begin{array}{c}
\text{Me} \cdots \text{O}^- \text{P}^- \text{OH} \\
\text{Me} \cdots \text{O}^- \text{POH} \\
\text{Me} \cdots \text{O}^- \text{POH} \\
\text{Me} \cdots \text{O}^- \text{POH}
\end{array}
\]

Pseudorotation has not been observed in biological systems.

How does ATP enable dehydration of carbonate?

Biotin Carboxylation
Biotin Carboxylation

- How does ATP enable dehydration of carbonate?
Biotin Carboxylation

How does ATP enable dehydration of carbonate?

Double inversion results in net retention of phosphate stereochemistry
How does ATP enable dehydration of carbonate?

\[
\begin{align*}
\text{HO}_3\text{CO}^- & \rightarrow \text{ADP} \rightarrow \text{HO}_3\text{CO}^- \rightarrow \text{biotin} \\
\text{HO}_3\text{CO}^- & \rightarrow \text{biotin} \rightarrow \text{biotin} \\
\text{HO}_3\text{CO}^- & \rightarrow \text{biotin} \rightarrow \text{biotin}
\end{align*}
\]
How does ATP enable dehydration of carbonate?

via:

biotin

di-anionic acylating agent

~70 ms lifetime

biotin
How does ATP enable dehydration of carbonate?
Biotin Carboxylation

How does ATP enable dehydration of carbonate?

via:

may potentially lead to waste of ATP through CO₂ emission
Biotin Carboxylation

How does ATP enable dehydration of carbonate?
Biotin Carboxylation

- Isotope exchange could arise from breakdown of carboxyphosphate

Biotin Carboxylation

- Isotope exchange could arise from breakdown of carboxyphosphate

Fragmentation of carboxyphosphate is potentially a reversible process

Fragmentation would lead to scrambling of labelled bicarbonate or phosphate through phosphate rotation prior to recombination

Biotin Carboxylation

Alternative mechanism for the formation of carboxybiotin

Biotin Carboxylation

Alternative mechanism for the formation of carboxybiotin

Model system for the intermediacy an orthocarbonate adduct at high pH

Consistent with enzyme wherein a base of $pK_a = 6.6$ is required from kinetic studies

Role of Biotin in Acetyl-CoA Carboxylase

- Biotin mediates the transfer of CO₂ from solution to acetyl-CoA
Biotin Transcarboxylation

- Carboxylation of acetyl CoA: mechanistic pathways

Biotin Transcarboxylation

Analysis of transcarboxylation indicates a stepwise process

Elimination rather than carboxylation indicates process is stepwise

Rate equivalent to that of ATP hydrolysis

**Biotin Transcarboxylation**

- Analysis of transcarboxylation indicates a stepwise process

\[
\text{CoA-S-CH}_2\text{CO}_2\text{H} \xrightarrow{\text{propionyl-CoA carboxylase}} \text{CoA-CH}_2\text{CO}_2\text{H}
\]

ATP, HCO_3^{-}

Elimination rather than carboxylation indicates process is stepwise

Rate equivalent to that of ATP hydrolysis

- Decarboxylation also hypothesized to occur as a result of enzyme mediated distortion