Four categories of enzymes

1. Group transfer reactions
2. Oxidations/reductions
3. Eliminations/isomerizations/rearrangements
4. Reactions that make or break C-C bonds

How do enzymes mediate biological oxidation/reduction reactions?

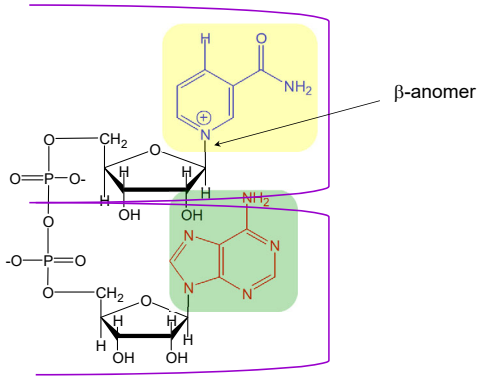
- e^- transfer through amino acid side chains?
- Use of redox cofactors
  - Organic (NAD+, FAD, lipoic acid, pterin, etc)
  - Metalloorganic (heme, chlorophyll, etc)
  - Inorganic (Fe, Cu, etc)

Two electron oxidations/reductions (transfer of e^- pairs) are the norm in enzyme-catalyzed reactions

- Two single electron steps (free radical int.)
  - Quinoid coenzyme mediation
  - Transition metal mediation
- A single two electron step
  - Hydride transfer $:H^- = 2e^- + H^+$

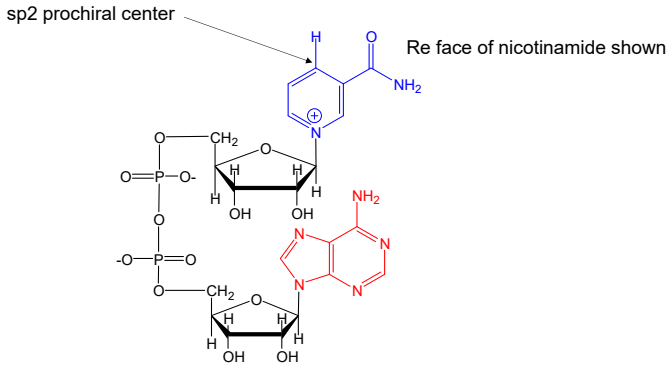
NAD+

Nicotinamide Adenine Dinucleotide



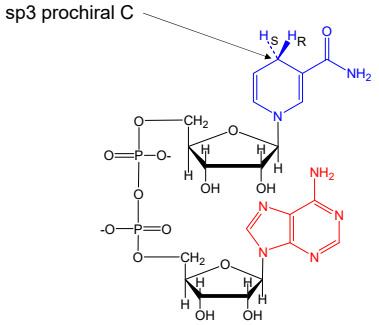
NAD+

Nicotinamide Adenine Dinucleotide



NADH

Reduced Nicotinamide Adenine Dinucleotide



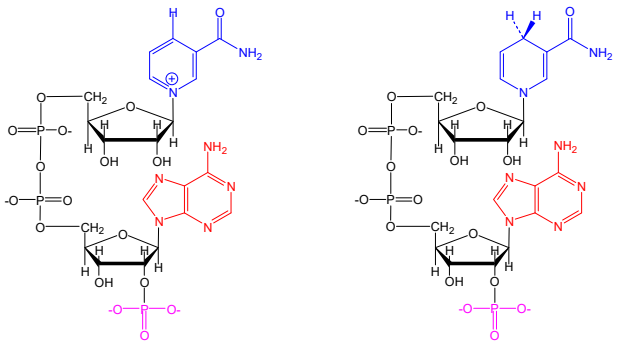
Chemical properties of NADH

- Reduction potential = -320 mV
- Air stable in aq. Solutions (kinetic stability)
- An enzyme substrate, not an integral cofactor

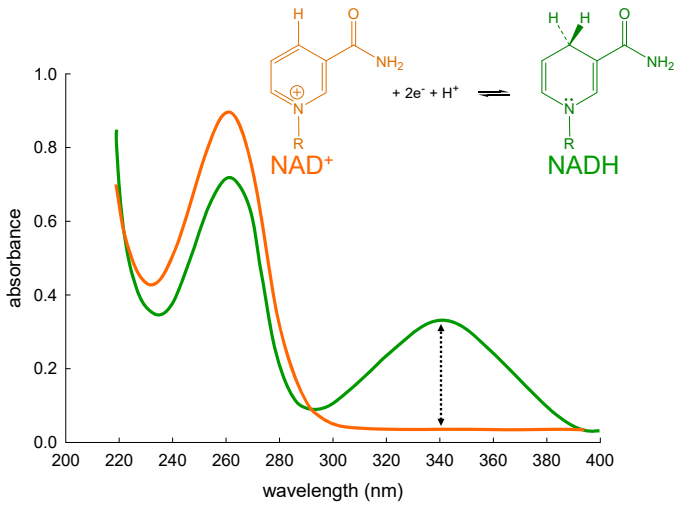
  (exception- masked redox chemistry)
- Follow oxidation/reduction at 340 nm
- NADP$^+$ and NADPH for biosynthesis
- Re vs. Si face hydride transfer
- Scope of reactions:
  - Redox
  - Masked redox
  - nonredox

NADP+ and NADPH

Nicotinamide Adenine Dinucleotide Phosphate



Spectral properties of NADH and NAD+

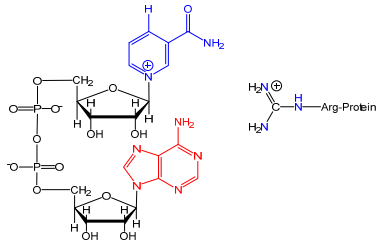


Scope of reactions: dehydrogenation examples

- Alcohol dehydrogenation — Lactate DH, malate DH, alcohol DH
- Amine dehydrogenation — Glutamate DH
- Aldehyde dehydrogenation — Aldehyde DH, G3P DH*
- Oxidative decarboxylation — Isocitrate DH
- Dihydrolipoyl DH (E3 of PDH) $NAD^+ + FADH_2 \rightarrow NADH + FAD + H^+$

Scope of reactions

- Redox
- Masked redox
- Non redox



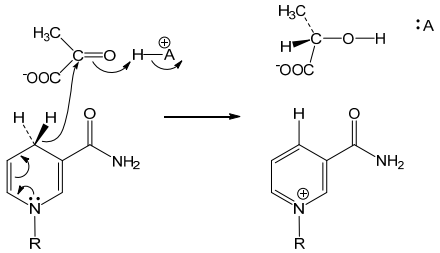
Lactate dehydrogenase

- Catalyzes the interconversion of pyruvate and L-lactate
- Oxidation of L-lactate: The ProR H of the nicotinamide ring is transferred to pyruvate to form L-lactate
- Reduction of pyruvate: the H of L-lactate is transferred to the Re face of the nicotinamide ring

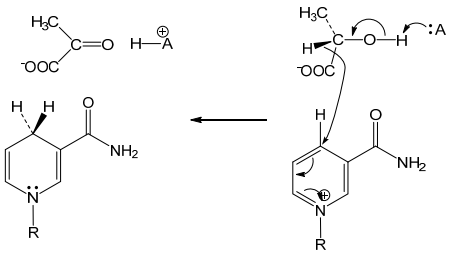
$$\text{L-lactate} + NAD^+ \rightleftharpoons \text{pyruvate} + NADH + H^+$$

Lactate dehydrogenase mechanism

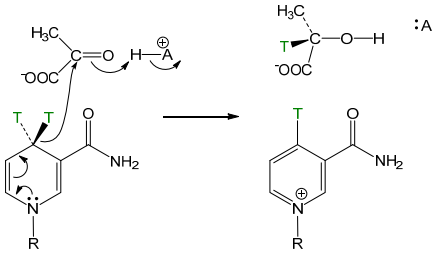
Reduction:



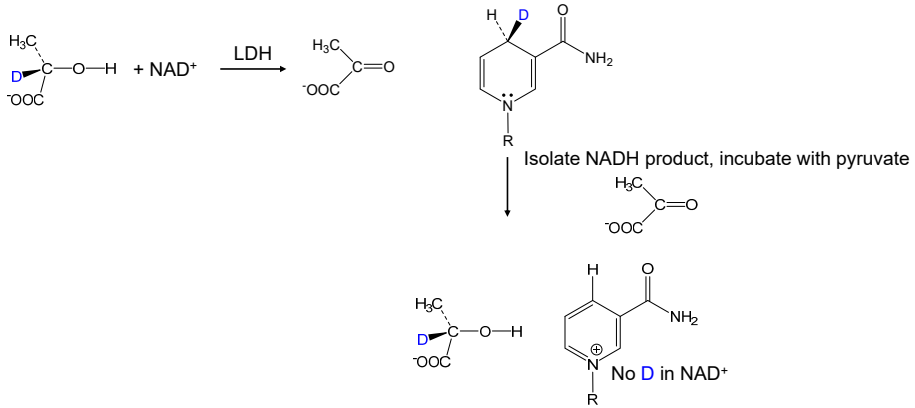
Oxidation:



Evidence for hydride transfer?

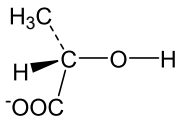


Stereospecificity of hydride transfer: NAD+/NADH



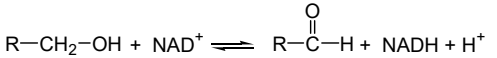
Lactate dehydrogenase (LDH)

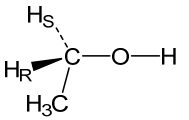
- Lactic acid is an α–hydroxyacid; pyruvic acid is an α–ketoacid
- LDH is specific for using and forming L-lactate



Liver alcohol dehydrogenase (ADH)

- α_2 dimer
- 2 Zn^{2+}/dimer
- Reversible oxidation of 1° and 2° alcohols

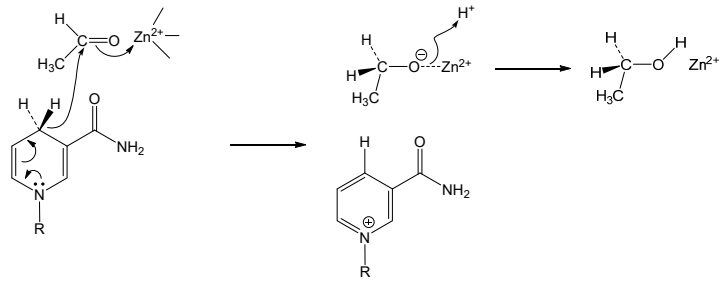




- For 1 alcohols, ADH exhibits specificity for ProR H

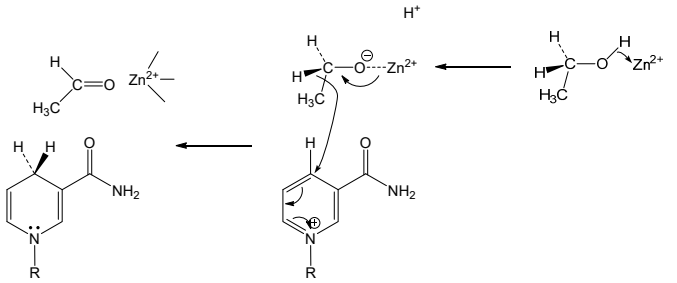
Liver alcohol dehydrogenase mechanism

Reduction:

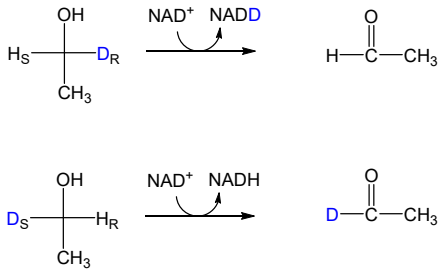


Liver alcohol dehydrogenase mechanism

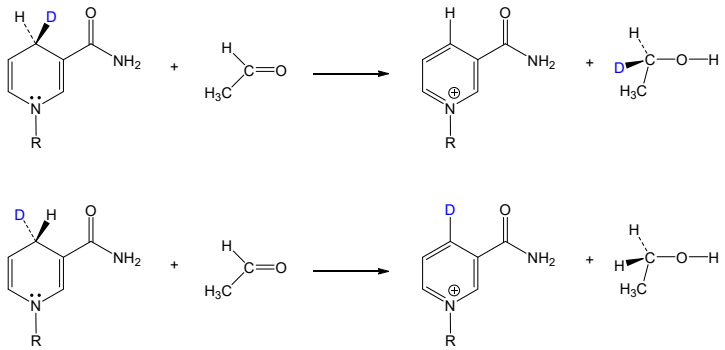
Oxidation:



Stereospecificity of hydride transfer: Which hydrogen of ethanol is transferred?

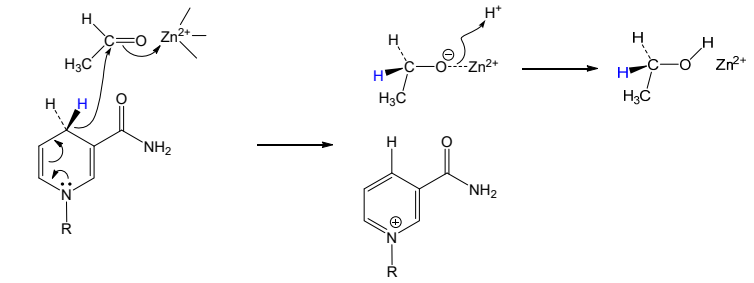


Stereospecificity of hydride transfer: Which hydrogen of NADH is transferred?



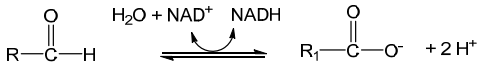
Liver alcohol dehydrogenase mechanism

Reduction:

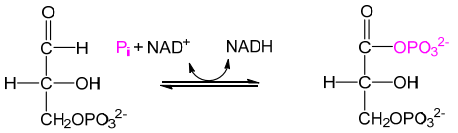


Aldehyde dehydrogenase

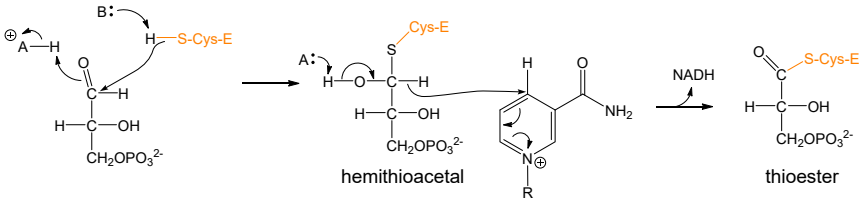
- Classic: hydrolysis produces a carboxylic acid

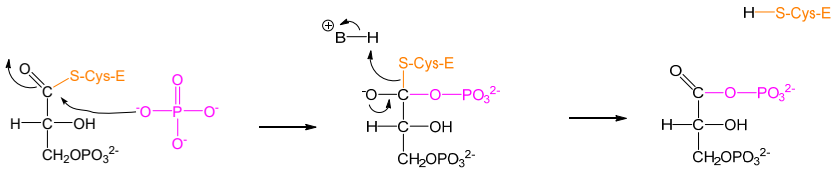


- Glycolysis: phosphorolysis produces an acyl phosphate



GAPDH mechanism



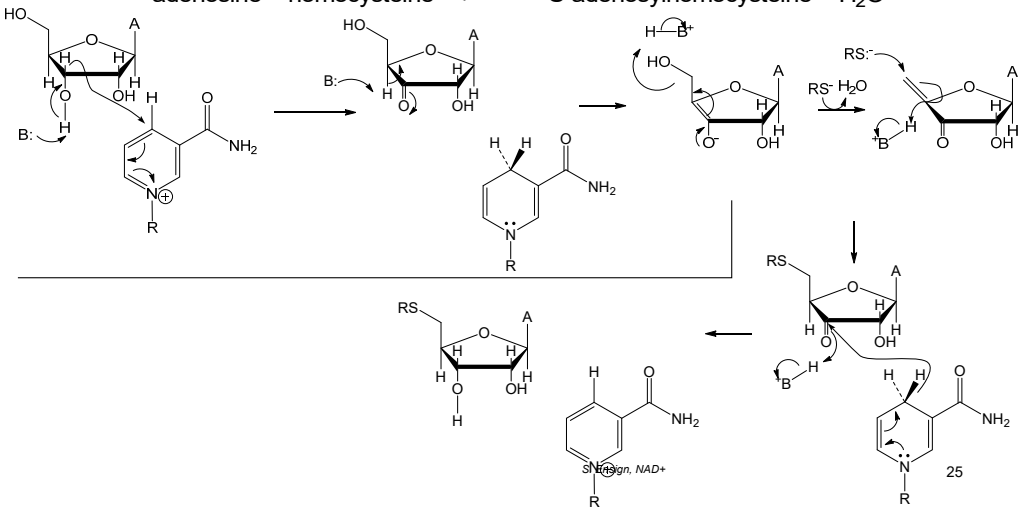


Masked redox chemistry

- Enzymes contain tightly bound NAD^+ (does not dissociate)
- Enzyme-bound NAD^+ undergoes reduction/oxidation
- Prototype enzymes:
  - UDP-glucose epimerase
  - S-adenosylhomocysteine synthase

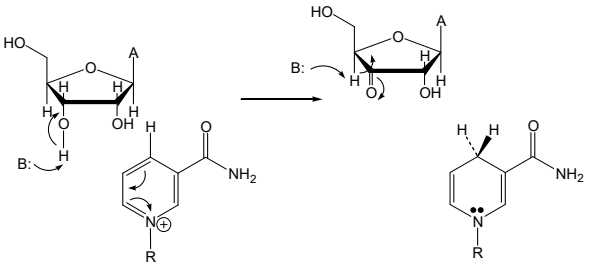
S-adenosylhomocysteine synthase

adenosine + homocysteine $\rightleftharpoons$ S-adenosylhomocysteine + H_2O



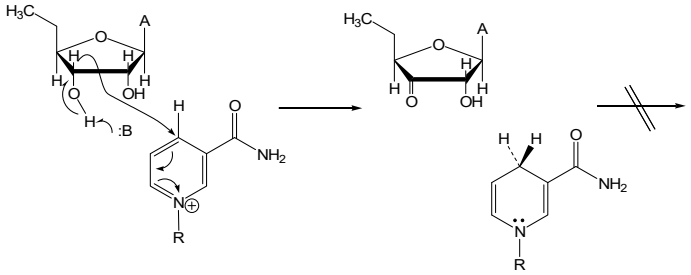
S-AdoHC synthase : evidence for masked redox chemistry?

(1) Carry out reaction with adenosine, E~NAD+, but without homocysteine



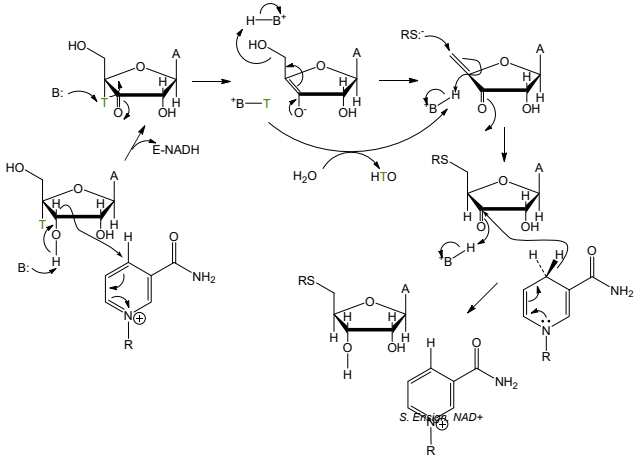
S-AdoHC synthase : evidence for masked redox chemistry?

(2) Carry out reaction with an adenosine analog where no complete chemistry can occur (e.g. 5'-deoxyadenosine)



S-AdoHC synthase : evidence for masked redox chemistry?

(3) Carry out reaction with adenosine labelled in either the 3' or 4' position with T; ask whether T is exchanged with solvent



S-AdoHC synthase : evidence for masked redox chemistry?

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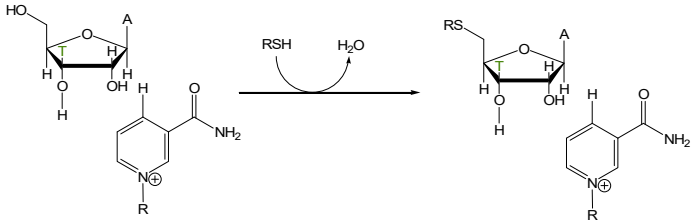
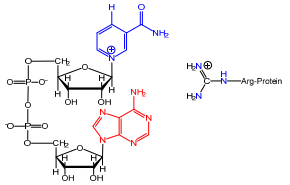


Nonredox roles for NAD+: ADP-ribosylation

- NAD glycohydrolase
- Protein ADP-ribosylating enzymes



Nonreversible reactions

- Diptheria toxin
- Cholera toxin
- Nuclear poly ADPR polymerases

Reversible ADP-ribosylation

- Only known to occur in one enzyme system
- Reversible ADP-ribosylation inactivates/activates enzymatic activity (compare reversible phosphorylation, adenylylation)
- Which enzyme is regulated by reversible ADP-ribosylation?

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Activating Factor for the Iron Protein of Nitrogenase from *Rhodospirillum rubrum*

Abstract. *As isolated from* Rhodospirillum rubrum, *the iron protein of nitrogenase has little or no activity. It can be activated by incubating it with a trypsin-sensitive, oxygen-labile component (activating factor) plus adenosine triphosphate and a divalent metal ion. After activation, the iron protein retains its nitrogenase activity when the activating factor is removed.*

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5 April 1976; revised 14 June 1976

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Vol. 82, pp. 3173–3177, May 1985
Biochemistry

Covalent modification of the iron protein of nitrogenase from *Rhodospirillum rubrum* by adenosine diphosphoribosylation of a specific arginine residue

(photosynthetic bacterium/modified peptide/nitrogen fixation)

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Communicated by R. H. Burris, January 22, 1985

ABSTRACT **Nitrogenase in *Rhodospirillum rubrum* is inactivated *in vivo* by the covalent modification of the Fe protein with a nucleotide. The preparation of two modified peptides derived from proteolytic digestion of the inactive Fe protein is described. The modifying group is shown to be adenosine diphosphoribose, linked through the terminal ribose to a guanidino nitrogen of arginine. The structural features were established by using proton and phosphorus NMR, positive- and negative-ion fast atom bombardment mass spectrometry, and fast atom bombardment/collisionally activated decomposition mass spectrometry. Spectral methods along with chromatographic analysis and sequential degradation established the sequence of the modification site of Fe protein as Gly-Arg(ADR-ribose)-Gly-Val-Ile-Thr. This corresponds to the sequence in the Fe protein from *Azotobacter vinelandii* for amino acid residues 99 to 104.**

MATERIALS AND METHODS

Growth of *R. rubrum* and Purification of Fe Protein. The conditions for cell growth and collection have been previously described, along with methods for the purification of inactive Fe protein (7).

Preparation and Purification of H_2ΔMG. These procedures have also been described previously (8).

Preparation and Purification of a Modified Hexapeptide from Inactive Fe Protein. Purified Fe protein was treated with DNase and RNase (0.1% each) for 15 min and was chromatographed on a 4.5 × 19 cm Sephadex G-25 column in 100 mM ammonium formate buffer, pH 8.5. This and all subsequent steps were performed aerobically. Fractions containing the Fe protein were collected. Subtilisin (Sigma) was added to the Fe protein solution (1% subtilisin) and proteolysis was allowed to continue for 16 hr.

Reversible ADP-ribosylation of nitrogenase Fe protein