

Introduction to Metabolism

Metabolic Pathways

Organic Reaction Mechanisms

Experimental Approaches to the Study of Metabolism

Thermodynamics of Phosphate Compounds

Oxidation-Reduction Reactions

Thermodynamics of Life

Metabolic Pathways

Series of consecutive enzymatic reactions that produce specific products.

Metabolites - reactants, intermediates, products

Anabolism - biosynthesis

Few metabolites (**pyruvate, acetyl-CoA, citric acid cycle intermediates**) serve as starting materials

Catabolism - degradation

Diverse substances converted to common intermediates, which lead to **acetyl-CoA**

Four characteristics of metabolic pathways:

1. Metabolic pathways are irreversible
2. Every metabolic pathway has a first committed step
3. All metabolic pathways are regulated
4. Metabolic pathways in eukaryotic cells occur in specific cellular locations

Organic Reaction Mechanisms

Chemical logic

Heterolytic bond cleavage - carbanion + proton or carbocation + hydride ion

Homolytic bond cleavage - radicals

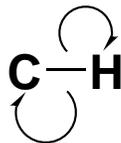
Nucleophiles - electron rich (amine, thiolate)

Electrophiles - electron deficient (H^+ , metal ion, carbonyl carbon)

Arrow convention



electron pair



single electron

Organic Reaction Mechanisms

Group transfer reactions

The group transfers that occur in biochemical systems involve the transfer of an electrophilic group from one nucleophile to another

Acyl group transfer - chymotrypsin

Phosphoryl group transfer - hexokinase

Glycosyl group transfer - lysozyme

Organic Reaction Mechanisms

Oxidation-reduction

Involves the loss or gain of electrons

Use of coenzymes NADH and FADH₂

Transfer one or two electrons at a time

Terminal electron acceptors for different organisms (O₂,
NO₃⁻, S, Fe³⁺)

Organic Reaction Mechanisms

Eliminations, isomerizations, and rearrangements

Elimination reactions result in the formation of a double bond between two single-bonded saturated atoms (eliminated groups are H_2O , NH_3 , ROH , RNH_2)

Three mechanisms

concerted

stepwise through a carbocation

stepwise through a carbanion

Two possible stereochemical courses

trans (anti) eliminations (most prevalent)

cis (syn) eliminations

Isomerization reactions involve the intramolecular shift of a H atom, which changes the location of double bond

Racemization is an isomerization reaction in which a H atom shifts position at the only chiral center so as to invert that center

If there is more than one chiral center, this is denoted an **epimerization**

Rearrangement reactions break and reform C-C bonds so as to rearrange the C skeleton

Organic Reaction Mechanisms

Reactions that make and break C-C bonds

Degradative and biosynthetic metabolism utilize both C-C bond making and breaking reactions

Stabilization of intermediates

Addition of nucleophilic centers to electrophilic centers

Experimental Approaches to the Study of Metabolism

Understanding metabolic pathways

Sequence of reactions

Mechanism of reaction

Control or regulatory mechanism

Use of metabolic inhibitors - build up of intermediates prior to the inhibited reaction

Use of growth studies - supply exogenous intermediate in the presence or absence of inhibitor, in genetically altered (natural or engineered) organism

Use of biochemical genetics - study naturally occurring organisms for defective processing of substances, manipulate organisms for absence or return of function

A very simple example:



No growth or production of D if you supply A or B

Growth and production of D if you supply C

which step is blocked?

Experimental Approaches to the Study of Metabolism

Isotopes in biochemistry

Isotopes - atoms with same number of protons but different number of neutrons, same chemical properties

Isotope effects on reaction rates are limited to H, D, T

Use labeled atoms as probes for nuclear magnetic resonance (NMR) spectrometry, electron paramagnetic resonance spectrometry (EPR), Mössbauer, vibrational spectroscopy

Use radiolabeled atoms for proportional counting, liquid scintillation counting, and autoradiography (1st order decay of radioactive isotopes)

Isotopes are used to establish precursor-product relationships

Experimental Approaches to the Study of Metabolism

Isolated organs, cells, and subcellular organelles

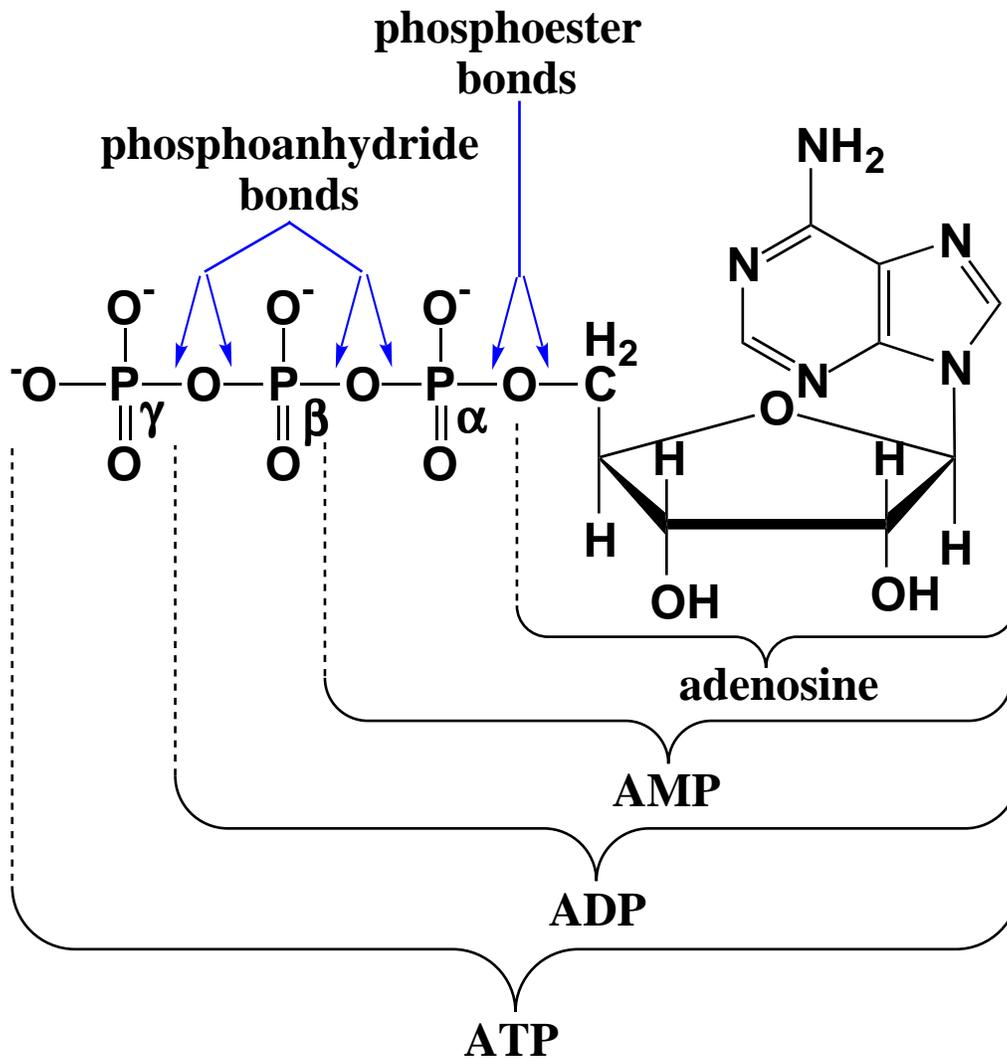
Need to look at specific locations for metabolic pathway relationships

Know functions of cellular organelles

Thermodynamics of Phosphate Compounds

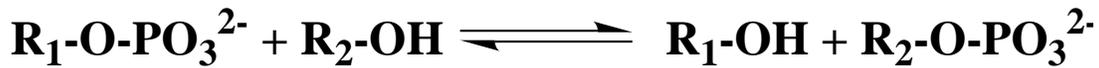
Endergonic processes of living (biosynthesis) are driven by exergonic reactions of nutrient oxidation (catabolism)

Adenosine triphosphate (ATP^{4-}) - form of energy currency

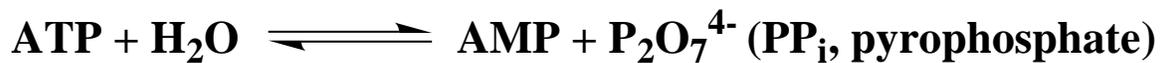
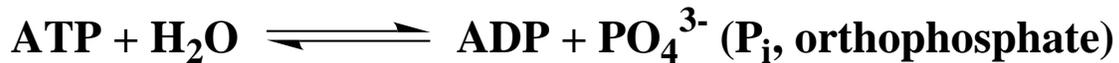


Thermodynamics of Phosphate Compounds

Phosphoryl-transfer reactions



Most important are hydrolysis of ATP



Key Concept:

Couple exergonic reactions to endergonic reactions to drive them to completion (this includes the regeneration of ATP)

Bioenergetic utility of phosphoryl-transfer reactions:

kinetic stability to hydrolysis

capacity to transmit relatively large amounts of ΔG°

ΔG of a reaction varies with total concentration of reactants and products, pH, ionic strength (for ATP also [metal ion])

$$\Delta G = \Delta G^\circ + RT \ln \left\{ \frac{[\text{ADP}^{3-}][\text{P}_i^{3-}]}{[\text{ATP}^{4-}]} \right\}$$

but ATP, ADP, AMP all have protonation constants

Net result: $\Delta G \sim -50 \text{ kJ}\cdot\text{mol}^{-1}$ for ATP hydrolysis in cell

Thermodynamics of Phosphate Compounds

Rationalizing the "energy" in "high-energy" compounds

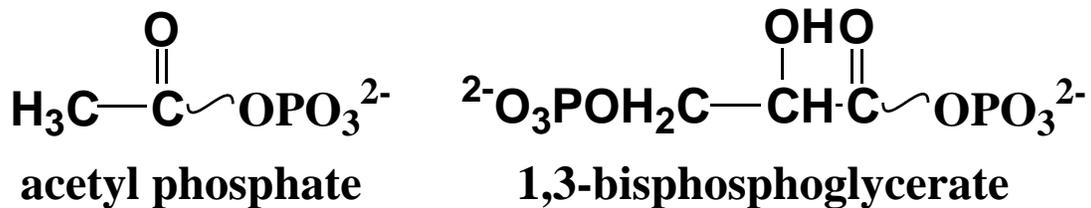
High-energy bonds - more negative than $-25 \text{ kJ}\cdot\text{mol}^{-1}$

Factors responsible for "high-energy" character of phosphoanhydride bonds:

1. Resonance stabilization less in phosphoanhydride bond than in hydrolysis product
2. Greater electrostatic repulsion in phosphoanhydride compared to hydrolysis product
3. Smaller solvation energy of a phosphoanhydride bond compared to hydrolysis product

Other high-energy compounds:

acyl phosphates



enol phosphates - phosphoenolpyruvate

phosphoguanidines - phosphocreatine, phosphoarginine

Thermodynamics of Phosphate Compounds

The role of ATP

Energy conduit between "high-energy" phosphate donors and "low-energy" phosphate acceptors

ATP consumption:

1. Early stages of nutrient breakdown (formation of "low-energy" phosphate compounds)
2. Interconversion of nucleoside triphosphates
$$\mathbf{ATP + NDP \rightleftharpoons ADP + NTP}$$
3. Physiological processes - muscle contraction, ion transport
4. Additional phosphoanhydride cleavage in highly endergonic reactions - inorganic pyrophosphatase

ATP formation:

1. Substrate-level phosphorylation
2. Oxidative- and photophosphorylation
3. Adenylate kinase reaction



Rate of ATP turnover (at rest) is $\sim 3 \text{ mol (1.5 kg) \cdot h}^{-1}$

Phosphocreatine is a high-energy reservoir for ATP formation in muscle and nerve cells



$$\Delta G^{\circ'} = +12.6 \text{ kJ} \cdot \text{mol}^{-1}$$

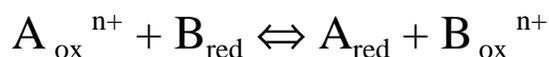
Oxidation and Reduction Reactions

Reactions that involve the transfer of electrons

In photosynthesis, CO_2 is reduced and H_2O is oxidized to yield carbohydrates. This is powered by photons (light energy).

In aerobic metabolism, the photosynthetic reaction is reversed in order to harvest the stored energy in the form of ATP.

The Nerst equation



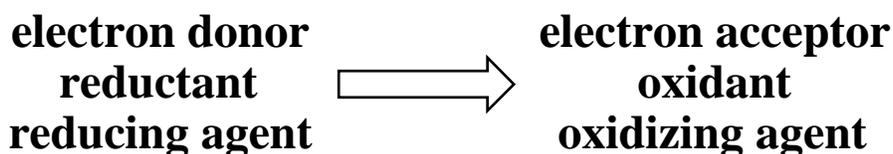
$$\Delta G = \Delta G^\circ + RT \ln \left\{ \frac{[\text{A}_{\text{red}}][\text{B}_{\text{ox}}^{n+}]}{[\text{A}_{\text{ox}}^{n+}][\text{B}_{\text{red}}]} \right\}$$

$$\Delta G = -nF\Delta\epsilon$$

($\Delta\epsilon$ = emf or redox potential)

$$F = (96,494 \text{ C}\cdot\text{mol}^{-1} = 96,494 \text{ J}\cdot\text{V mol}^{-1})$$

$$\Delta\epsilon = \Delta\epsilon^\circ + \frac{RT}{nF} \ln \left\{ \frac{[\text{A}_{\text{red}}][\text{B}_{\text{ox}}^{n+}]}{[\text{A}_{\text{ox}}^{n+}][\text{B}_{\text{red}}]} \right\}$$



$$\Delta\epsilon > 0 \rightarrow \Delta G < 0 \text{ (spontaneous reaction)}$$

Oxidation and Reduction Reactions

Measurements of redox potentials

Half-reactions are written as reductions

The more positive the standard reduction potential, the greater the tendency for the redox couple's oxidized form to accept electrons and become reduced

Protein components of redox enzymes play active roles in electron-transfer reactions by modulating the redox potentials of their bound redox-active centers

Concentration Gradients

A concentration gradient has a lower entropy (greater order) than a uniformly mixed solution and requires energy for its formation.

Energy may be harnessed from a concentration gradient to drive an endergonic reaction (i.e., ATP synthesis by H⁺ gradient)

Thermodynamics of Life

Living systems are never at equilibrium, they are open systems and obey the laws of thermodynamics

Conjugate flows result from thermodynamic forces:

diffusion - concentration gradient

electric current - electric field gradient

heat - temperature gradient

Nonconjugate flows (energy transduction) result from thermodynamic forces:

concentration gradient - electric current, heat, chemical reaction

electrical potential - concentration gradient, heat, chemical reaction

Living things maintain steady state

Biochemical reactions are highly specific

It is the role of an enzyme to selectively reduce the ΔG of activation of a chemically coupled reaction so that it approaches equilibrium faster than the more thermodynamically favored uncoupled reaction