INTRODUCTION TO BIOLOGICAL CHEMISTRY OF THE ELEMENTS
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Lecture 1. Introduction. Overview of elements used by biology; introduction to molecules and macromolecules.

Lecture 2. Biological chemistry of phosphorous

Lecture 3. Biological chemistry of sulphur.

Bibliography


PERIODIC TABLE

SCALE OF BIOCHEMISTRY
- Distance

Atoms/bonds  0.05-0.5 nanometres (nm)
Small molecules  0.2-1.0 nanometres (nm)
(X-ray crystallography, spectroscopy)

Proteins  2-20 nm
(X-ray crystallography → electron microscopy)

Cells  1000 – 100,000 nm (1-100 μm)
(electron microscopy → light microscopy)
SMALL MOLECULES COME FROM THE ENVIRONMENT OR ARE BUILT

BIG (MACRO) MOLECULES ARE BUILT FROM SMALL MOLECULES

MONOMER LINKAGES ARE FORMED BY CONDENSATION REACTIONS

LIMITS ON MACROMOLECULE SIZE?

MACROMOLECULE SIZE AND SHAPE - big molecules

LIMITS ON MACROMOLECULE SIZE?
NON-TRANSITION METALS

Interact ionically – stable in solution, no oxidation/reduction reactions

Bind strongly to proteins
structural + signalling

Zinc finger
Bind strongly to proteins
structural + signalling

Biological chemistry of the elements

TRANSITION METALS

Transition metal: chemically active electrons in the d-orbitals.

Fe : 1s²2s²2p⁶3s²3p⁶3d⁶4s²
Cu : 1s²2s²2p⁶3s²3p⁶3d⁹4s²

Multiple oxidation states – can take part in a wide variety of chemical reactions.

Interact both ionically and covalently – can bind strongly to a wide variety of ligands, including very small molecules.

Need to be “controlled” in biological systems.

THE VERSATILITY OF Fe AND Cu

These elements can play many different chemical roles within a metalloprotein:

Electron transfer (e.g. cytochrome c)

Transport oxygen (e.g. haemoglobin)

Redox catalyst (e.g. tyrosinase)

Regulation (e.g. aconitase)

PERIODIC TABLE

11 elements are essential to all forms of life
10 elements are essential to most forms of life
7 elements are essential to quite a few forms of life
~20 elements in the human body with a known role
(cf. 42 in a mobile phone)
PHOSPHORUS – GROUP V ELEMENT

- Valence -3 to +5
- Covalent bonding
- Readily oxidised in air
- Normally found as phosphate

Valence -3 to +5

Covalent bonding

Redox Properties of Phosphorus

\[ nE^\circ = -2.5 \text{ to } -1.25 \] for oxidation state -2 to 0

Reduction potential for oxidation state -3 to 0

Phosphorus is readily oxidised in air and normally found as phosphate.

Stable in oxidising and non-oxidising conditions (phosphate)

GEOMETRY OF PHOSPHATE

Phosphate ion

Tetrahedral E. P. G.

Molecular Geometry

Phosphate as Buffer Ion

Phosphoric acid

Dihydrogen phosphate

Hydrogen phosphate

Orthophosphate

\[ pK_a = 7 \]

Phosphate is an important buffer in plasma (1 mmol/l) and cells (10 mmol/l)

The ionisation state of phosphate can be a useful indicator of intracellular pH

PHOSPHATE AS BUFFER ION

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Phosphate is an important buffer in plasma (1 mmol/l) and cells (10 mmol/l).

The ionisation state of phosphate can be a useful indicator of intracellular pH.

INSOLUBLE CALCIUM PHOSPHATE IS THE BASIS OF BONE AND TOOTH ENAMEL

\[ 10 \text{ Ca}^{2+} + 6 \text{ PO}_4^{3-} + 2 \text{ OH}^- \rightarrow \text{Ca}_{10} \text{(PO}_4)_6 \text{(OH)}_2 \text{(s)} \]

Hydroxyapatite matrix

Protein fibres (collagen)

Replacement of OH with F gives more resilient compound

Must avoid CaF²⁻ in solution where phosphate occurs.

Observation of Intracellular pH by Nuclear Magnetic Resonance (NMR)

Intracellular pH rises in Xenopus (toad) eggs after fertilisation.

pH rise from 7.42 → 7.62

\[ ^{31}P \text{ NMR spectrum} \]

Before

After

Insoluble calcium phosphate is the basis of bone and tooth enamel.

Must avoid CaF²⁻ in solution where phosphate occurs.
CHEMISTRY OF PHOSPHORIC ACID

\[ \text{acid} + \text{alcohol} \rightarrow \text{phosphate ester} + \text{H}_2\text{O} \]

Glucose: \( \text{C}_6\text{H}_{12}\text{O}_6 \)

Glucose-6-phosphate: \( \text{C}_6\text{H}_{11}\text{O}_7^- \)

2 \(-\)ve charges added to glucose. Prevents glucose from crossing cell membrane, i.e. keeps glucose inside cell.

All intermediates in glycolysis are phosphorylated.

PROTEINS CAN BE PHOSPHORYLATED AS A CONTROL MECHANISM

Certain amino acids have OH side chains: serine (S), threonine (T), tyrosine (Y)

- (aliphatic)
- (aromatic)

Phosphorylation of insulin receptor switches it on.

Phosphorylation introduces high local concentration of \(-\)ve charge.

See also glycogen synthase (-)/phosphorylase (+).

OTHER PHOSPHATE ESTERS

\[ \text{R} \text{O} + \text{R} \text{O} \rightarrow \text{phosphodiester} + \text{R} \text{O} \text{P} \text{O} \text{R} \text{O} \text{P} \]

Phospholipid: \( \text{glycerol-fatty acid-phosphate} \)

DNA/RNA HAVE A PHOSPHODIESTER ‘BACKBONE’

\[ \text{R} \text{O} \text{H} + \text{R} \text{O} \text{H} + \text{R} \text{O} \text{H} + \text{R} \text{O} \text{H} \rightarrow \text{phosphodiester} \]

Each additional phosphate adds an additional \(-\)ve charge. Charge = length.

Useful in separating DNA by ELECTROPHORESIS.

PHOSPHORIC ACID ANHYDRIDES

\[ \text{H}_3\text{PO}_4 \rightarrow \text{pyrophosphoric acid} + \text{H}_2\text{O} \]

Acid anhydrides in water are thermodynamically unstable. i.e. hydrolysis will yield a lot of energy (~ 40kJ/mol).

Hydrolysis of pyrophosphates are very slow at room temperature ‘kinetically stable’. Therefore they can act as a ‘store of energy’.

PYROPHOSPHATES AND ENERGY

- Pyrophosphate is used by some organisms as an immediate energy source (e.g. to drive ion pumps in the purple bacterium \( R. \text{rubrum} \)).
- The major ‘immediate’ source of energy in cells is adenosine triphosphate (ATP).
### 3D Structure of ATP
- Phosphate groups
- Mg²⁺ ion
- Adenine ring
- Ribose

### ATP as an ‘Energy Currency’
- Catabolism:
  - Fermentations
  - Oxidation of glucose
  - Tricarboxylic acid cycle
  - Fatty acid oxidation
  - Photosynthesis
  - Oxidation of NADH, FADH₂ (by electron transfer chains)
- Anabolism:
  - Ion transport
  - Movement
  - Synthesis of macromolecules (DNA, RNA, proteins)
  - Information processing
  - Signalling

### ATP Provides Energy by Group Transfer, Rarely by Hydrolysis
**Glutamine Synthetase**

**Overall reaction:**
\[
\text{Glutamate} + \text{NH}_3 + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + \text{P}_i
\]

**Step 1:**
\[
\text{R} - \text{NH}_2 + \text{ATP} \rightarrow \text{R} - \text{NH_2} - \text{ADP} - \text{Pi}
\]

**Step 2:**
\[
\text{R} - \text{NH_2} - \text{ADP} - \text{Pi} \rightarrow \text{R} - \text{NH_2} + \text{NH}_3 + \text{ADP} + \text{Pi}
\]

**Mixed anhydride activated intermediate**

### ATP Provides Energy by Group Transfer, Rarely by Hydrolysis
**Aminoacyl-tRNA Synthetase**

**Overall reaction:**
\[
\text{amino acid} + \text{tRNA} + \text{ATP} \rightarrow \text{aminoacyl-tRNA} + \text{AMP} + \text{PP}_i
\]

**Step 1:**
\[
\text{amino acid} + \text{ATP} \rightarrow \text{aminoacyl-AMP} + \text{PP}_i
\]

**Step 2:**
\[
\text{aminoacyl-AMP} + \text{tRNA} \rightarrow \text{aminoacyl-tRNA} + \text{AMP}
\]

### ATP as a Short Term Energy Store
- Kinetically stable (hydrolysis <1% per day)
- Thermodynamically unstable (hydrolysis releases > 50kJ/mol for one anhydride bond)
- Two anhydride bonds (can drive very energy demanding reactions e.g. linking amino acid to tRNA)
- Anhydride = dehydrating agent (macromolecule synthesis involves condensation reactions)
- Source of phosphate groups – said to have a high phosphate transfer potential (e.g. in phosphorylation of glucose, proteins)

**Is adenine just a ‘handle’?**
*cf. NADH, FADH₂*

### N-P Bonds
**Adenylyl imidodiphosphate**
Artificial enzyme inhibitor for ATP-utilising enzymes.

**Creatine Phosphate (PCr)**
Naturally-occurring, short term energy reserve in muscle.
ATP used to drive muscle contraction – about 70% of all body ATP is in muscle.
ATP remains constant in muscle during severe exercise, pH and phosphocreatine (PCr) both fall and Pi goes up.
Regulatory mechanisms in the cell maintain ATP levels within a very narrow range.

**TOXICITY OF ARSENATE**
Arsenate is an analogue of phosphate.
Can be used by glyceraldehyde-3-phosphate dehydrogenase or mitochondrial ATP synthase in error.

\[
\begin{align*}
\text{ADP} + P_i & \rightarrow \text{ATP} \\
\text{ADP} + \text{As} & \rightarrow \text{ADP} + \text{As} \\
\text{ADP} + \text{As} & \rightarrow \text{HPO}_4^{2-} + \text{As} + \text{H}_2\text{O} \\
\end{align*}
\]

Thermodynamically unstable
Kinetically stable
Half life > 7 days
Thermodynamically unstable
Kinetically unstable
Half life about 7 sec
Production of heat but no energy stored

**KEY FACTS**
- Phosphorus is normally found in biology as phosphate (fully oxidised form), with 1 or 2 negative charges.
- Many metabolites (e.g. intermediates of glycolysis) are phosphorylated
- Protein phosphorylation is a method of controlling enzyme/receptor activity
- Anhydrides of phosphoric acid are useful energy sources in biology (PPi, ATP)
- ATP (energy currency) is common bridge between catabolism and anabolism
- ATP levels in cells are very strictly controlled
- ATP can act as dehydrating agent, phosphate donor, or source of energy by hydrolysis.

**MEASUREMENT OF ATP AND PCr BY \(^{31}\text{P NMR}\)**

ATP remains constant in muscle during severe exercise, pH and phosphocreatine (PCr) both fall and Pi goes up.
Regulatory mechanisms in the cell maintain ATP levels within a very narrow range.

**ATP IS TIGHTLY REGULATED**
- so eating it does you no good
**SULPHUR – GROUP VI ELEMENT**

- $1s^22s^22p^63s^23p^2$
  - 2 unpaired $p$ electrons
- Valence -2 to +6
- Covalent bonding
- Can be found free in nature as element (S0)
- In biology often found as RSH ($S^{-}$) and sometimes as sulphate ($SO_4^{2-}$)

**REDOX PROPERTIES OF SULPHUR**

- Stable in oxidising conditions (sulphate)
- Stable in oxidising and non-oxidising conditions (phosphate)

**SULPHATE IS A PERMANENT DIANION AT PHYSIOLOGICAL pH**

- Sulphate is not a buffer at physiological pH values, and is not found at significant concentrations in biological fluids.

**HANDLING SULPHATE IN CELLS**

- APS is used:
  - as a source of sulphate for transfer to saccharides;
  - to handle sulphate for reduction to sulphide.

**ADENOSINE PHOSPHOSULPHATE**

- Adenosine phosphosulphate (APS) is formed by the condensation of ATP with sulphate

**SULPHATED POLYSACCHARIDES OCCUR IN CONNECTIVE TISSUE**

- Chondroitin-4-sulphate
- Chondroitin-6-sulphate
- Dermatan sulphate
- Heparin sulphate
**Proteoglycans from Connective Tissue**

Different polymers have different physical properties, and are found in different locations. Negative charges ensure an open (gel) structure of mucus etc. Lung extracellular matrix.

**S(-II) in Amino Acids**

There are 2 amino acids containing sulphur:
- Cysteine (cys): contains a free SH group
  - Can be involved in cross-linking with other cys residues to form intra and inter protein cross links
  - Can be important at active site of enzymes – as a nucleophile
  - Reactive with metal ions (Fe, Zn, Hg, Pb)
- Methionine (met): contains -S-CH\(_3\) group
  - Used as first amino acid in translation of ALL proteins
  - S may be replaced by Se (in small quantities)
  - S-CH\(_3\) bond reactive – can be used as methyl donor

**Formation of Disulphide Bridges**

\[
\text{SH} + \text{HS} + \frac{1}{2} \text{O}_2 \rightarrow \text{S-S} + \text{H}_2\text{O}
\]

- This occurs spontaneously in an oxidising environment. BUT NOT INSIDE ANIMAL CELLS which is a reducing environment.
- Disulphide bridges occur only in extracellular proteins e.g. blood proteins, connective tissue proteins, etc, or in some organelles.
- These disulphide bonds are formed during protein synthesis in the endoplasmic reticulum, which provides an oxidising environment.
- Forming the correct disulphide bonds is a problem during protein folding.

**Proteins Containing Intersubunit Disulphide Bridges**

- Keratin (hair)
- Immunoglobulin (antibody)
- Haemoglobin (an intracellular protein)

Subunits held together by non-covalent interactions.

**Sulfur is Used to Bind Metal Ions to Proteins**

Zinc finger

- Zn\(^{2+}\) binds to four cys-SH groups forming a rigid core

**Sulfur Based Metal Clusters Use S\(_2^–\) Ions**

- Ferredoxin: Each Fe held in place by 1cys
- Each also bound to 3 S\(^2–\) ions
- Rather like having a crystal of iron sulphide in the middle of a protein – very primitive?
- Fe\(_7\)Mo\(_5\)S\(_9\).homocitrate cofactor of nitrogenase (FeMoco).

Within the protein, the terminal Fe is coordinated by Cys and the Mo is coordinated by His.
CHEMISTRY OF –SH GROUP

- SH group can be deprotonated to act as a nucleophile.

\[
\text{RS}-\text{H} \quad \text{RS}^- + \text{H}^+ \\
\text{pK}_a \approx 8 \\
\text{S}^- \text{is a good nucleophile.}
\]

In a protein active site, other groups can help deprotonate Cys.

- SH group reacts like alcohols (e.g. with acids to give thioesters) but the products are less stable.

Thioesters are thermodynamically unstable, rapidly hydrolysed by water (cf. R-COO-Me which is stable).

If thioesters can be kept kinetically stable they could act as a temporary ‘energy store’

THIOESTERS PROVIDE A LINK BETWEEN OXIDATION AND HIGH ENERGY PHOSPHATE

Chemistry of the enzyme glyceraldehyde-3-phosphate dehydrogenase. Responsible for the first ATP made in glycolysis.

COENZYME A

CoA + Fatty acid + ATP → Fatty acyl CoA + AMP + PPi

• Coenzyme A is used as intracellular carrier for fatty acids
• Fatty acids are oxidised bound to CoA (acetyl CoA is an intermediate)
• Fatty acids can be transferred from CoA to acceptor (glycerol P; some proteins)

SULFUR ISOTOPES AND ATMOSPHERIC OXYGEN

• \(^{32}\text{S} - 95\% \quad ^{34}\text{S} - 4.2\% \quad ^{33}\text{S} - 0.75\%
• Normal chemical reactions discriminate against heavy isotopes.
• \(^{33}\text{S} / ^{34}\text{S}\) ratio constant in rocks back to \(2.5 \times 10^9\) years,
• Before this, ratio may be very high (or very low) in different rocks

Time series of deviations of \(^{34}\text{S}\) concentrations from the MFL

SULFUR ISOTOPES AND ATMOSPHERIC OXYGEN

• Deviation from mass fractionation can arise from non-chemical reactions (e.g. photolysis by UV light which produces free radicals).
• Conclude that more than 2.4 billion years, there were lots of photolytic reactions in atmosphere involving \(\text{H}_2\text{S}\) and \(\text{SO}_2\) – but after this, photolysis was prevented.
• \(\text{O}_3\) in atmosphere produces the ozone layer which filters out UV, i.e. \(\text{O}_3\) appeared in the atmosphere 2.4 billion years ago.
• Where did it come from? Photosynthetic cyanobacteria evolved 2.9 billion years ago, and produce oxygen.
• Is there a time anomaly??

CATALYTIC MECHANISM OF CYSTEINE PROTEASE

• Cysteine protease

Enzyme active site

Activated nucleophile

Unstable thioester intermediate

RAPAIN – protease from pineapple

Biological chemistry of the elements
Key points

- Sulphur in biology mainly found as S⁰ oxidation state.
- Occurs in the amino acids cysteine and methionine.
- Cysteine’s SH group can form disulphide bridges with cys on other parts of the same protein chain, or between protein chains.
- Cysteine’s SH group (and methionine S) is important in complexing to metal ions, e.g. Fe, Cu, Zn.
- Cysteine’s SH group can act as a key residue in enzyme active sites, e.g. as a nucleophile, participating in a thioester.
- The potential for oxidation of −SH groups is important in their function.
- S⁰ (as sulphate) is used in biological systems, normally as part of sulphated polysaccharides.