INTRODUCTION TO BIOLOGICAL CHEMISTRY OF THE ELEMENTS

Dr Mark R. Wormald

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

J.J.R. Fraústo da Silva and R.J.P. Williams (2001) “The Biological Chemistry of the Elements”, pub. Oxford University Press.

D.A. Harris (1996) in “Principles of Medical Biology: Cell Chemistry and Physiology, Part II”, ed Bittar & Bittar.

PERIODIC TABLE

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57 La*	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89 Ac~	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 ---	111 ---	112 ---		114 ---		116 ---		118 ---

Lanthanide Series*	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
Actinide Series~	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

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37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
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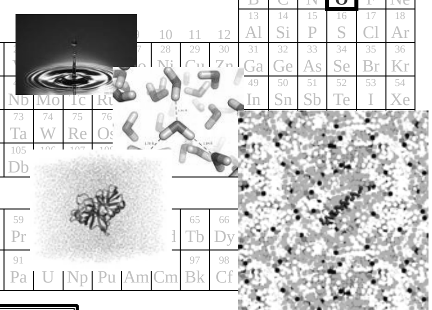
Water



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Organic molecules
Macromolecules

SCALE OF BIOCHEMISTRY - Distance

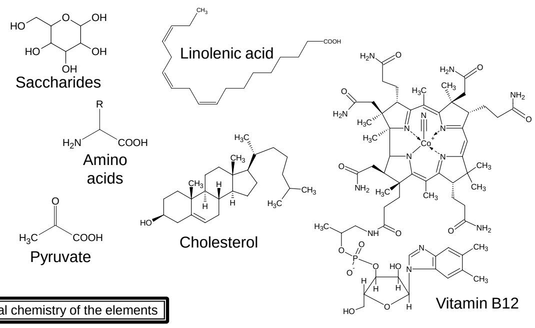
Atoms/bonds *0.05-0.5 nanometres (nm)*
Small molecules *0.2-1.0 nanometres (nm)*
(X-ray crystallography, spectroscopy)

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

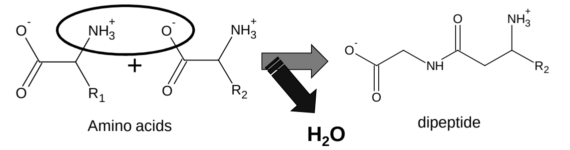
Cells *1000 – 100,000 nm (1-100 μm)*
(electron microscopy → light microscopy)

SMALL MOLECULES COME FROM THE ENVIRONMENT OR ARE BUILT

H^+
O_2
CO_2
HCO_3^-
NO



BIG (MACRO) MOLECULES ARE BUILT FROM SMALL MOLECULES



In aqueous environment, equilibrium well over to left

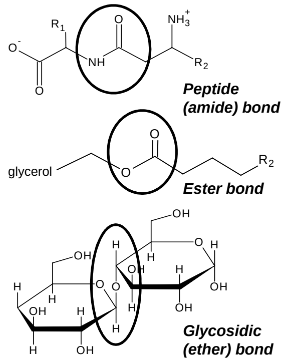
Dehydrating agent needed to drive to right
Dehydration = energy input

MONOMER LINKAGES ARE FORMED BY CONDENSATION REACTIONS

Amino acid + amino acid
$R_1NH_3^+ + R_2COO^-$ → H_2O

Fatty acids + glycerol
$R_1OH + R_2COO^-$ → H_2O

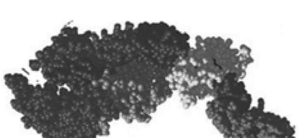
Sugar + sugar
$R_1OH + R_2OH$ → H_2O

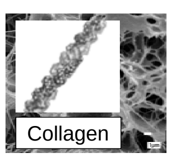


LIMITS ON MACROMOLECULE SIZE?

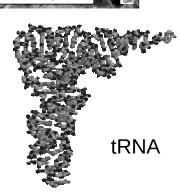
Macromolecule	Monomer	No. of residues
Protein	Amino acid	100-5,000
RNA	Nucleic acid	100-15,000
DNA	Nucleic acid	>10,000,000
Polysaccharide	Monosaccharide	10,000-50,000,000

MACROMOLECULE SIZE AND SHAPE
- big molecules


Myosin head


Collagen

Information retrieval is necessary to specify sequence (with the possibility of errors).


tRNA

LIMITS ON MACROMOLECULE SIZE?

Macromolecule	Monomer	No. of residues
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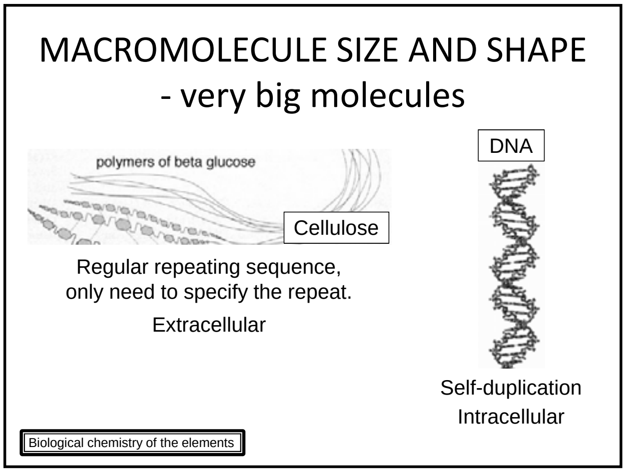
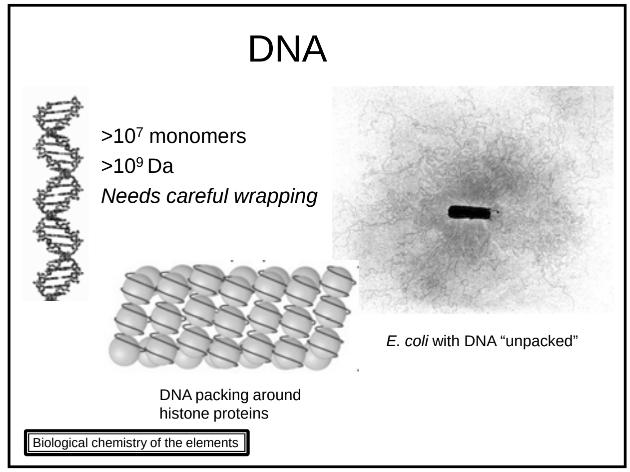
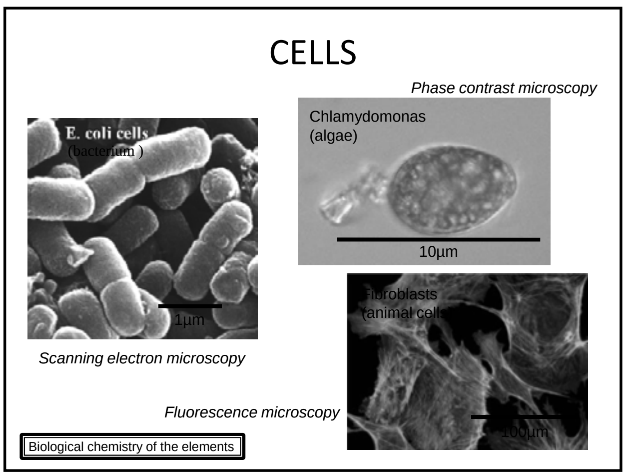
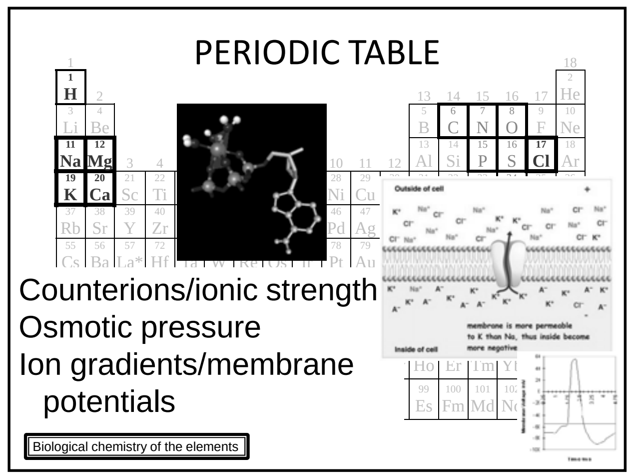
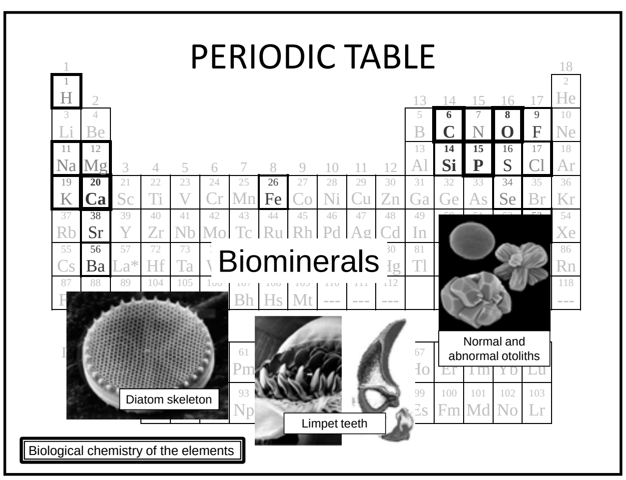
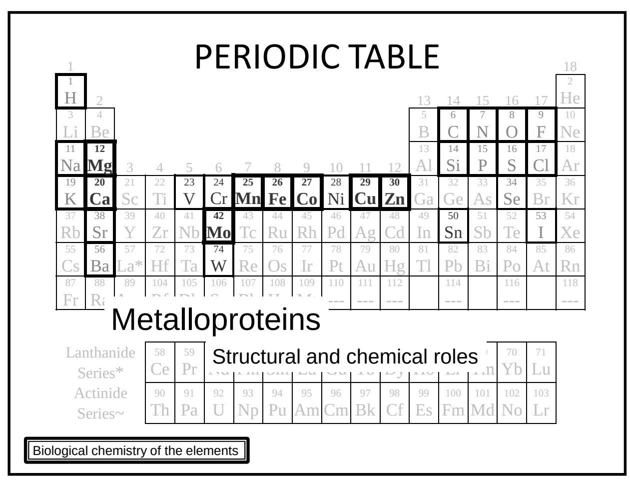

MACROMOLECULE SIZE AND SHAPE
- very big molecules
polymers of beta glucose
Cellulose
Regular repeating sequence,
only need to specify the repeat.
Extracellular
DNA
Self-duplication
Intracellular
Biological chemistry of the elements


DNA
>10^7 monomers
>10^9 Da
Needs careful wrapping
E. coli with DNA "unpacked"
DNA packing around
histone proteins
Biological chemistry of the elements


CELLS
Phase contrast microscopy
E. coli cells
(bacterium)
Chlamydomonas
(algae)
10µm
1µm
Fibroblasts
(animal cells)
Scanning electron microscopy
Fluorescence microscopy
100µm
Biological chemistry of the elements


PERIODIC TABLE
Counterions/ionic strength
Osmotic pressure
Ion gradients/membrane
potentials
Biological chemistry of the elements

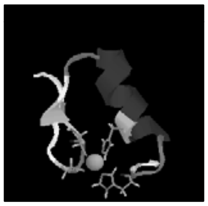

PERIODIC TABLE
Biominerals
Normal and
abnormal otoliths
Diatom skeleton
Limpet teeth
Biological chemistry of the elements

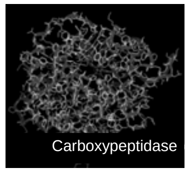

PERIODIC TABLE
Metalloproteins
Structural and chemical roles
Lanthanide
Series*
Actinide
Series~
Biological chemistry of the elements

NON-TRANSITION METALS

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

Bind strongly to proteins
structural + signalling





Polarise ligands
enzyme active sites

TRANSITION METALS

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

Fe : $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^6} 4s^2$

Cu : $1s^2 2s^2 2p^6 3s^2 3p^6 \mathbf{3d^9} 4s^2$

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:

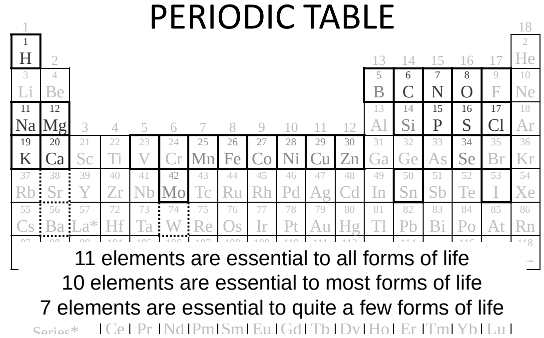
Electron transfer (e.g. cytochrome c)

Transport oxygen (e.g. haemoglobin)

Redox catalyst (e.g. tyrosinase)

Regulation (e.g. aconitase)

PERIODIC TABLE

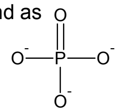


11 elements are essential to all forms of life
10 elements are essential to most forms of life
7 elements are essential to quite a few forms of life

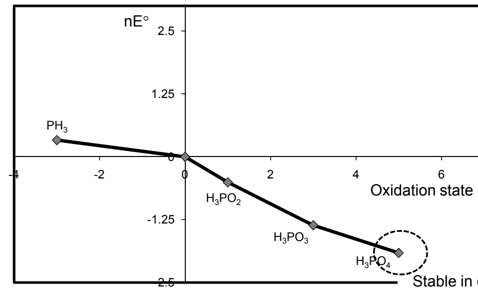
~20 elements in the human body with a known role
(cf. 42 in a mobile phone)

PHOSPHORUS – GROUP V ELEMENT

- $1s^2 2s^2 2p^6 3s^2 3p^3$
  3 unpaired *p* electrons
- Valence -3 to +5
- Covalent bonding
- Readily oxidised in air
- Normally found as phosphate

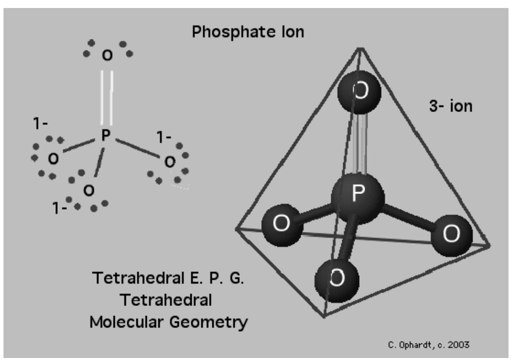


REDOX PROPERTIES OF PHOSPHORUS

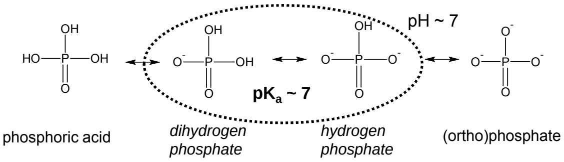


Stable in oxidising and non-oxidising conditions (phosphate)

GEOMETRY OF PHOSPHATE



PHOSPHATE AS BUFFER ION



(inorganic) phosphate often written as
Pi

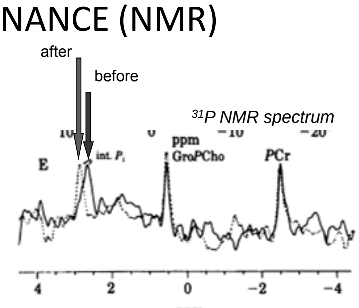
Phosphate is an important buffer in plasma (1 mmol/l) and cells (10 mmol/l)

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

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



INSOLUBLE CALCIUM PHOSPHATE IS THE BASIS OF BONE AND TOOTH ENAMEL

$$10\,Ca^{2+}_{(aq)} + 6\,PO_4{}^{3-}{}_{(aq)} + 2\,OH^-_{(aq)} \rightarrow Ca_{10}(PO_4)_6(OH)_{2\,(s)}$$

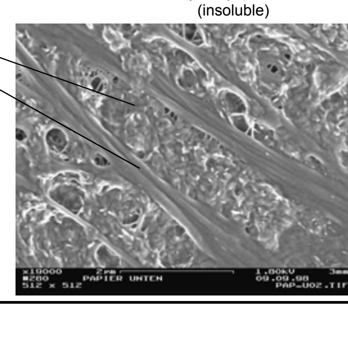
hydroxyapatite (insoluble)

Hydroxyapatite matrix

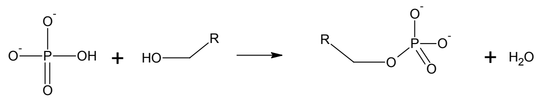
Protein fibres (collagen)

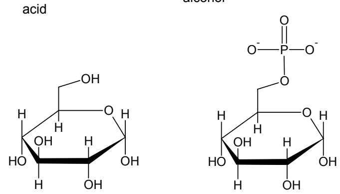
Replacement of OH^- with F^- gives more resilient compound

Must avoid Ca^{2+} in solution where phosphate occurs



CHEMISTRY OF PHOSPHORIC ACID



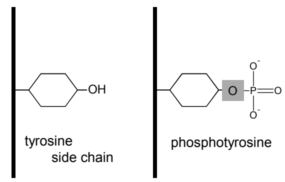


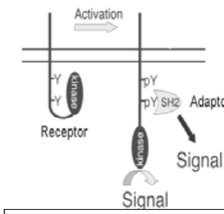
2 –ve charges added to glucose.
Prevents glucose from crossing cell membrane, i.e. keeps glucose inside cell.

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)



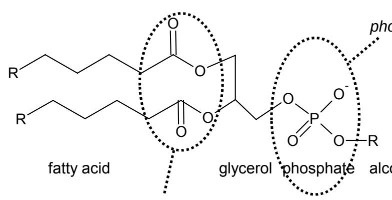


phosphorylation of insulin receptor switches it on

Introduces high local concentration of –ve charge

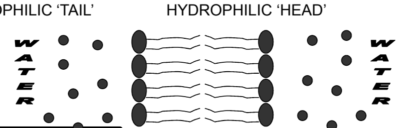
See also glycogen synthase (-)/phosphorylase (+)

OTHER PHOSPHATE ESTERS

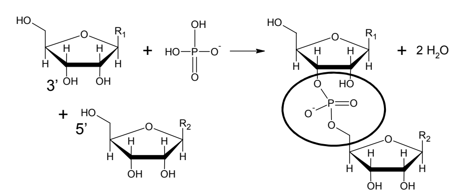


phospholipid

LIPOPHILIC 'TAIL' HYDROPHILIC 'HEAD'



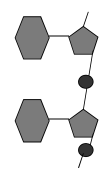
DNA/RNA HAVE A PHOSPHODIESTER 'BACKBONE'



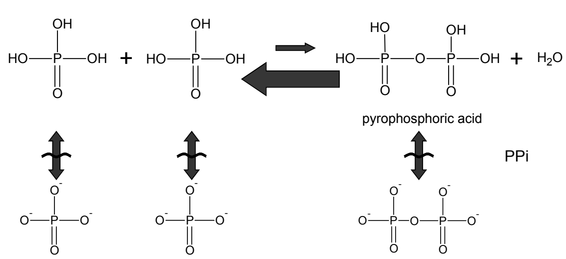
Each additional phosphate adds an additional –ve charge.

Charge α length

Useful in separating DNA by ELECTROPHORESIS



PHOSPHORIC ACID ANHYDRIDES

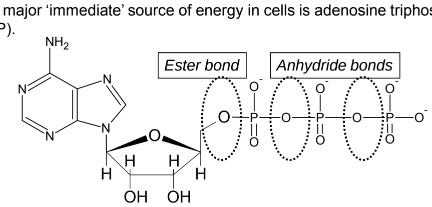


Acid anhydrides in water are thermodynamically unstable i.e. hydrolysis will yield a lot of energy (~ 40kJ/mol).

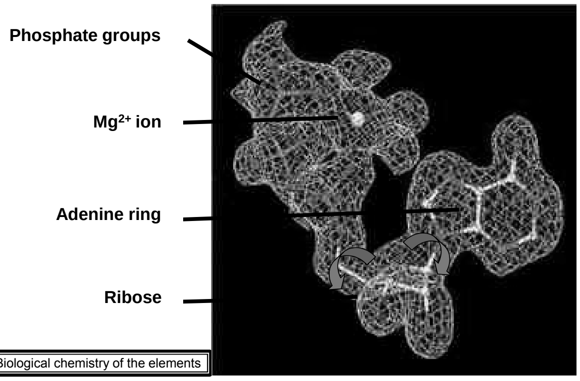
Hydrolysis of pyrophosphates are very slow at room temperature 'kinetically stable'.
Therefore they can act as a 'store of energy'.

PYROPHOSPHATES AND ENERGY

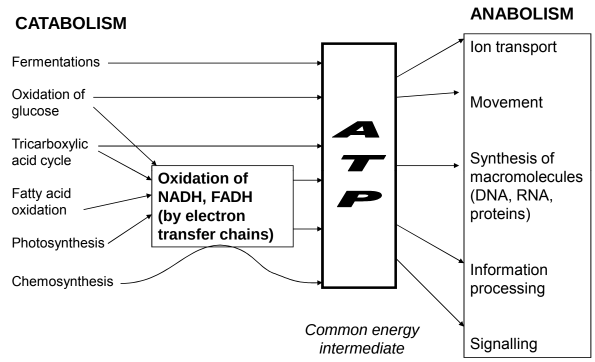
- Pyrophosphate is used by some organisms as an immediate energy source (e.g. to drive ion pumps in the purple bacterium *R. rubrum*).
- The major 'immediate' source of energy in cells is adenosine triphosphate (ATP).



3D STRUCTURE OF ATP



ATP AS AN 'ENERGY CURRENCY'

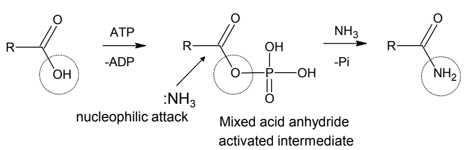


ATP PROVIDES ENERGY BY GROUP TRANSFER, RARELY BY HYDROLYSIS

Glutamine synthetase

Overall reaction:

Glutamate + NH_3 + ATP → glutamine + ADP + P_i



ATP PROVIDES ENERGY BY GROUP TRANSFER, RARELY BY HYDROLYSIS

Aminoacyl-tRNA synthetase

Overall reaction:

amino acid + tRNA + ATP → aminoacyl-tRNA + AMP + PP_i

Step 1: amino acid activation

amino acid + ATP → aminoacyl-AMP + PP_i

Step 2: transfer to tRNA

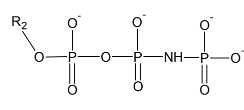
aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP

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)

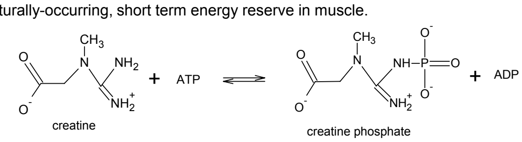
Is adenine just a 'handle'?
cf. NADH, $FADH_2$

N-P BONDS



Adenylyl imidodiphosphate
Artificial enzyme inhibitor for ATP-utilising enzymes.

Creatine phosphate (PCr)
Naturally-occurring, short term energy reserve in muscle.

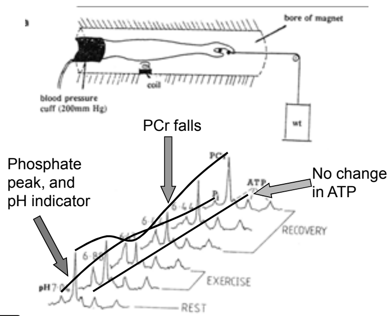


MEASUREMENT OF ATP AND PCr BY ^{31}P NMR

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.



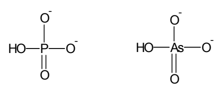
ATP IS TIGHTLY REGULATED

- so eating it does you no good

Base de crètaine

TOXICITY OF ARSENATE

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



P_i **As_i**

ADP + P_i → ATP (→ ADP + P_i)

Thermodynamically unstable
Kinetically stable
Half life > 7 days

ADP + As_i → ADP~As → ADP + As_i

Thermodynamically unstable
Kinetically unstable
Half life about 7 sec

Production of heat but no energy stored

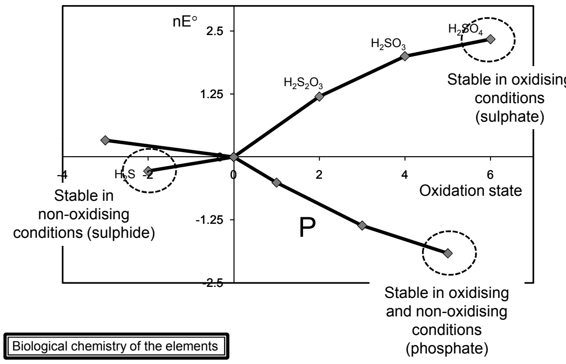
KEY FACTS

- 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 (PP_i, 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.

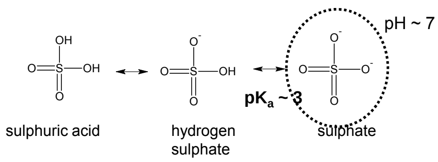
SULPHUR – GROUP VI ELEMENT

- $1s^2 2s^2 2p^6 3s^2 3p^4$
  2 unpaired *p* electrons
- Valence -2 to +6
- Covalent bonding
- *Can be found free in nature as element (S^0)*
- *In biology often found as RSH (S^{-II}) and sometimes as sulphate (S^{VI})*

REDOX PROPERTIES OF SULPHUR

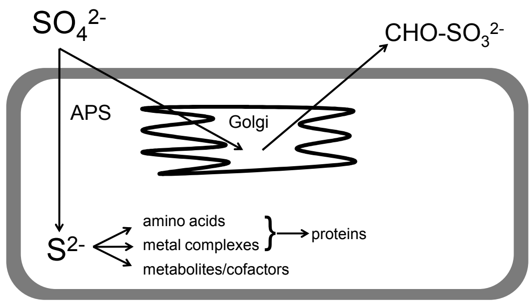


SULPHATE IS A PERMANENT DIANION AT PHYSIOLOGICAL pH



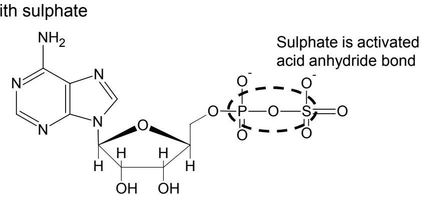
Sulphate is not a buffer at physiological pH values, and is not found at significant concentrations in biological fluids.

HANDLING SULPHATE IN CELLS



ADENOSINE PHOSPHOSULPHATE

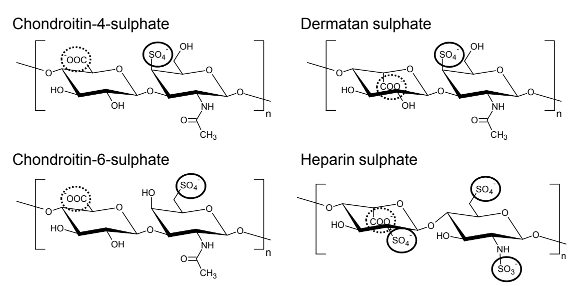
Adenosine phosphosulphate (APS) is formed by the condensation of ATP with sulphate



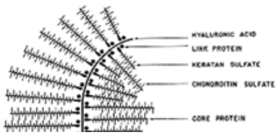
Sulphate is activated in an acid anhydride bond

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

SULPHATED POLYSACCHARIDES OCCUR IN CONNECTIVE TISSUE

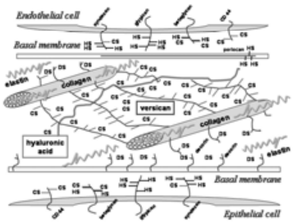


PROTEOGLYCANS FROM CONNECTIVE TISSUE



Negative charges ensure open (gel) structure of mucus etc.

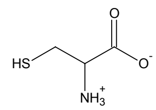
Different polymers have different physical properties, and are found in different locations



Lung extra-cellular matrix

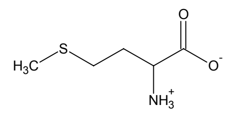
S(-II) IN AMINO ACIDS

There are 2 amino acids containing sulphur



Cysteine (cys) - contains a free SH group

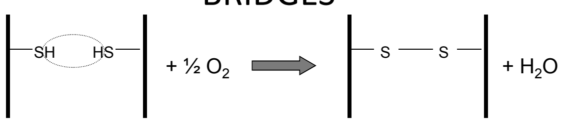
- Can be involved in cross-linking with other cys residues to form intra and inter protein cross links
- Can be important at active site of enzymes – as a nucleophile
- Reactive with metal ions (Fe, Zn, Hg, Pb)



Methionine (met) – contains -S-CH_3 group

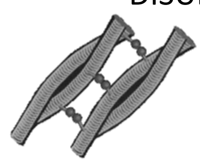
- Used as first amino acid in translation of ALL proteins
- S may be replaced by Se (in small quantities)
- S-CH_3 bond reactive – can be used as methyl donor

FORMATION OF DISULPHIDE BRIDGES

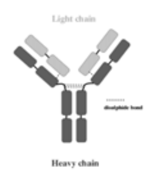


- This occurs *spontaneously* in an oxidising environment, BUT NOT INSIDE ANIMAL CELLS which is a reducing environment.
- Disulphide bridges occur only in *extracellular proteins* e.g. blood proteins, connective tissue proteins, etc, or in some organelles.
- These disulphide bonds are formed during protein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

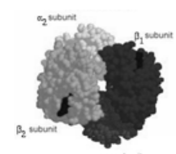
PROTEINS CONTAINING INTERSUBUNIT DISULPHIDE BRIDGES



Keratin (hair)



Immunoglobin (antibody)

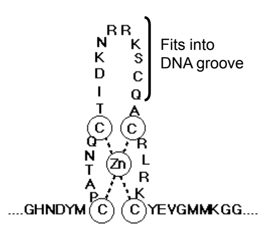


Haemoglobin

(an intracellular protein)

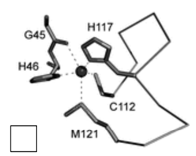
Subunits held together by non-covalent interactions

SULPHUR IS USED TO BIND METAL IONS TO PROTEINS

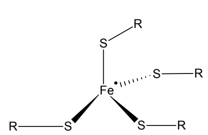


Zinc finger

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

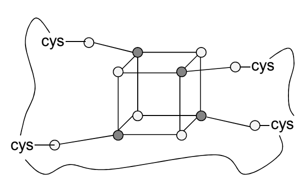


Active site of azurin (Cu)



Rubredoxin ($Fe(Cys)_4$)

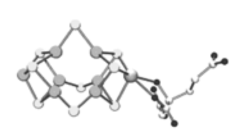
SULPHUR BASED METAL CLUSTERS USE S^{2-} IONS



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_7MoS_9.homocitrate cofactor of nitrogenase (FeMoco).

Within the protein, the terminal Fe is coordinated by Cys and the Mo is coordianted by His.

CHEMISTRY OF –SH GROUP

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

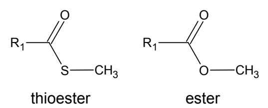
$$R\text{—}SH \xrightarrow{pK_a \sim 8} R\text{—}S^- + H^+$$

(cf. pK_a of R-OH is ~ 16)

S^- is a good nucleophile.

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

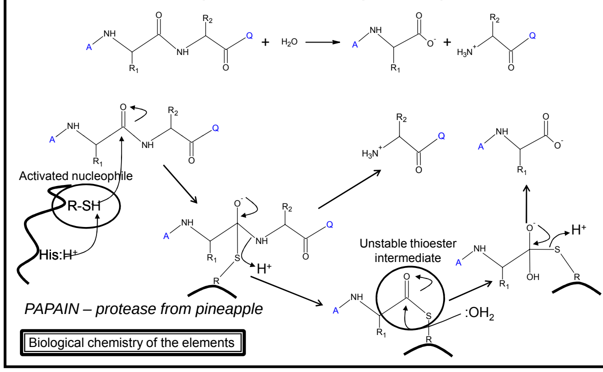
-SH group reacts like alcohols (e.g. with acids to give thioesters) but the products are less stable.



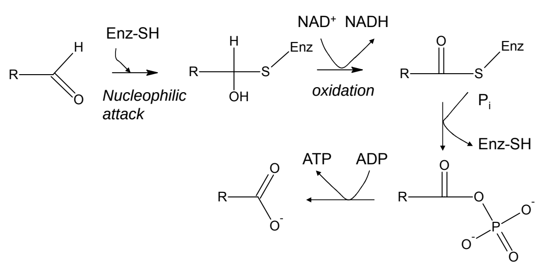
Thioesters are thermodynamically unstable, rapidly hydrolysed by water (cf. R-COO-Me which is stable).

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

CATALYTIC MECHANISM OF CYSTEINE PROTEASE

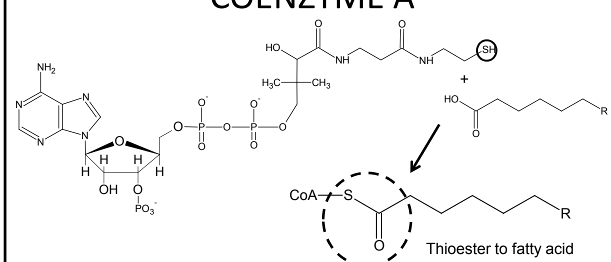


THIOESTERS PROVIDE A LINK BETWEEN OXIDATION AND HIGH ENERGY PHOSPHATE



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

COENZYME A



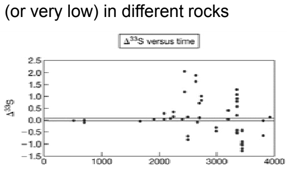
CoA + Fatty acid + ATP → Fatty acyl CoA + AMP + PP_i

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

Time series of deviations of ^{33}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 H_2S and SO_2 – but after this, photolysis was prevented.
- O_2 in atmosphere produces the ozone layer which filters out UV, i.e. O_2 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??*

Key points

- Sulphur in biology mainly found as S^{-II} 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.
- S^{VI} (as sulphate) is used in biological systems, normally as part of sulphated polysaccharides.