Phosphorus - the Giver of Life

G. Michael Blackburn

Krebs Institute, Sheffield University

Department of Molecular Biology and Biotechnology

M. Bowler et al., New J Chem., 2010, 34, 784
“What really interests me is whether God could have created the world any differently; in other words, whether the demand for logical simplicity leaves any freedom at all.”

Albert Einstein

“How there’s life, there’s phosphorus”

Sir Alexander Todd, Kyoto Lecture, 1982

Life on earth depends on Phosphorus: uniquely!

Global Issues
1. The variety of roles of phosphate in life: are they endless?
2. How did phosphorus get to earth? Are we a lucky Planet?
3. Morocco’s centrality for phosphate and its global future – sustainable or not?

Molecular Issues
4. The paradox: extreme structural stability vs. functional reactivity
5. Is there any alternative to phosphate?
6. How has protein crystallography resolved this central paradox of life?
1. Multiple Major Roles for Phosphate in Life

**The RNA World** - life likely started with RNA preceding DNA and proteins - for both genome and catalysts

**Genome stability** - DNA structure demands ultra-stable phosphate diesters - and repair processes on demand for survival

**Lipids** - cell membranes utilise phospholipids as essential stable components

**Skeletal structure** - calcium phosphate (apatite) as a key mineral component

**Compartmentalisation** – ionised phosphate esters are unable to cross membranes and can be trafficked

**Energetics** - “Energy-rich phosphates” especially Adenosine Triphosphate [ATP]

**Organelle membrane recognition** - phosphoinositols

**Signalling** – most catalytic proteins become phosphorylated by Kinases to modulate their activity and second messengers, cAMP and cGMP, are vital for cell signalling

**GTP hydrolysis** is the core of cell signalling in man (30 s⁻¹)
2. Galactic & Earth Origin of Phosphorus

- Needs nuclear explosion of a supernova, SNe
- Supernova must be $\geq 8$ x bigger than our Sun
- 10% of stars in Milky Way big enough for SNe

- SNe ends with production of Fe and Ni, as further nuclear reactions of these elements are endothermic
- Hence galactic phosphorus ends up as $\text{Fe}_3\text{P}$ and $\text{Ni}_3\text{P}$

\[ 16\text{O} + 16\text{O} \rightarrow 31\text{P} + p + 7.7 \text{ MeV} \]
\[ 28\text{Se} + 4\text{He} \rightarrow 31\text{P} + p \]
\[ 32\text{S} + \gamma \rightarrow 31\text{P} + n \]
\[ 27\text{Al} + 4\text{He} \rightarrow 31\text{P} \]

Cassiopeia A Supernova remnant

![Cassiopeia A Supernova remnant](image)

![Diagram of P, N, O, S, H abundances](image)

- Human Body P elevated $10^5$
- Relative Abundance (normalized to Si)
- Solar
- Comets/meteors

3. Use of Phosphate and its Future

Increase in European Phosphate Imports

The 28 member countries of the EU have less than 1% of the production capacity and less than 1% of the reserves of world phosphate rock.

World phosphate production

World rock phosphate production vs world population

World population
3B. World use of Phosphate and its Bleak Future

- Phosphate rock, like oil, is a non-renewable resource.
- Approx. 50-100 years remain of high quality reserves.
- Phosphate rock production will peak c.a 2030.
- After 2035, demand will exceed supply.

(Dr. Diana Cordell, Sydney Tech.Uni)

‘Life can multiply until all the phosphorus is gone, and then there is an inexorable halt which nothing can prevent. We may be able to substitute nuclear power for coal, and plastics for wood, and yeast for meat, and friendliness for isolation – but for phosphorus there is neither substitute nor replacement’. Isaac Asimov “Life’s Bottleneck” 1974
4. Phosphate – Beauty in the Eye of the Beholder!

Benguérir phosphate mine morocco

K N P fertilizer

Crops with (left) and without (right) fertilizer

Triple Phosphate, $\text{NH}_4^+\text{Mg}^{2+}\text{PO}_4^{3-}$

Phosphoric Acid

Phosphate Triester
uncharged, unstable

Phosphate Diester

Phosphate Monoester monoanion
dianion

Dialkyl Pyrophosphate: Coenzymes NADH, CoA

Alkyl Triphosphate: ATP, GTP, dATP, dCTP etc.
Part 2. How did Evolution select Phosphorus for its Multiple Roles in Life?

4. Does Stability of its Mono- and Di-Esters make it Unique?

5. Is there an Alternative Element to Phosphorus for Life?

6. How has Evolution achieved the necessary Dynamics for its Multiple uses of Phosphate Esters?
4. The Central Paradox of Phosphate Esters: Stability vs Mobility

How good are Enzymes for the Hydrolysis of Phosphate Esters?

Enzymatic Hydrolysis of Phosphate Mono-esters in Water at 37 °C

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_{\text{cat}}$ s$^{-1}$</th>
<th>$k_{\text{cat}}/K_m$ M$^{-1}$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI</td>
<td>Phosphorylase Pase-α</td>
<td>39</td>
<td>4 x 10$^6$</td>
</tr>
<tr>
<td>FBPase</td>
<td>Fructose 1,6-BP</td>
<td>21</td>
<td>1.5 x 10$^7$</td>
</tr>
<tr>
<td>IP</td>
<td>Inositol 1-P</td>
<td>22</td>
<td>3 x 10$^5$</td>
</tr>
<tr>
<td>No Enz</td>
<td>Methyl Phosphate</td>
<td>2 x 10$^{-20}$ (k$_{\text{uncat}}$)</td>
<td></td>
</tr>
</tbody>
</table>

Thus, $k_{\text{cat}}/k_{\text{uncat}} = 10^{21}$

Uncatalysed reactions $t_{1/2}$ in water at 25 °C

- MeOPO$_3^-$ hydrolysis $10^{12}$ years
- (RO)$_2$PO$_2^-$ hydrolysis $10^8$ years
- Peptide hydrolysis 450 years
- Triose phosphate isomerisation 2 days
- CO$_2$ hydration 5 sec

Lad, C; Williams, N.H.; Wolfenden, R.; PNAS, 2003, 100, 5607-10
4. Stability - There is No Alternative to Phosphate Di- and Mono-Esters

<table>
<thead>
<tr>
<th>Element</th>
<th>Oxyacid</th>
<th>Abundance</th>
<th>( pK_{a1} )</th>
<th>( pK_{a2} )</th>
<th>( pK_{a3} )</th>
<th>Diester ( t_{1/2} ) y</th>
<th>Diester Charge</th>
<th>Bond Cleaved</th>
<th>Monoester ( t_{1/2} ) y</th>
<th>Monoester Charge</th>
<th>Bond Cleaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>( \text{H}_3\text{PO}_4 )</td>
<td>Low</td>
<td>2.1</td>
<td>7.2</td>
<td>13.1</td>
<td>10^8 y</td>
<td>1 -ve</td>
<td>P–O</td>
<td>10^{12} y</td>
<td>2 –ve</td>
<td>P–O</td>
</tr>
<tr>
<td>S</td>
<td>( \text{H}_2\text{SO}_4 )</td>
<td>Medium</td>
<td>&gt;0</td>
<td>2.0</td>
<td>–</td>
<td>1.7 h</td>
<td>0</td>
<td>C–O</td>
<td>1100 y</td>
<td>1 –ve</td>
<td>C–O</td>
</tr>
<tr>
<td>As</td>
<td>( \text{H}_3\text{AsO}_4 )</td>
<td>Medium</td>
<td>2.2</td>
<td>7.0</td>
<td>11.5</td>
<td>&lt;2min</td>
<td>1 –ve</td>
<td>As–O</td>
<td>6 min</td>
<td>2 –ve</td>
<td>As–O</td>
</tr>
<tr>
<td>Si</td>
<td>( \text{H}_4\text{SiO}_4 )</td>
<td>High</td>
<td>9.5</td>
<td>&gt;13</td>
<td>–</td>
<td>&lt;1min</td>
<td>0</td>
<td>Si–O</td>
<td>&lt; 1 min</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

- **Dialkyl sulfates**: non-ionic, excellent alkylating agents, totally unfitted for a structural role in biology

- **Monoalkyl sulfates**: anionic, used in biology for compartmentalization, lipid structure, signaling etc. BUT too high energy of formation (it takes 2 ATP to make one sulfate ester). (Richard Wolfenden & Yang Yuan, PNAS, 2006).

- Similarities between arsenic acid and phosphoric acid raise the possibility that arsenate can replace the phosphate in biological molecules such as the hexose phosphates and adenosine triphosphate. Condensed arsenates such as \( \text{K}_4\text{As}_2\text{O}_7 \) are known in the solid state. BUT, the As–O–As in these compounds is extremely unstable in water. Thus, species containing an arsenic-oxygen-arsenic group cannot be present in aqueous media. (Westheimer, Science 1987).

- **Neutral esters of arsenic** acid such triorganyl arsenate can only be made in non-aqueous conditions. The As–O–C bond is unstable in water.

- **Mono- and diesters of arsenic acid have never been isolated** and arsenic acid esters are very much more easily hydrolyzed than phosphoric acid esters.

- **Silicic acid** is more abundant in nature than phosphoric acid and is tetravalent. It is non-viable because its esters hydrolyze far too rapidly to survive. Silicic acid is also too weak an acid: \( pK_{a1} \sim 9.5 \). Moreover, its diesters cannot ionize in neutral solution to carry a negative charge.
6. What makes Phosphate Esters so Stable?

Alexander Todd’s appraisal of the suitability of phosphate
“Let’s try to summarise what Nature needs to facilitate all she has to do with carbon-based building bricks.
1) She needs a strong acid capable of forming anhydrides which can be used for energy storage and transport.
2) The acid must be tribasic so that it may act as a link between two molecules of groups and still have one free acidic hydroxyl for further reaction.
3) The strength of the acid is important since it permits use in carbon-carbon bond synthesis.

Frank Westheimer’s more perceptive line
“Phosphoric acid is specially adapted for its role in nucleic acids because it can link two nucleotides and still ionize; the resulting negative charge serves both to stabilize the diesters against hydrolysis and to retain the molecules within a lipid membrane…. Phosphates with multiple negative charges can react by way of the monomeric metaphosphate ion, \( \text{PO}_3^- \), as an intermediate.

No other residue appears to fulfil the multiple roles of phosphate in biochemistry.”

6. How has Evolution achieved the Necessary Dynamics?

**Five Fundamentals** in Catalysis of Phosphoryl Group Transfer (PO$_3$)$_-$

1. Optimize Geometry – “In-Line” Phosphoryl Transfer

2. Charge Balance – Neutralize the “Anionic Shield”

3. Desolvation – Activate the nucleophile and the electrophile

4. Bring the Reactants close together – “Associative Mechanism”
6.1 “In-Line” Attack of Nucleophile is Universal in Biology

In the natural state, A = B = C = oxygen-16, so the phosphorus atom is Pro-Pro-chiral. Make the phosphorus chiral and study the stereochemistry of the reaction:

Make A = oxygen-18, B = oxygen-17, C = oxygen-16 (Knowles, Lowe, others)

RESULTS: **Inverting Enzymes** (Hexokinase, Glycerol kinase, all kinases) use one-step phosphoryl transfer

**“Retaining Enzymes”:**
- Phosphoglycerate mutase (histidine)
- Alkaline phosphatase (serine)
- β-Phosphoglucomutase (aspartate)
- Tyrosine phosphatase (cysteine)

use two-step phosphoryl transfer.

**NB** Retention of configuration at phosphorus always means a 2-step reaction, both steps being inversion.

Dynamic” Phosphate aligned by 8 hydrogen bonds “and Mg coordination
6.2 “In-Line” Attack is manifest in Protein Crystallography

**Pentacovalent Phosphorus Intermediate of a Phosphoryl Transfer Reaction,**

**RhoA.RhoGAP-GDP-MgF$_3^-$**

**hPGK-MgF$_3^-$**

**UvrD-DNA-ADP-MgF$_3^-$**
6.4 Multiple “In-Line” Attack in Metal Fluoride Complexes

Tetrafluoroaluminate Anion, $\text{AlF}_4^-$

102 Examples in the PDB. All octahedral with 2 apical oxygen ligands. (1995 - date)

RhoA-GTPase
Rittinger K, et al., Nature 1997, 1.68 Å

Beryllium Trifluoride Anion, $\text{BeF}_3^-$

111 Examples in the PDB. All tetrahedral mimics of stable phosphate dianion (1995-date).

Myosin.ADP.BeF₃
Rayment I. et al., Biochemistry 1995, 2.0Å

Aluminum Trifluoride, $\text{AlF}_3^0$

The $\text{AlF}_3^0$ Structure initially assigned for phosphoserine phosphatase is, in fact, $\text{MgF}_3^-$

52 PDB entries for tbp AF3 complexes. We have shown 5 are MGF using $^{19}\text{F}$ NMR. Another has been disproved by Al-free crystallisation. All 46 remaining are highly suspect. All are really MGF.
6.5. Charge Balance is used to overcome the Anionic Shield

Adenosine 5’-triphosphate has to "overcome anionic repulsion of 4 -ve vs 2 -ve charges. Yet it achieves a rate acceleration of 10^12!"

Charge Balance sums the Pauling Atomic charges inside a sphere of radius \( r \) centered on phosphorus.

Charge balance for cAPK, calculated as MgF\(_3^−\). Neutral to 13.5 Å

**6.8. Bring the Reactants close together – “Associative Mechanism”**

**Association and Dissociation** described in terms of (a) Bond Lengths or (b) Bond Orders. As the connection is an exponential function (Linus Pauling) they offer very different interpretations of the same situation.

In **Bond Length** terms:
- In a **Dissociative process** (upper) the AO--P bond substantially lengthens before the P—OB bond forms;
- A **Concerted process** has bond making synchronous with bond breaking.
- In an **Associative process** (lower) there is P-OB bond forming in advance of AO—P bond breaking.

The question is: “How to Bring the Reactants close together – against the anionic shield?”

---

β-Phosphoglucomutase (βPGM) as a Working Example

βPGM is a classical small molecule mutase whose task is to interconvert β-D-Glucose 1-Phosphate (βG1P) into D-Glucose 6-Phosphate (G6P). The enzyme has two domains that undergo extensive opening and closing motions during the catalytic cycle.
β-Phosphoglucomutase (βPGM) 2-Step Mechanism Resolved by Synthetic Analogues

βPGM is a classical small molecule mutase whose task is to interconvert β-D-Glucose 1-Phosphate (βG1P) into D-Glucose 6-Phosphate (G6P)

- Substitute these labile oxygens by carbons

![Diagram of βPGM enzyme with substrate and products]

- S-Diastereoisomer binds >100 better than R-epimer


- MgF$_3^-$ is isosteric and isoelectronic with the phosphoryl group, PO$_3^-$.
  Structure by $^{19}$F NMR and x-ray of protein complex.
CONCLUSIONS

1. The unique properties of Phosphate are:
   (a) Highly stable diesters
   (b) Ultra-stable monoesters
   (c) Water solubility
   (d) Anionic character for mono-and di-esters
   (e) Acceptable energy of formation of R–O–P bonds
   (f) Significant release of energy on hydrolysis of P–O–P bonds
   (g) Adequate elemental availability throughout the Universe through SNe

2. No other element has esters of its oxyacids with the required stability, anionic character, and water-solubility to support life / and low pK_a. Sulfates, arsenates, and silicates all fail to meet these requirements.

3. In magnesium, phosphate anions have found the perfect complement to modulate their behaviour.

4. Evolution has found a surprisingly easy route to catalyse phosphoryl group transfer – by overcoming the “Anionic Shield” with complementary charge in the transition state.

5. Protein crystallography has revealed ground state, transition state analogue, and product complexes for phosphoryl transfer reactions that have enormously advanced our understanding of how enzymes work.

6. It thus seems inevitable that Wherever there is Life in Our Universe, there will be Phosphate; its esters and anhydrides fulfilling comparable roles in structure, signalling, and energetics.
All Thanks Go To

Debabrata Bhattasali (Dalhousie)
Nicky Baxter
David Berkowitz (Nebraska)
Wolfgang Bermel (Bruker)
Matt Bowler (EMBL)
Matt Cliff (Manchester)
Jo Griffin
Andrea Hounslow

David Jakeman (Dalhousie)
Yi Jin (York)
Xiao-Xia Liu (Dallas)
Erika Pellegrini (EMBL)
Jon Waltho
Edwin Webster (Memphis)
Mike Williamson