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Chemistry and mechanism of phosphatases, diesterases and triesterases

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In 1987 the late Frank Westheimer wrote an article for Science magazine entitled, “Why Nature Chose Phosphates” [1]. Westheimer summarized the unique chemistry of phosphate esters and anhydrides that uniquely suited them for roles in many aspects of biology, from highly stable nucleic acids, to energy rich molecules like ATP, and a host of phosphorylated intermediate metabolites and small molecules. At that time the ubiquitous role of phosphorylation in regulation, mediated by the synergistic activities of kinases and phosphatases, was not yet appreciated. The remarkable stability of phosphate esters makes them well suited for their roles in biology.

Physical organic chemists have shown that the hydrolysis of phosphate monoesters, particularly the dianions that are the substrates of most phosphatases, is one of the slowest uncatalyzed reactions of biological relevance, despite being thermodynamically favorable [2]. As a result, phosphatases are some of the most catalytically proficient enzymes known, in terms of enzymatic rates relative to the corresponding uncatalyzed reaction. Considerable study has been devoted to discerning the mechanisms by which phosphate esters react both in solution, and by enzymes. The results have been periodically summarized in reviews as knowledge has advanced in recent decades [3–9]. The more recent reviews give comprehensive summaries of our current understanding of the mechanisms of phosphoryl transfer, how enzymes catalyze these reactions, and of the mechanistic tools used to answer these questions. The 2011 review by Lassila et al. does a particularly good job of explaining the central questions in the field and the methodologies used in mechanistic analysis, in a way that is accessible to non-specialists, while also exhaustively reviewing and discussing a very large body of experimental data [9]. The articles in this special section describe and analyze recent developments in uncatalyzed reactions, as well as enzymatic reactions of phosphatases, phosphodiesterases, and phosphotriesterases.

Some of the most interesting recent work in the phosphatase field has explored the ability of these enzymes to catalyze the hydrolysis of different, but related, substrates. The phenomenon of catalytic promiscuity has been increasingly observed and reported in the literature, and phosphatases are proving to be some of the most adept hydrolases exhibiting this property. The article by Mohamed and Hollfelder points out that while catalytic promiscuity was once thought to be little more than a curiosity, it is now recognized as a means for the evolution of new enzymatic activities. Many phosphatases exhibit promiscuity, and it is not yet clear what factors are the crucial ones that endow some enzymes, but not

others, with this property. Their article, “Efficient, crosswise catalytic promiscuity among enzymes that catalyze phosphoryl transfer,” reviews the current state of known promiscuity among these enzymes, some of which exhibit rate accelerations of six to seventeen orders of magnitude for both their native and promiscuous reactions.

The second article examines promiscuity by a particular enzyme: “Promiscuity comes at a price: Catalytic versatility vs. efficiency in different metal ion derivatives of the potential bioremediator GpdQ,” by Daumann et al. Nominally a diesterase, GpdQ is highly tolerant of replacements at its dinuclear metal center, which affect not only its catalytic efficiency, but may have mechanistic consequences as well.

The next article continues the bioremediation theme. “Development of metal-ion containing catalysts for the decomposition of phosphorothioate esters” by Brown and Edwards describes metal-based catalysts that catalyze the decomposition of phosphorothioate triesters. Such compounds are in widespread use as pesticides in agriculture, and related compounds were developed as chemical weapons. The safe destruction of these compounds is a topic of current environmental interest. Their article describes the energetics involved and the mechanisms by which these catalytic species work, with special consideration given to the role of the metal ions.

Given the fact that phosphotriesters do not occur naturally and were introduced into the environment only in the mid-20th century, it is remarkable that a class of enzymes has been identified that efficiently hydrolyzes them. Phosphotriesterases have been identified in several bacteria, as well as in squid, and in mammals. Some of these enzymes may have arisen from a promiscuous activity of lactonases. The article “Catalytic mechanisms for phosphotriesterases” by Raushel and Bigley describes the common features, and the differences, of known phosphotriesterases. Their article considers both structural details and common features of the catalytic mechanisms among these enzymes, which together can give insights into their possible evolutionary origin.

New insights into the uncatalyzed reactions of phosphotriesters are the subject of the next article, “New light on phosphate transfer from triesters,” by Nome, Kirby, and Mora. These authors use some of the classical tools of physical organic chemistry to probe the title reactions, but with a twist. Traditionally, linear free energy relationships, one of the classic tools for mechanistic analysis, focus on the effect the entering and leaving groups have on the reaction rate. These researchers have shown that reactivity in phosphotriesters also depends strongly on the non-leaving groups (the two “spectator” substituents on the phosphoryl group).

The last two articles delve into two areas of intense current interest in phosphatase chemistry. Understanding the molecular details of how post-translational modifications regulate the activity of proteins is a major frontier. Protein-tyrosine phosphatases utilize a conserved cysteine nucleophile that possesses an unusually low pK_a due to its highly positively charged environment in the active site. The ability of this residue to exist as a thiolate anion at physiological pH makes it a more powerful nucleophile, but also susceptible to oxidation. The article, “Redox regulation of protein tyrosine phosphatase

activity by hydroxyl radical” by Zhang and Meng presents evidence that such oxidation provides a means to regulate the activity of these enzymes *in vivo*.

The final article, “P–N bond protein phosphatases” by Attwood, points to a significant recent development in our understanding of biological phosphorylation. The reversible phosphorylation of proteins and other biological molecules on oxygen is well known. Proper assessment of phosphorylation on nitrogen in biological systems has been hindered by the lability of the P–N bond, often resulting in its hydrolysis under conditions commonly used for protein isolation. Compared to the enzymes that regulate the phosphorylation state of serine, threonine, and tyrosine, little is known about the corresponding enzymes that may be involved in nitrogen phosphorylation and dephosphorylation. This contribution reviews the current state of knowledge regarding reversible protein phosphorylation on arginine, lysine, and histidine, and the enzymes involved.

The consistently high number of articles appearing in the literature reporting on the biological consequences of phosphorylation makes it apparent that much remains to be learned about how this modification affects and controls biological processes, and of the underlying molecular interactions. The ability of nature to use phosphorylation in so many different ways, with more probably yet to be discovered, is truly amazing.

I am grateful to all of the authors for their fine contributions to this special section. I also thank Paul Cook for extending the original invitation to edit this venture, and to Andy Deelen and Sandra Tokashiki in the editorial office for their assistance in manuscript handling and keeping all of us moving along to complete our tasks.

Biography

Alvan C. Hengge was born and grew up in Cincinnati, Ohio. After obtaining his B.S. from the University of Cincinnati he taught high school chemistry and physics from 1975 to 1982 at Robert A. Taft High School. He then returned to the University of Cincinnati for graduate school, obtaining a Ph. D. in Organic Chemistry in 1987 in the laboratory of R. Marshall Wilson studying the reactions of triazolidinedione ylides. This was followed by an NIH postdoctoral fellowship in the lab of W. W. Cleland at the University of Wisconsin in the Institute for Enzyme Research, where he studied the biochemistry and enzymology of phosphoryl and acyl transfer. This was followed by several years as an Assistant Scientist in the Cleland laboratory. He joined the faculty at Utah State University in 1996, where he is now a Professor in the Department of Chemistry and Biochemistry, and has been Department Head since 2009. His research focuses on investigations, through the eyes of a

chemist, of the mechanisms of biologically important reactions, particularly phosphate and sulfate ester chemistry. He has authored a number of reviews on phosphoryl transfer, including articles in *Chemical Reviews*; *Accounts of Chemical Research*; *Comprehensive Natural Products II: Chemistry and Biology*; *Advances in Physical Organic Chemistry*; and the *Encyclopedia of Catalysis*.

References

1. Westheimer FH. Why nature chose phosphates. *Science*. 1987; 235:1173–1178. [PubMed: 2434996]
2. Lad C, Williams NH, Wolfenden R. The rate of hydrolysis of phosphomonoester dianions and the exceptional catalytic proficiencies of protein and inositol phosphatases. *Proc Natl Acad Sci U S A*. 2003; 100:5607–5610. [PubMed: 12721374]
3. Knowles J. Enzyme-catalyzed phosphoryl transfer reactions. *Annu Rev Biochem*. 1980; 49:877–919. [PubMed: 6250450]
4. Thatcher GRJ, Kluger R. Mechanism and catalysis of nucleophilic substitution in phosphate esters. *Adv Phys Org Chem*. 1989; 25:99–265.
5. Cleland WW, Hengge AC. Mechanisms of phosphoryl and acyl transfer. *FASEB J*. 1995; 9:1585–1594. [PubMed: 8529838]
6. Hengge, AC. Transfer of the PO_3^{2-} group. In: Sinnott, M., editor. *Comprehensive Biological Catalysis: A Mechanistic Reference*. Academic Press; San Diego, CA: 1998. p. 517-542.
7. Hengge AC. Mechanistic studies on enzyme-catalyzed phosphoryl transfer. *Adv Phys Org Chem*. 2005; 40:49–108.
8. Cleland WW, Hengge AC. Enzymatic mechanisms of phosphate and sulfate transfer. *Chem Rev*. 2006; 106:3252–3278. [PubMed: 16895327]
9. Lassila JK, Zalatan JG, Herschlag D. Biological phosphoryl-transfer reactions: understanding mechanism and catalysis. *Annu Rev Biochem*. 2011; 80:669–702. [PubMed: 21513457]