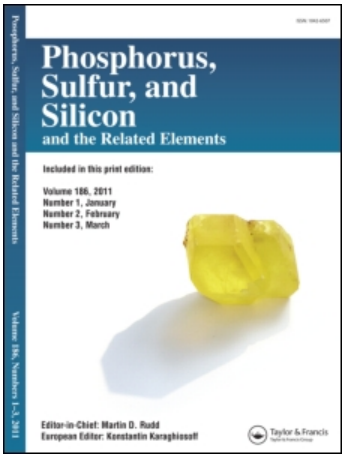

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Phosphomonoesterase Enzymes That Utilize Histidine or Cysteine as Nucleophiles in $S_N2(P)$ Reactions

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PHOSPHOMONOESTERASE ENZYMES THAT UTILIZE HISTIDINE OR CYSTEINE AS NUCLEOPHILES IN $S_N2(P)$ REACTIONS

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Abstract The past decade has seen a striking development in studies of the mechanisms used by a group of phosphomonoesterase enzymes that have been broadly defined as "acid" phosphatases on the basis of the pH optima that they exhibit with certain substrates. There is now convincing evidence that this apparent kinetic similarity masks the mechanistic use, by these enzymes, of at least two quite different nucleophilic residues in order to achieve catalysis in the hydrolysis of phosphate monoesters. The general properties of these enzymes are reviewed together with a summary of the extensive range of mechanistic information that is now available. This includes kinetic data that implicates the occurrence of a common rate limiting step, burst titration kinetics, stereochemical studies with phosphomonoesters that are chiral at phosphorus, trapping of covalent phosphoenzyme intermediates, and the identification of enzyme nucleophilic residues by covalent modification, trapping, spectroscopic and other experimental means. Finally, experiments are described that illustrate the use of active site-directed mutagenesis to explore the mechanisms of these enzymes.
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INTRODUCTION

The enzymes known as acid phosphatases were defined historically on the basis of an observed pH optimum in the dilute acid region, usually measured with *p*-nitrophenyl phosphate. However, this substrate possesses an unusually low pK_{a2} which in turn causes the pH "optimum" measured in typical experiments to be anomalously low when compared with that measured using more representative, physiologically-occurring substrates[1]. In fact, the two major types of acid phosphatases to be discussed here are highly active against phosphomonoester substrates even at neutral pH. The first type includes human prostatic acid phosphatase[1], which is representative of a group of mostly dimeric[2] non-metallo enzymes[3] having a subunit size of approximately 354 residues,

and which possess the active site sequence ArgHisGlyXArgXPro that may be regarded as characteristic of these enzymes[4]. Mammalian tissues contain several genetically distinct acid phosphatases[5]. Thus, human and bovine liver contains not only a high molecular weight (100 kDa) lysosomal enzyme related to the prostatic type, but also a structurally distinct, low molecular weight (18 kDa) enzyme[6,7] that has at least one active site sulfhydryl group[8-10]. This brief review examines some of the evidence developed in our laboratory showing that these two types of enzymes, although having virtually identical pH-rate profiles,[1,11] in fact proceed with the involvement of two distinct active site nucleophilic groups, namely histidine and cysteine.

Common Rate Limiting Step. An important early indication that at least some acid phosphatases catalyzed reactions by a mechanism that could involve a relatively stable covalent intermediate was the finding by others that a common V_{max} value was observed for the hydrolysis of a range of substrates having widely varied (alkyl, aryl, acyl) leaving groups[12]. The low molecular weight enzymes appear somewhat more sensitive to the nature of the leaving group, but still exhibit effectively constant V_{max} values with phenolic substrates having *meta* and *para* substituents covering a broad range of Hammett sigma constants[13].

Phosphatase-catalyzed Exchange of ^{18}O-labeled Phosphate Ion. The tendency with hydrolytic enzymes such as phosphatases is to focus on the hydrolysis direction, but useful information may often be obtained by studying the reverse reaction. For example, the reverse of phosphoenzyme hydrolysis would be the reaction of phosphate ion with enzyme, and would be accompanied by the release of a molecule of water.

$$E + P_i \rightleftarrows E \cdot P_i \rightleftarrows E\text{-}P + H_2O$$

The relatively slow phosphatase-catalyzed exchange reactions can be easily measured because ^{18}O-labeled phosphate ion is easily synthesized[14] and the loss of ^{18}O from phosphate ion can be quantitated by using normal ^{16}O-water as a medium in conjunction with either mass spectrometry or ^{31}P NMR spectroscopy. This has been accomplished with human prostatic acid phosphatase[15] and the low molecular weight cytoplasmic enzymes[16].

Burst Titration Kinetics. In their most frequently encountered form, burst titration kinetic experiments involve spectroscopic measurements

of the transient (pre-steady state) formation of the first released product. Because of experimental limitations involving amounts of material, solubility, chromophoric sensitivity and absorbance by unreacted substrate and impurities, it turned out to be much more difficult to perform such experiments with acid as opposed to alkaline phosphatases.[17] Finally, by working at pH 7 and at reduced temperature (4.5°C) it was possible to provide definitive evidence for an initial stoichiometric burst of *p*-nitrophenol when *p*-nitrophenyl phosphate and low molecular weight phosphatase were mixed together[16].

Phosphotransfer and Partition Experiments. Small alcohols that are more nucleophilic than water may cause a marked increase in the rate of disappearance of substrate. This is consistent with the formation of a relatively stable phosphoenzyme intermediate whose hydrolysis is normally the rate determining step, but whose disappearance is increased by the attack of alternate nucleophiles. Such experiments have been recently described in detail for the case of the low molecular weight cytoplasmic enzyme[16].

Stereochemistry of Phospho Group Transfer. By using phosphomonoesters that are chiral at phosphorus, it was possible to establish the stereochemical integrity with which a phospho group is transferred from an alcohol or phenol leaving group to a new acceptor. This has been successfully accomplished with a low molecular weight cytoplasmic enzyme[18] and with human prostatic acid phosphatase[19]. In both cases, the stereochemistry of transfer from chiral phenyl [$^{16}O,^{17}O,^{18}O$]phosphate to propanediol was established to occur with complete overall retention of configuration around phosphorus. This is most simply interpreted as being the result of two $S_N2(P)$ reactions, with the first being the formation of a covalent phosphoenzyme intermediate and the second inversion reaction occuring upon reaction of the phosphoenzyme with the nucleophilic alcohol.

Phosphoenzyme Trapping. By trapping the phosphoenzyme intermediates, the nucleophilic groups have been identified to be histidine and cysteine for the high and low molecular weight types of enzymes, respectively.[20-22]

Site-directed Mutagenesis. Both the prostatic-type and the low molecular weight cytoplasmic-type enzymes have been cloned and sequenced.[4,22,23] Site-directed mutagenesis experiments have been used to confirm the specific identities of the nucleophilic histidine and cysteine residues, respectively (K. Ostanin, J. P. Davis and R. L. Van Etten, manuscripts in preparation.)

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