Stereochemical Constraints on the Catalytic Hydrolysis of Organophosphate Nerve Agents by Phosphotriesterase

Suk-Bong Hong & Frank M. Raushel

a Department of Chemistry, Texas A & M University, College Station, Texas, 77843, USA
b Department of Chemistry, Texas A & M University, College Station, Texas, 77843, USA

Published online: 17 Mar 2008.

To cite this article: Suk-Bong Hong & Frank M. Raushel (1999) Stereochemical Constraints on the Catalytic Hydrolysis of Organophosphate Nerve Agents by Phosphotriesterase, Phosphorus, Sulfur, and Silicon and the Related Elements, 144:1, 521-524, DOI: 10.1080/10426509908546296

To link to this article: http://dx.doi.org/10.1080/10426509908546296
Stereochemical Constraints on the Catalytic Hydrolysis of Organophosphate Nerve Agents by Phosphotriesterase

SUK-BONG HONG and FRANK M. RAUSHEL

Department of Chemistry, Texas A&M University, College Station, Texas, 77843, USA

The chiral specificity of the bacterial phosphotriesterase for enantiomeric substrates has been examined. Paraoxon (diethyl p-nitrophenyl phosphate) is the best substrate for this enzyme but this protein is quite tolerant of significant alterations to this structure. One or both of the two ethyl groups in paraoxon can be replaced with various combinations of methyl, isopropyl, or phenyl substituents. The individual enantiomers, as well as racemic mixtures, were tested as substrates for the phosphotriesterase. The enantiomeric preference of the phosphotriesterase depends on the absolute configuration at the phosphorus center and the steric bulk of the alkyl substituents. The kinetic constants for the enzyme-catalyzed hydrolysis of the $S_P$-(-)-enantiomer of ethyl phenyl p-nitrophenyl phosphate were 1–2 orders of magnitude greater than those for the corresponding $R_P$-(+)-enantiomer. Similar differences were also obtained for other pairs of enantiomers.

Keywords: phosphotriesterase; organophosphorus nerve agents; acetylcholinesterase; stereochemistry

INTRODUCTION

Organophosphorus compounds are among the most toxic substances known. These reagents are used in chemical weapons, including the nerve agents sarin, soman and VX in addition to the insecticides diazinon, parathion and coumaphos. The organophosphorus compounds are effective reagents because of their specific inhibition of nerve functions through the inactivation of the enzyme acetylcholine esterase. Millions of pounds of organophosphate pesticides are used each year in the U.S. It has been estimated that thousands of people receive some form of organophosphorus poisoning annually in the United States alone\footnote{1}. Human exposure to excess levels of
these compounds has also been shown to exert delayed chronic cholinergic and neurologic toxicity. Organophosphorus nerve agents were developed during World War II and have been stockpiled as chemical weapons. The threat of chemical warfare agents was recently observed in the Gulf War and the sarin gas attack in the Tokyo subway system. According to U.S. Army sources, disposal of the U.S. chemical weapons stockpile may cost an estimated $12 billion. Because of the concerns about the impact of environmental contamination by toxic organophosphorus compounds, it is necessary to develop safe and efficient detoxification systems for medical and environmental uses.

The phosphotriesterase (PTE) from *Pseudomonas diminuta* is a zinc metalloenzyme that catalyzes the hydrolysis of an extensive array of organophosphate neurotoxins\(^2,3\). Recent studies have shown that this enzyme is a highly efficient catalyst for the hydrolysis of organophosphorus nerve agents. The turnover numbers for the best substrates approach \(10^4\) s\(^{-1}\), and \(k_{cat}/K_m \approx 10^5\) M\(^{-1}\) s\(^{-1}\). It has been shown that two zinc ions are critical for maximal catalytic activity of the enzyme. The native zinc ions can be substituted with a variety of divalent cations including Co\(^{2+}\), Ni\(^{2+}\), Cd\(^{2+}\), and Mn\(^{2+}\). The catalytic mechanism for the enzymatic hydrolysis of organophosphates such as paraoxon (diethyl p-nitrophenyl phosphate) proceeds via a direct S\(_{N}\)2-like displacement of the leaving group by an activated water molecule, resulting in an inversion of configuration at phosphorus\(^4\).

The recent three-dimensional structure of phosphotriesterase with a bound substrate analog has shown that the active site is located at the carboxy-terminal end of the \(\alpha\beta\)-barrel\(^5\). The regions of the active site that interact with the substituents liganded to the phosphorus center have been shown to vary in size. Therefore, the stereochemical arrangement of the alkyl or aryl substituents attached to the phosphorus core will affect the proper orientation of the substrate bound within the active site. This may result in the stereoselective hydrolysis of chiral substrates. In fact, it has been shown that phosphotriesterase is capable of differentially recognizing the individual enantiomers of chiral substrates\(^4,6\). The enzyme hydrolyzes only the \(S_p\)-enantiomer of \(O\)-ethyl \(O\)-(4-nitrophenyl) phenylphosphonothioate (EPN). The enzyme also preferentially hydrolyzes the \(S_p\)-isomers of \(O,S\)-dimethyl \(N\)-acyetylphosphoramoidothioate (acephate) and \(O,S\)-dimethyl phosphoramoidothioate (methamidophos).
RESULTS and DISCUSSION

In order to explore the substrate specificity of the phosphatase, a large number of racemic and enantiomeric phosphatases were examined. The two ethyl groups in paraoxon were replaced with various combinations of methyl, isopropyl, and phenyl groups. The enantiomeric phosphotriesters were prepared using L-proline methyl ester as chiral reagents.[7] Reactions of phosphotriesterase with racemic and chiral phosphotriesters were followed spectrophotometrically by monitoring the appearance of p-nitrophenol at 400 nm in 100 mM 2-(A-r-cyclohexylamino) ethanesulfonic acid (CHES) buffer, pH 9.0. All kinetic parameters were determined from a fit of the data to the Michaelis-Menten equation. The hydrolysis of the racemic phosphotriesters catalyzed by phosphotriesterase showed an initial fast release of approximately 50% of the total p-nitrophenol. The fast phase was followed by a much slower release of the remaining p-nitrophenol. When the reaction was half-complete, the reaction mixture was quenched and then extracted with chloroform. The chloroform-extracted material was determined to be the (+)-enantiomer upon measurement of the specific optical rotation when racemic ethyl phenyl p-nitrophenylphosphate was used as a substrate. Further kinetic analysis of the time courses demonstrated that the (-)-enantiomers were hydrolyzed 1-2 orders of magnitude faster than (+)-enantiomers. When the ethyl group in paraoxon is replaced with longer and more branched alkyl chains, such as isopropyl, 1,2,2-trimethylpropyl or 3,3-dimethylbutyl substituents, there is a large decrease in both $k_{cat}$ and $k_{cat}/K_m$.

Examination of the crystal structure of PTE with a bound substrate analog indicates that there are three binding pockets that can accommodate substituents of the substrate. On the basis of X-ray crystallography and molecular modeling studies, the leaving group sits in a very hydrophobic cluster of amino acids and is directed towards the solvent. Those regions of the active site that interact with the two other substituents for the chiral substrate may play a major role in ligand association.

Assignment of the absolute configuration of the preferred substrates is necessary for a more complete understanding of how the substrate binds to the active site and the potential role of amino acid residues in binding and catalysis. Treatment of (+)-ethyl p-nitrophenyl phenyl phosphate with sodium methoxide gave (-)-ethyl methyl phenyl phosphate which has previously been determined to be of the $S_p$-configuration. In this reaction, the methoxide displaces the p-nitrophenyl group from the (+)-ethyl p-nitrophenyl phenyl phosphate with inversion of configuration at phosphorus. Therefore (+)-ethyl p-nitrophenyl phenyl phosphate was assigned as the $R_p$-configuration. The $S_p$-(-)-isomer is thus the preferred substrate for phosphotriesterase.
A preliminary investigation showed that $R_p$-(+) -ethyl $p$-nitrophenyl phenyl phosphate is a more potent inhibitor of human recombinant acetylcholinesterase than its enantiomer. The bacterial phosphotriesterase and human recombinant acetylcholinesterase therefore show the opposite stereoselectivity toward phosphotriester enantiomers. Chiral organophosphorus nerve agents differ greatly in their biological activity. For example, $S_p$-(-)-sarin (isopropyl methylphosphonofluoridate) has a $10^3$-fold higher activity than the $R_p$-(-)-isomer for the inactivation of acetylcholinesterase\textsuperscript{[8]}. These results suggest that phosphotriesterase would catalyze the hydrolysis of $R_p$-(+) -sarin faster than ($S_p$)-(-)-sarin.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (GM 33894).

References