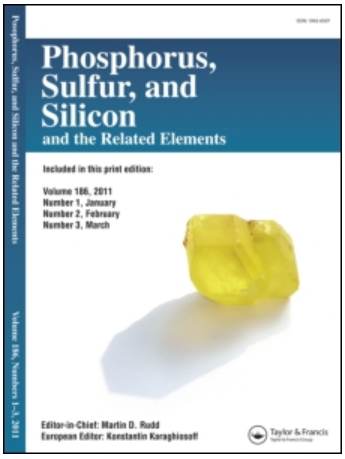

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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To cite this Article Broomfield, Clarence A.(1996) 'Transition State Analogs for Catalytic Antibodies', Phosphorus, Sulfur, and Silicon and the Related Elements, 109: 1, 233 — 236
To link to this Article: DOI: 10.1080/10426509608545133
URL: http://dx.doi.org/10.1080/10426509608545133

Phosphorus, Sulfur, and Silicon, 1996, Vols. 109-110, pp. 233-236
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TRANSITION STATE ANALOGS FOR CATALYTIC ANTIBODIES

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ABSTRACT

The first antibodies that behave as enzymes (abzymes) appeared in the literature almost ten years ago and in the intervening years abzymes have been made that catalyze a variety of reactions, including ester hydrolysis, carbonate hydrolysis, amide hydrolysis and aminolysis. It would be useful to have available an enzyme that catalyzes the hydrolysis of organophosphorus anticholinesterases (OPA Hydrolase). Such enzymes are ubiquitous in nature, but are generally very inefficient.One approach to the development of an improved OPA Hydrolase is to produce an abzyme with this activity. This involves the synthesis of a pentacoordinate (trigonal bipyrimid) transition state analog that is stable enough to be attached to a protein carrier and then remain in the body of an animal long enough to elicit antibodies. It is apparent that this is not a simple matter; the only elements that have pentavalent forms are in the vanadium or nitrogen families. While some of those compounds are trigonal bipyramids they are universally unstable in water. Today's challenge is to find ways to make pentacoordinate transition state analogs that can be attached to protein carriers and have a reasonable lifetime in an aqueous environment.

The idea that enzymes differ from antibodies primarily by virtue of the fact that antibodies bind the ground state of their "substrates" while enzymes are designed to bind transition states was first noted by Pauling nearly 50 years ago (1). While that was an interesting observation it was largely ignored for two decades until Jencks suggested that selected reactions could be promoted if antibodies could be found that would stabilize the rate-controlling transition state (2). Again, this was an interesting proposal that could not be practically implemented until a homogeneous population of antibodies could be developed. This key step was taken by Kohler and Milstein (3) with the development of *in vitro* monoclonal antibodies, a population of antibodies with a single, specific molecular structure that could be grown in large numbers.

Thus, by 1975 the tools were available to test the hypothesis that antibodies could be produced that would act as catalysts, and in 1986 Tramontano in Lerner's lab (4) and Pollack in Schultz's lab (5) described the first catalytic antibodies, the former designed to catalyze the hydrolysis of an ester and the latter taking advantage of a natural antibody to catalyze the hydrolysis of a particular carbonate that has a structure similar to the natural ligand. Since that time the field has grown rapidly. There are now reports in the literature of abzymes with novel specificities and reaction mechanisms ranging from hydrolysis of esters and amides to oxidations, isomerization and β-elimination. The

development of a specific abzyme appears to be particularly useful to catalyze those reactions for which there are no natural enzymes.

Several years ago we conceived the idea of protecting soldiers against nerve agent poisoning by pretreating them with scavengers that would reside in the blood stream for an extended period of time. The best candidates were proteins which would be able to neutralize nerve agents between the time of exposure and their arrival at their target molecule. This concept was proven by treating animals with enzymes. Administration of certain enzymes, e.g. butyrylcholinesterase (6), which are known to react very rapidly and irreversibly with nerve agents, followed by exposure to soman, sarin or VX produced protection. It was shown that both rodents and nonhuman primates could tolerate several LD_{50} doses of these nerve agents without measurable side effects when so treated. Furthermore, pretreatment of mice with an OP hydrolyzing enzyme protected them against at least twice the dose of soman that was lethal to all controls (7). Obviously, the catalytic scavenger has many advantages over the stoichiometric scavenger, but there are no OP hydrolyzing enzymes in nature that are efficient enough to afford the required protection at reasonably low doses. One proposal for developing an adequate enzyme is the production of a catalytic antibody, compatible with the human body, that will catalyze the hydrolysis of the organophosphorus nerve agents (8).

While the concept of making catalytic antibodies against nerve agents is attractive, there are several difficulties to be resolved in its accomplishment. The most important of these is the design and synthesis of appropriate haptens that approximate the transition state for organophosphate hydrolysis. While easily made tetrahedral phosphorus compounds are reasonable transition state analogs for carbon chemistry, the transition state for organophosphate hydrolysis is probably pentacoordinate, and presumably, based on what is known about enzyme inhibition kinetics (9), a trigonal bipyrimid [Fig 1.]. This is a problem. Pentacoordinate phosphorus compounds are highly unstable,

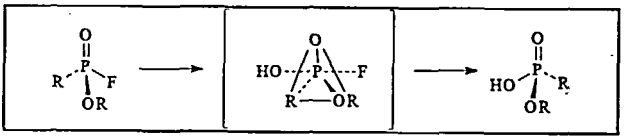


Fig. 1. The generally accepted formation of a trigonal bipyrimidal transition state from the tetrahedral ground state of pentavalent phosphorus compounds upon attack by a nucleophile.

particularly in aqueous solutions. Dr. Moriarty has experienced some success at increasing the stability of such compounds (10) (c.f. Moriarty et al., this volume) but to do so he had to add bulky groups [Fig.2]. As can be seen in the model, that bulk might prevent recognition of the geometry around the phosphorus and thus make it very difficult to obtain antibodies that will catalyze the hydrolysis of organophosphorus esters or acid anhydrides.

Transition State Analogs

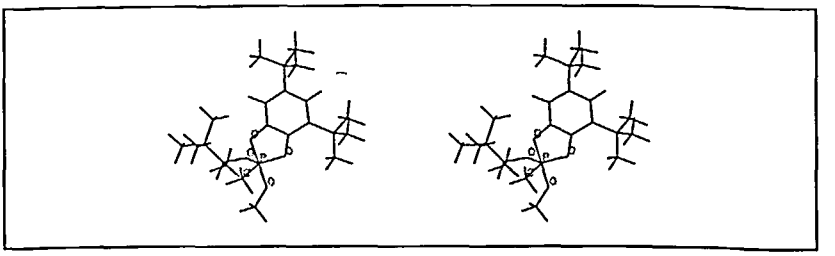
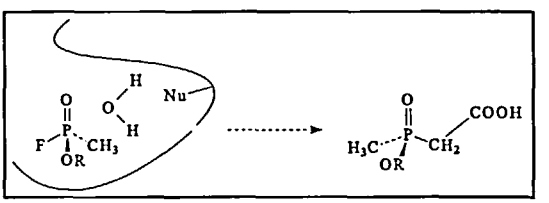


Fig 2. Three dimensional structure of a pentacoordinate transition state analog, stabilized by ring formation and bulky substituents.

One proposal for getting around the instability of pentacoordinate phosphorus transition state analogs was to use a different element that could form the same, trigonal bipyrimidal geometric configuration. Unfortunately, there are very few elements (only those in the Nitrogen or Vanadium series plus a few rare earths) that have pentavalent forms and fewer that have been shown to form trigonal bipyrimids. Furthermore, most of the pentacoordinate organic compounds of these elements are even more unstable than the phosphorus compounds. Dr. Crans and others, notably Ray et al. (11) and Wlodawer et al.(12), have circumvented this difficulty by making coordinate complexes with vanadium that approximate specific transition state structures. Dr. Crans will elaborate on those ideas (Crans, this volume).



3. Schematic design of a bait-and-switch hapten for phosphate ester/anhydride hydrolysis. Note stereospecificity requirements for this hydrolysis scheme. If specific binding sites for R and -OR' groups are induced in the antibody attention must be given to the chirality of the hapten.

Another strategy for eliciting catalytic antibodies, not dependent upon a transition state analog, has been termed "bait and switch". This mechanism involves the design and synthesis of haptens that have the ability to elicit in the antibodies specific combining site residues that might act as chemical groups in catalysis [Fig. 3.]. As can be seen in the figure, the ideal hapten presents both orienting binding sites and an appropriately positioned polar group so that in at least some of the resulting antibodies there will be a nucleophilic center that will be able to catalyze the hydrolysis of the organophosphorus compound. Only one such clone is needed; it can then be expanded into large colonies and its gene isolated. Once the gene of a catalytic molecule is in hand it can be

manipulated to change specificity and other characteristics. It should be pointed out that, in the case of the bait-and-switch hapten, it is necessary to consider the chirality of the desired substrate. In the case of soman, for example, only the $P_{(R)}$ isomers are highly toxic; the others are relatively poor cholinesterase inhibitors (13). If it is desired to destroy the toxic isomers preferentially it would be advantageous to know the absolute configuration of the target substrate and to construct a hapten with the same configuration.

Finally, the "best" hapten would be one that combines transition state stabilization with induction of a catalytic site, thus mimicking the best enzymes. This is a challenge for phosphorus synthesis chemists; it is easy to determine what is needed, but more difficult to synthesize the desired structure. I hope that our discussions in this meeting will produce strategies to attempt the construction of these difficult structures and will ultimately result in a catalytic antibody that will catalyze the rapid destruction of organophosphorus nerve agents. That would be a genuine contribution to world safety.

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