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An Approach to Sequence-Specific Antibody Proteases

The Use of Haptens Mimicking Both a Transition State and a Distorted Ground State

ROBERT M. SMITH, PING YUAN, DAVID P. WEINER, CARYN R. DUTTON, AND DAVID E. HANSEN*

Department of Chemistry, Amherst College, Amherst, MA 01002

ABSTRACT

We describe here a novel strategy for the isolation of antibodies with sequence-specific protease activity: the synthesis of dipeptide haptens in which the targeted peptide bond has been replaced by a ring-strained or torsionally strained hydroxyethylene transition-state analog. Thus, the analogs mimic both a peptide bond in a distorted, reactive conformation and the transition state for peptide bond hydrolysis. In order to obtain sequence-specific antibody proteases, these analogs have been flanked with additional amino acid residues in preparation for immunization. In particular, we have synthesized peptides containing analogs such as 2-*cis*-amino-3-*cis*-hydroxycyclobutane carboxylic acid and *endo*-(3-amino-2-hydroxy)bicyclo[2.2.1]-heptane-7-anti-carboxylic acid. We have also prepared a series of peptide derivatives containing analogs, such as 2-[3-amino-2-oxo-1-azetidinyl]-3-methylbutanoic acid, in which the targeted peptide bond has been incorporated into a β-lactam ring. Since the "peptide bond" has been left intact, these species mimic only a distorted ground state. At present, antibodies are being elicited against a number of the above peptide derivatives.

Index Entries: Antibody catalysis; protease activity; ground-state strain; cyclobutanol; [2.2.1]bicycloheptanol; β-lactam.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

In 1984, Lerner (*1*) predicted that sequence-specific antibody proteases might ultimately be obtained, and at present, the isolation of such antibodies is an endeavor that attracts intense interest (*2,3*). Antibody proteases have potential for use in the treatment of diseases in which foreign structural or catalytic proteins are exposed to circulating antibodies, as is the case, for example, in many cancers (*4*) and infections (*5*); in addition, these antibodies may find application in a variety of biochemical analyses.

To date, however, no general method for the isolation of antibody proteases has been developed, and we describe here a new strategy: the elicitation of antibodies against dipeptide analogs in which the targeted peptide bond has been replaced by a ring-strained or torsionally strained hydroxylethylene transition-state analog. Thus, our analogs mimic a peptide bond in a distorted, reactive conformation as well as the transition state for peptide bond hydrolysis. To explore further the role of ground-state strain in catalysis, we have also synthesized dipeptide analogs in which the targeted peptide bond has been incorporated into a β-lactam ring. Because the "peptide bond" has been left intact, these species mimic only a distorted ground state. In order to obtain sequence-specific antibody proteases, we have flanked these dipeptide analogs with additional amino acid residues in preparation for immunization. Note that since many antibodies raised against peptides crossreact with cognate sequences in globular proteins (*6*), one can, in principle, obtain an antibody protease by immunizing with an appropriate peptide analog. (Although such crossreactivities have been questioned [*7*], recent studies indicate that the phenomenon is real [*8,9*].)

Our approach is an extension of the now prevalent use of transition-state analogs to elicit antibody catalysts, an approach that first succeeded in 1986 when groups led by Lerner and Tramontano (*10*) and by Schultz (*11*) independently demonstrated monoclonal antibodies that bind tetrahedral phosphorous analogs can catalyze the hydrolysis of esters and carbonates, respectively. In the intervening seven years, well over 40 additional examples of antibody catalysts have appeared, and new strategies, such as the use of charged immunogens and the covalent attachment of cofactors, have been employed (*12*). Over ten examples of antibody-catalyzed ester hydrolysis alone have been reported (*12*).

Nevertheless, peptidolytic antibodies have never been obtained by immunizing with a transition-state analog (for example, *see* Pollack et al. [*13*]), possibly because peptides are far more difficult to hydrolyze than are esters or carbonates. Using tetrahedral phosphorus derivatives, Janda et al. (*14*) have, however, elicited an antibody that catalyzes the hydrolysis of a *p*-nitroanilide substrate, an activated amide, and Martin et al. (*15*) have, as reported at this symposium, obtained an antibody that catalyzes the hydrolysis of a terminal, primary amide. This latter result is

especially significant since the substrate is an unactivated amide. In a related system, the Benkovic group (*16*) has reported the antibody-catalyzed rearrangement of an asparagyl-glycyl-containing peptide to a mixture of the aspartyl-glycyl and isoaspartyl-glycyl derivatives; a cyclic bifunctional transition-state analog was utilized to elicit the antibodies. This reaction provides an elegant means of deactivating asparagyl-glycyl-containing peptides and again demonstrates that antibodies can, in the absence of cofactors, expel the basic leaving group ammonia.

Although traditional transition-state analogs have so far failed to elicit antibody peptidases, two groups have utilized other strategies to obtain such antibodies. Iverson and Lerner (*17*) isolated antibodies with sequence-specific peptidase activity by immunizing with a Co^{3+}(triethylenediamine) exchange-inert peptide complex, and Paul and coworkers have reported that polyclonal human autoantibodies (*18,19*), as well as a mouse monoclonal antibody (*20*), catalyze the sequence-specific cleavage of vasoactive intestinal peptide. These two successes have not, however, led to the isolation of an antibody protease, a feat that has yet to be accomplished by any means.

RESULTS

We have designed and synthesized a variety of hydroxyethylene transition-state analogs in which the peptide bond mimicked has also been distorted into a reactive conformation. Specifically, we have completed the synthesis of the dipeptide analogs **1** and **2**, in which the peptide bond mimicked is ring-strained, and the analogs **3–5**, in which the peptide bond mimicked is torsionally strained.

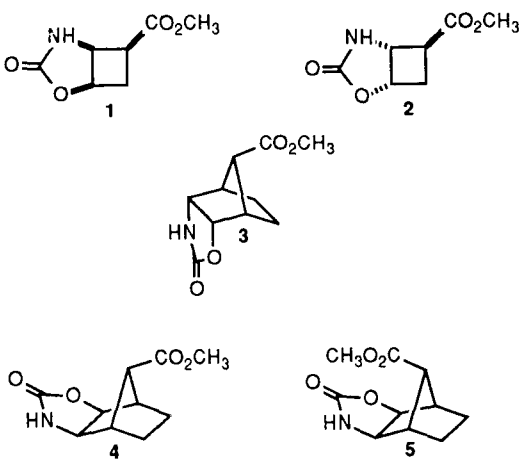


Figure 1 indicates the alignment of analogs **1** and **3** with the correspond ing glycyl-glycyl dipeptide. We have also reproduced the syntheses of the

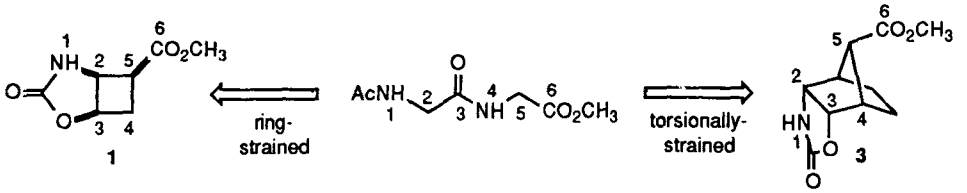
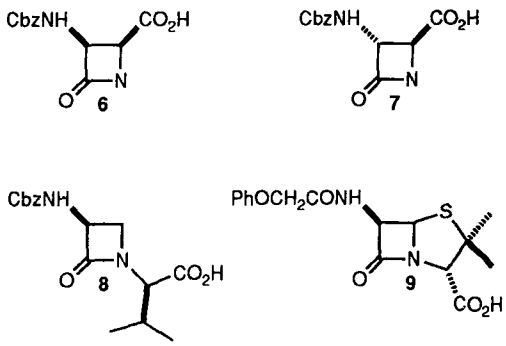
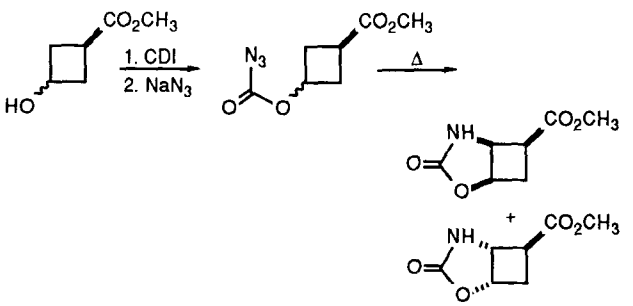


Fig. 1. Alignment of analogs **1** and **3** with the corresponding glycyl-glycyl dipeptide.

β-lactam-containing dipeptide analogs **6–8**, each of which mimics a ring-strained ground state, and have purchased penicillin V, **9**, which mimics both a ring-strained and torsionally strained peptide ground state. With the exception of the β-lactam derivatives **8** and **9**, which are optically pure, all of the above analogs were obtained as racemic mixtures.



The analogs **1–5** were each synthesized from the corresponding hydroxy ester following a modification of the strategy of Lowe and Swain (*21*). Specifically, the hydroxy ester was treated with 1,1′-carbonyldiimidazole (CDI), followed by sodium azide to generate the azidocarbonate. This material was thermolyzed in methylene chloride to yield the *O*-acylnitrene, which upon insertion into a neighboring carbon–hydrogen bond gave the corresponding cyclic carbamate, as is shown in Eq. (1) for the synthesis of the cyclobutanol derivatives **1** and **2** (these derivatives separated on silica gel chromatography).



Eq. (1)

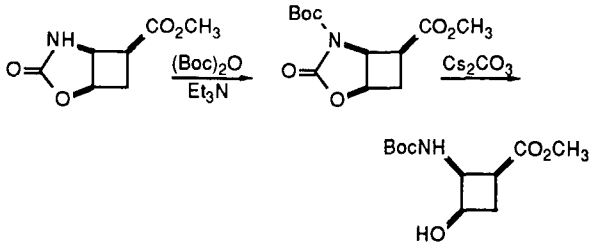
The hydroxy ester precursors to **1** and **2**, methyl *cis*- and *trans*-3-hydroxycyclobutanecarboxylate, were synthesized using a modification of the procedure of Caputo and Fuchs (*22*); the hydroxy ester precursors to **3** and to **4**, methyl *endo*- and *exo*-2-hydroxy-*anti*-7-bicyclo[2.2.1]heptanecarboxylate, were synthesized as described by Flury and Grob (*23*); and the hydroxy ester precursor to **5**, methyl *exo*-2-hydroxy-*syn*-7-bicyclo[2.2.1]-heptanecarboxylate, was synthesized using a modification of the procedure of Swartz (*24*).

The β-lactam derivatives **6** and **7**, *cis*- and *trans*-3-amino-4-carboxy-2-azetidinone, were prepared following the procedure of Kishimoto et al. (*25*) and of Finkelstein et al. (*26*), and the β-lactam derivative **8**, 2-[3-amino-2-oxo-1-azetidinyl]-3-methylbutanoic acid, was synthesized as described by Duranti and Bonifazi (*27*). Penicillin V, **9**, was obtained from Sigma Chemical Co.

In order to elicit sequence-specific antibodies, the dipeptide analogs must be flanked with additional amino acid residues prior to immunization. Our choice of peptide sequence was guided by an earlier report of Schultz and coworkers (*13*) on monoclonals obtained against a phosphonate-containing tripeptide derivative. These antibodies catalyze the hydrolysis of the corresponding depsipeptide with a rate acceleration of just 100-fold, far less than observed in many other antibody-catalyzed hydrolysis reactions. The authors commented, "with relatively large transition state analogs such as these tripeptides, the tetrahedral phosphonate probably contributes proportionally less to the overall binding energy of the hapten to the antibody." We therefore decided to flank our analogs with only one amino acid residue on each terminus. In addition, all of the antibody catalysts obtained by the Schultz group against the aforementioned phosphonate derivative, which was epimeric at a center corresponding to phenylalanine, recognized the depsipeptide substrate containing D-phenylalanine. Presumably, as the authors point out, this result is because of the enhanced immunogenicity of D-amino acids. Thus, we chose to flank our analogs with D-amino acids, specifically D-phenylalanine, D-tyrosine, and/or D-valine; these amino acid residues are particularly immunogenic (*28*). Furthermore, the incorporation of D-amino acids lessens the possibility that the corresponding peptide substrates will be cleaved by traces of contaminating proteases that may remain in the purified antibodies. Of course, the antibodies raised against these analogs will not crossreact with native proteins; nevertheless, we believe the increase in immunogenicity worth that loss. Clearly, we will incorporate any analogs that elicit an antibody peptidase (against the corresponding D-amino acid-containing substrate) into longer sequences containing only L-amino acids. Antibodies obtained against these derivatives may well crossreact with, and cleave, proteins.

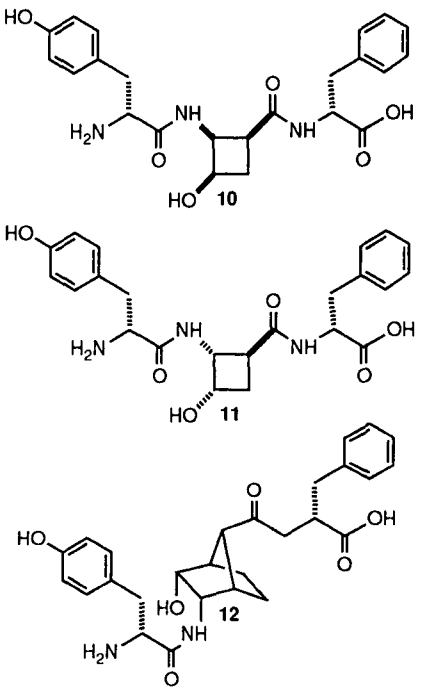
To date, we have synthesized peptides containing the analogs **1–3** and **6–9**. For the cyclobutanol analogs **1** and **2**, and the bicycloheptanol analog **3**, the methodology of Ishizuka and Kunieda (*29*) was employed

first to convert each into the corresponding *t*-butoxycarbonylamino (Boc) hydroxy ester, as is shown in Eq. (2) for analog **1**.



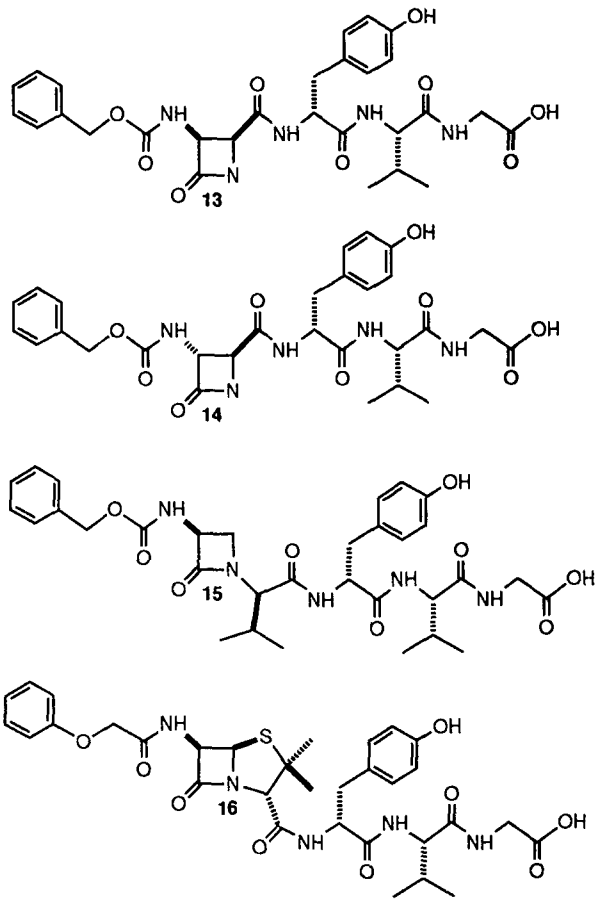
Eq. (2)

Using standard coupling procedures (*30*), these Boc hydroxyl ester derivatives were then incorporated into the peptide sequence D-tyrosyl-[dipeptide analog]-D-phenylalanine to yield the derivatives **10–12**.



Initially, we had hoped also to incorporate our β-lactam derivatives into the same sequence, but we discovered that hydrogenolysis of the carbobenzoxy (Cbz) group of the derivative **8** followed by peptide coupling proceeded in low yield, presumably because the deprotected β-lactam ring rearranges to the oxopiperazine derivative (*27*). However, given that the Cbz group is hydrophobic and aromatic, and that it is isosteric with the side chain of phenylalanine, we simply extended the peptide only at the carboxy terminus. Analogously, we left intact the Cbz group on the derivatives **6** and **7**, and the phenoxyacetyl group on penicillin V (in this latter case, of course, we had no choice). Thus, in a single step, the free

carboxy terminus of each β-lactam derivative was coupled to the tripeptide D-tyrosyl-valyl-glycine to yield the derivatives **13–16**.



In preparation for immunization, we have conjugated each of the above peptide derivatives, with the exception of **12**, to the carrier proteins bovine serum albumin and keyhole limpet hemacyanin (*31*). The cyclobutanol derivatives **10** and **11** were coupled via their free amino terminus to iminothiolane-modified carrier protein (*32*) using the heterobifunctional linker *N*-succinimidyl-6-maleimidocaproate. The β-lactam derivatives **13–16** were coupled via their *N*-hydroxysulfosuccinimide esters (*33*). Note that diastereomeric mixtures of the peptide derivatives were not separated before conjugation to carrier protein. Although we have synthesized and characterized the peptide **12**, our initial attempts to conjugate it to carrier protein failed; we are presently repeating this procedure. Monoclonal antibodies are currently being raised against the above conjugates.

The monoclonals obtained against the cyclobutanol peptide derivatives **10** and **11** will be assayed for hydrolytic activity with the substrate *N*-acetyl-D-tyrosyl-glycyl-glycyl-D-phenylalanine; monoclonals against the bicycloheptanol derivative **12** against *N*-acetyl-D-tyrosyl-glycyl-glycyl-

D-phenylalanine, as well as against *N*-acetyl-D-tyrosyl-glycyl-L-prolyl-D-phenylalanine and *N*-acetyl-D-tyrosyl-glycyl-D-prolyl-D-phenylalanine; monoclonals against the β-lactam derivatives **13** and **14** assayed with Cbz-glycyl-glycyl-D-tyrosyl-valyl-glycyl-carboxamide; monoclonals against the β-lactam peptide derivative **15** assayed with Cbz-glycyl-D-valyl-D-tyrosyl-valyl-glycyl-carboxamide; and monoclonals against the penicillin V peptide **16** derivative assayed with *N*-phenoxyacetyl-glycyl-glycyl-D-tyrosyl-valyl-glycyl-carboxamide. Note that in the peptide substrates corresponding to the derivatives **10–12**, the *N*-acetyl group corresponds to the truncated linker *N*-succinimidyl-6-maleimidocaproate, whereas in the substrates corresponding to the derivatives **13–16,** the carboxamide functionality corresponds to the truncated amide bond in the carrier protein lysyl side chain.

DISCUSSION

The goal of the research described here is the isolation of monoclonal antibodies with sequence-specific peptidase and, ultimately, protease activity. Our primary strategy is to elicit antibodies against dipeptide analogs in which the targeted peptide bond has been replaced by a ring-strained or torsionally strained hydroxyethylene transition-state analog. Thus, the analogs mimic both the peptide bond in a distorted, reactive conformation and the transition state for peptide bond hydrolysis. Antibodies raised against these derivatives may effect catalysis both by destabilizing the bound peptide substrate and by stabilizing the transition state. Antibodies will also be elicited against peptides incorporating β-lactam rings, derivatives that mimic only ground-state strain. In particular, we are raising monoclonal antibodies against peptides containing the cyclobutanol derivatives **1** and **2**, the [2.2.1]bicycloheptanol derivatives **3–5**, and the β-lactam derivatives **6–9**. Sequence specificity will be achieved by flanking each of these analogs with additional amino acid residues.

In an important precedent for the design of our analogs, the Schultz group (*34*) has shown that an antibody raised against an undistorted hydroxyethylene transition-state analog catalyzes the hydrolysis of both esters and carbonates; furthermore, the catalytic efficiency of this antibody is only slightly less than that of an antibody raised against the corresponding phosphonate derivative. Similarly, in a recent study of an antibody raised against a bifunctional phosphinate, hydroxyethylene transition-state analog, the Benkovic group (*35*) has estimated that the phosphinate group is only four to seven times more efficacious in eliciting hydrolytic activity than is the hydroxyethylene group.

The role of transition-state stabilization in enzyme catalysis is well recognized and well analyzed (*36*), but the importance of ground-state

strain has received less attention. Nonetheless, as comprehensively discussed by Albery and Knowles (*37*), ground-state strain and transition-state stabilization are inextricably linked and cannot be deconvoluted. In perhaps the most direct exegesis on ground-state destabilization, Jencks has stated (*38*):

> It has been pointed out that strain and destabilization of a bound substrate do not increase the second-order rate constant for an enzyme reaction, k_{cat}/K_m. . . Destabilization of the enzyme-substrate (ES) complex has no effect on the difference in energy between E + S and the transition state, $ES^{\ddagger}$, which corresponds to k_{cat}/K_m. It might therefore be concluded that strain and destabilization are not important for enzyme catalysis.
>
> Nothing could be further from the truth. Destabilization of the ES complex is absolutely essential for enzymatic catalysis. It is true that *small* changes in this destabilization have no effect on k_{cat}/K_m, but destabilization is essential for the large increase in rate that enzymes bring about. . .
>
> To obtain catalysis, the Gibbs free energy of the ES and EP complex *must* be increased so that the transition state can be reached easily from ES and EP. This represents destabilization of the ES complex. . . which can occur by physical strain, desolvation, and other mechanisms. This destabilization must be relieved in the transition state.

Fersht (*39*) has also insightfully analyzed the role of strain in enzymatic catalysis:

> Although strain may be manifested in some cases by a genuine distortion of the substrate, it is likely that strain will generally be distortionless. This could be due either to the substrate and the enzyme having unfavorable interactions that are relieved in the transition state, or to the transition state having additional binding interactions that are not realized in the enzyme-substrate complex. In both cases there would be forces that *tend* to distort the substrate toward the transition state. . . The term strain has a specific meaning in physics and engineering: it implies that an object is physically distorted. Its companion term, stress, means that an object being subjected to forces is not distorted by them. *Using these precise physical terms, it is probably apt to say that in the enzyme–substrate complex, the enzyme is often strained, whereas the substrate is often stressed* [original emphasis].

Regardless of whether a substrate is stressed or strained, it is destabilized to an equal degree: in the strain mechanism, the substrate is distorted and then the reaction occurs; in the stress mechanism, the geometrical changes equivalent to ground-state distortion occur concomitantly with approach of the transition state. In either case, catalysis results, and as such, we will simply use the term "strain" even though, as Fersht points out, "stress" is likely more accurate.

As Jencks noted in 1969 (*40*), in a statement true to this day, "The main reason that more attention has not been paid to the strain-distortion hypothesis is that it is difficult to devise experimental tests for its evaluation..." Indeed, the data are scarce, but perhaps the best experimental evidence for the use of strain in enzymatic catalysis comes from recent work on peptidyl prolyl *cis–trans* isomerases. These enzymes, most notably cyclophilin and FK506-binding protein (FKBP), catalyze the *cis* to *trans* isomerization of peptidyl prolyl bonds (*41*). (In addition, cyclophilin and FKBP are the immediate cellular targets of the immunosuppressants cyclosporin A and FK506, respectively [*41*].) Although the initially proposed mechanism for cyclophilin-catalyzed isomerization involved nucleophilic addition of a cysteine sulfhydryl to the peptide carbonyl group, followed by carbon–nitrogen bond rotation and expulsion of the cysteine (*42*), recent work has indicated that no enzymic side chains are directly involved in catalysis. For example, as Harrison and Stein concluded in 1990 (*43*):

> Taken together, our results support a mechanism involving catalysis by distortion in which [cyclophilin] binds and stabilizes a transition state...The energetic cost for -Ala—Pro- bond distortion is reflected in the large entropy of activation ($-T\Delta S$ = 13.2 kcal/mol at 300 K) that is required to distort and constrain the substrate to a conformation that allows optimal interaction with the enzyme.

A similar mechanism has been proposed for catalysis by FKBP (*44*). The fact that the cyclosporin A and FK506, which inhibit the *cis–trans* isomerase activity of cyclophilin and FKBP, respectively, each mimic a twisted peptide bond (*45*) strongly supports this proposed mechanism.

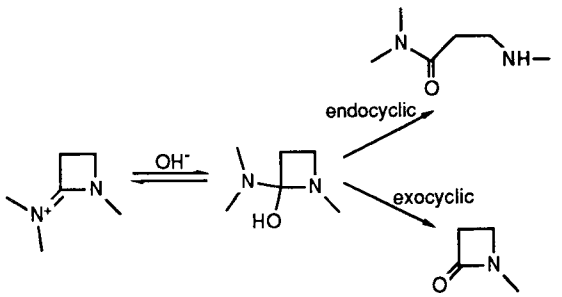
Some evidence exists that antibodies can effect ground-state strain. For example, based on free energy of activation data, both the Hilvert (*46*) and Schultz (*47*) groups reported that their "chorismate mutase" antibodies may effect catalysis via substrate strain, and a recent communication suggests that on binding to an anti-single-stranded-DNA monoclonal antibody, single-stranded DNA undergoes a significant structural change (*48*). Finally, note that a comparison of several crystal structures of antibody Fab fragments with the corresponding Fab–antigen complexes has revealed that both antibody and antigen structures can change on complex formation (*49*). That is, many antibodies are flexible and, in this regard, appear **not** to be fundamentally different from enzymes.

In summary, the role of substrate strain in catalysis has been theoretically elucidated and, to a lesser extent, experimentally verified. We may now ask what rate accelerations we might expect with antibodies raised against our ring-strained and torsionally strained analogs. To answer this question, we will consider the direct amide counterpart to each analog and ascertain its reactivity.

For the ring-strained cyclobutanol (**1** and **2**) and β-lactam (**6–9**) derivatives, the obvious strained counterpart is a β-lactam itself. For monocyclic lactams, the rates of acid- and base-catalyzed hydrolysis are slightly faster

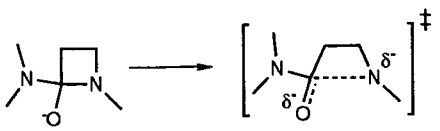
than for acyclic amides, and these rate accelerations can be accounted for by the release in ring strain on nucleophilic addition to the lactam carbonyl group—that is, as a result in the change in hybridization from sp^2 to sp^3 (*50*). For penicillin V, the rate of hydrolysis is significantly faster than that of a monocyclic β-lactam. Page (*50*) has cogently argued, however, that this acceleration is due primarily to the low pK_a (5.2) of the expelled thiazolidine amine leaving group. In short, although the strain energy in a β-lactam ring is approx 26 kcal/mol (*50*), its rate of hydrolysis is only slightly faster than that for a corresponding acyclic amide, and thus the probability of eliciting an antibody peptidase with a ring-strained β-lactam or cyclobutanol derivative may appear small.

However, a recent series of experiments by Page et al. (*51*) mitigate against this conclusion. These workers observed that under basic conditions, cyclic amidinium derivatives undergo exocyclic and endocyclic carbon–nitrogen bond fission (Eq. [3]) at approximately equal rates.



Eq. (3)

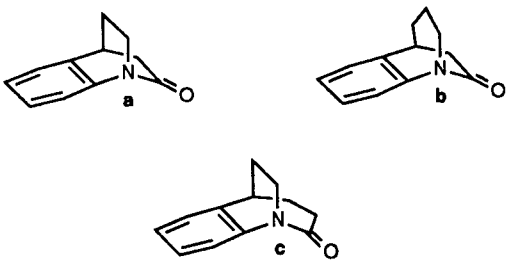
That is, even though the leaving groups are essentially the same, relief of ring strain has virtually no effect in the product-determining step. After ruling out entropic and stereoelectronic effects, the authors concluded that endocyclic ring fission was unexpectedly slow because of constraints in the transition state imposed by the four-membered ring itself: as the carbon–nitrogen bond begins to lengthen and break, two ring bond angles become acute, as is shown in Eq. (4), leading to an increase in ring strain in the transition state.



Eq. (4)

The essential point here is that a peptide distorted by an antibody raised against a β-lactam derivative will indeed be angle-strained, but not ring-strained, and hence not subject to the constraint above. Thus, a rate acceleration far greater than that for a β-lactam itself may well be observed in these antibody-catalyzed reactions.

In contrast to β-lactam derivatives, amides that have been torsionally strained hydrolyze orders of magnitude faster than their unstrained



counterparts: for example, the bicyclic amide derivatives **a–c** undergo base-catalyzed hydrolysis 2.6×10^8, 6.0×10^7, and 1.7×10^7 times faster, respectively, than the analogous acyclic species (*52*). The energy required to twist formamide 90° has been theoretically (*53*) and experimentally (*54*) determined to between 15–20 kcal/mol (whereas that for a peptide bond is presumably slightly higher), and thus, unlike with the lactams discussed above, a considerable proportion of the strain energy is translated into a rate acceleration. Although none of our torsionally distorted bicycloheptanol analogs exactly mimics the bicyclic derivatives **a–c**, each does mimic a peptide bond twisted in a similar manner, and the corresponding peptides should hydrolyze equally quickly.

CONCLUSION

We have proposed a novel strategy for the isolation of sequence-specific antibody peptidases and, ultimately, proteases. Antibodies are being elicited against transition-state analogs in which the peptide bond mimicked has also been distorted—either through ring or torsional strain —into a reactive conformation. Thus, the antibodies obtained should effect catalysis by both destabilizing the bound peptide substrate and stabilizing the transition state. To explore further the role of ground-state strain in antibody catalysis, we are also eliciting antibodies against β-lactam-containing dipeptide analogs, species that mimic only a distorted ground state. Our ultimate goal is to generate therapeutically useful antibody peptidases and proteases, such as, for example, an antibody that selectively hydrolyzes an autocrine growth factor or a viral coat protein.

ACKNOWLEDGMENTS

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DISCUSSION

D. Hansen

Green: Your presentation emphasizes the importance of the design and synthesis of antigenic haptens. An innovative approach is required in

this area to generate antibody structures capable of achieving more challenging chemistries than has been possible until now. You pointed out that the phosphonate group would play an important role, but because of the difficulty of synthesis, you chose not to incorporate that group. The phosphonate group may be crucial because of the charge rather than the tetrahedral aspect of the transition state. You seem to have taken care of the tetrahedral aspect with the hydroxyethylene group. The fact that substrates generally do not provoke catalytic antibody formation may be because of the absence of charge, and not so much because of their planar structure. The electrostatics of the catalysis, for example oxyanion stabilization, may be a critical aspect.

Hansen: We have made a variety of diastereomers of each of our analogs. If we get a lukewarm antibody catalyst from one of these analogs, our plan is to then synthesize the phosphonate derivative of that particular species. We want to screen a lot of geometries at the hydroxyethylene level, which can be done relatively easily, and then make the phosphonate derivative of the most promising analog.

Paul: Your estimate of the half-life of a peptide bond is about 100-fold greater than an earlier experimentally determined estimate of 7 yr derived using a tetrapeptide conjugated to a solid phase. Can you account for the discrepancy?

Hansen: The estimate of 7 yr came from study of a tetrapeptide, phe-phe-phe-gly, with the C-terminal gly being radiolabeled (*1*). The authors looked for release of radiolabeled gly from the solid support and estimated that the half-life for hydrolysis of the terminal phe-gly peptide bond was 7 yr. The authors also say that they saw no hydrolysis of the internal bonds like the phe–phe bonds, which would release labeled phe-gly or phe-phe-gly. Their assay was 100-times more sensitive than necessary to pick up a half-life of 7 yr. If one were to generalize, although the phe–gly exopeptide bond hydrolysis has a half-life of 7 yr, the endopeptide bond hydrolysis has a longer half-life. One could speculate about charge playing a role in rapid exopeptide bond hydrolysis. Our number of 500 yr agrees with the inferred number of 700 yr for endopeptide bond hydrolysis. I think these values are going to be dependent on the peptide sequence. Buffer effects also need to be looked at, and rates need to be determined under the conditions used for an antibody-catalyzed reaction, so that one can really determine a value for the uncatalyzed reaction.

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