Predicting Antibody Catalyst Selectivity from Optimum Binding of Catalytic Groups to a Hapten

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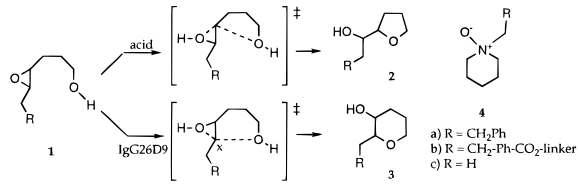
The immunological response produces amino acid residues in the variable regions of antibodies to give tight binding of the antigen. When a hapten closely resembles the transition state of a reaction, the antibody binding site may also bind the transition state strongly, resulting in antibody catalysis.[1] During the last ten years, there have been many brilliant demonstrations of hapten-induced production of antibody catalysts.[2−4]

We demonstrate a theoretical method to explore the relationship between optimum binding of a hapten and the catalytic groups which cause acceleration, and show how this method was used to predict which reaction a catalytic antibody would catalyze when confronted with a new substrate.

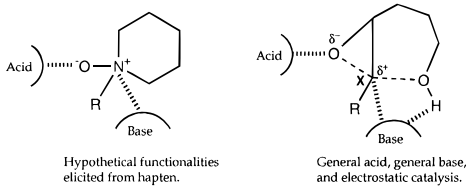
Computational Methods. Reactant, transition state, and product structures were located with GAUSSIAN 92[5]. Transition structures were optimized at the RHF/6-31G* level and tested with harmonic frequency analysis. Energy calculations at the MP2 level of theory were done by single-point calculations with the 6-31+G* basis set on RHF/6-31G*-optimized geometries. Constrained optimizations with the RHF/6-31G* basis set were performed for interactions of haptens or transition states with added catalytic groups. Energies were evaluated with MP2/6-31G* single-point calculations.

Results and Discussion. The cyclization of protonated oxirane **1a** occurs to give tetrahydrofuran **2** with aqueous acid in solution. In the presence of antibody IgG26D9,[6] elicited by hapten **4** (linker = site of attachment to protein), the major product becomes the normally disfavored tetrahydropyran **3**[7] (Scheme 1). Ab initio quantum mechanical studies showed that the six-membered transition state leading to **3** has more S_N1 character, reflected in the longer CO bond lengths and greater positive charge at the carbon undergoing substitution (carbon X in Scheme 1).[8] We hypothesized that the antibody selectively stabilizes the higher energy six-membered transition state by electrostatic stabilization of the higher partial positive charge. We report a computational test of this hypothesis and make a prediction about selectivity that the antibody should show with another substrate.

Scheme 1



Scheme 2



Epoxide opening requires either acid catalysis at the epoxide oxygen, base catalysis at the hydroxyl, or both. Both acid and base catalysis in solution give **2**. Scheme 2 shows schematically the arrangement of stabilizing groups of the hapten which could selectively stabilize the disfavored transition state.

Concurrent general acid/base catalysis (Scheme 2) is a common catalytic motif for lysozymes and other hydrolytic enzymes.[1b,9] Hapten **4** might induce binding groups that can utilize this motif to catalyze formation of pyran **3** selectively. We tested whether these appropriately oriented groups could provide selective stabilization of the six-membered transition state. Stabilizing groups were placed around the hapten, and their positions were optimized. Whereas nature tries millions of combinations and selects for the most effective, we began by testing only two possible catalytic motifs. A molecule of formic acid or methanol was placed near the oxygen of the hapten model **4c**, and the hydrogen-bond was optimized. A formate ion was placed on the other side of the molecule; this anion provides electrostatic stabilization to the positive end of the *N*-oxide dipole. The positions of these groups with respect to the hapten were then optimized. The results are shown in Figure 1. Not surprisingly, formic acid makes a stronger hydrogen bond than methanol to the hapten oxide, as reflected by the shorter OH distance of 1.57 Å for the formic acid complex versus 1.78 Å for the methanol complex. The formate molecule stabilizes the positive end of the NO dipole by electrostatic interaction.

Next, we tested the ability of this induced arrangement of functional groups to act as a catalyst.[10] The positions of the functional groups were fixed, and the hapten was replaced by the transition states previously found, minus the proton catalyst.[8] This proton is now supplied by the theozyme proton donor, and the position of this proton is optimized. Each transition state geometry was held constant, but the position of the transition state was optimized with respect to the catalytic group (see Figure 1[11]).

In each complex there is a strong hydrogen bond between the acid or alcohol and the epoxide oxygen; in addition, the formate is positioned to interact both with the alcohol hydrogen and the backside of the partially positive carbon. Both theozymes stabilize the six-membered transition state more than the five-membered one. There are stronger hydrogen bonds to the epoxide oxygen in the six-membered transition state, and

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(2) For reviews on catalytic antibodies, see: (a) Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659. (b) Mayforth, R. D. *Designing Antibodies*; Academic Press, Inc.: San Diego, CA, 1993. (c) Schultz, P. G.; Lerner, R. A. *Acc. Chem. Res.* **1993**, *26*, 391. (d) Janda, K. D.; Chen, Y.-C. *The Pharmacology of Monoclonal Antibodies*; Rosenberg, M., Moore, G. P., Eds.; Springer-Verlag: New York, 1994; pp 209−242.

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(7) (a) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734. (b) Baldwin, J. E.; Kurse, L. I. *J. Chem. Soc., Chem. Commun.* **1977**, 233. (c) Baldwin, J. E.; Thomas, R. C.; Kruse, L. I.; Silberman, L. *J. Org. Chem.* **1977**, *42*, 3846. (d) Baldwin, J. E.; Lusch, M. J. *Tetrahedron* **1982**, *19*, 2939.

(8) Na, J.; Houk, K. N.; Shevlin, C. G.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 8453.

(9) For example, see: Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman and Co.: New York, 1985.

(10) The theoretical catalyst, or theoretical enzyme, has been nicknamed a "theozyme".

(11) Calculations on both five-membered complexes for more than 200 optcycles produce the complexes shown. After the first 100 steps, the optimizations showed an oscillating pattern between optcycles, but no real change in structure or energy occurs. Both six-membered complexes are energy-minimized structures.

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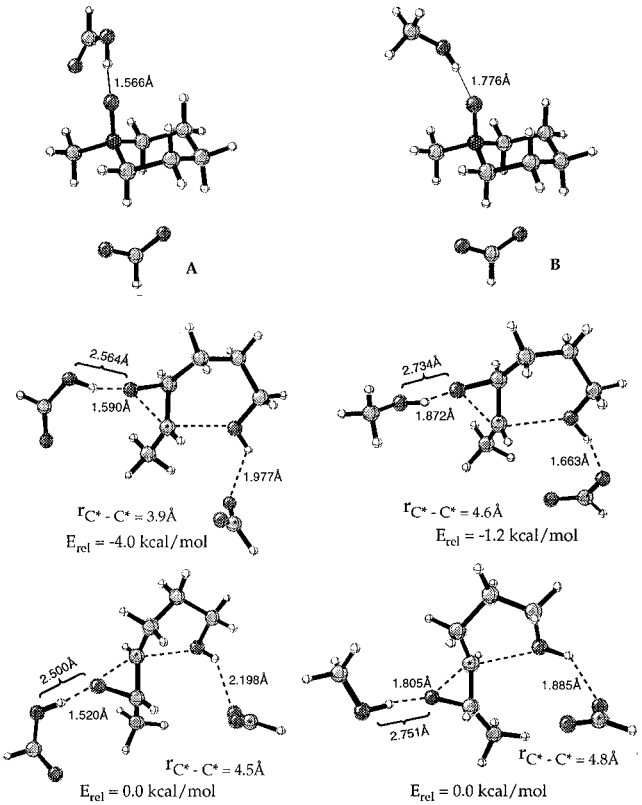
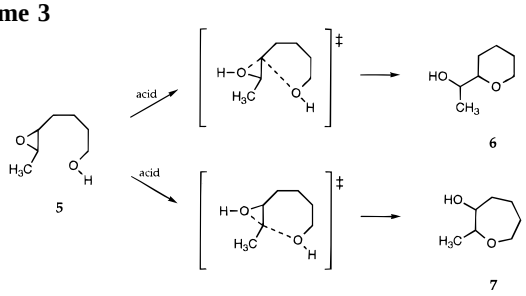


Figure 1. *N*-Oxide hapten complexed by stabilizing groups: (A) formic acid/formate; (B) methanol/formate. Shown below are complexes of the transition states of 5-exo- and 6-endo-epoxide openings of *trans*-4,5-epoxyhexan-1-ol stabilized by the theozymes.[11]

Scheme 3



the formate is better positioned to stabilize the larger partially positive charge as well.

In support of our previous hypothesis,[8] these results show that functionalities elicited in response to the charge distribution of the hapten can account for the observed selectivity by the antibody IgG26D9. Since the mechanism of catalysis we propose is based on electrostatic stabilization and not on preferential hydrophobic complementarity to a six-membered ring, we believe the antibody should also selectively catalyze other endo-epoxide openings, regardless of ring size. To test this hypothesis, we have studied the acid-catalyzed epoxide openings of *trans*-5,6-epoxyheptan-1-ol (Scheme 3).

Under normal acid-catalyzed conditions, the homologous epoxide **5** undergoes the favored 6-exo opening to form product **6**.[12] Will the arrangement of catalytic groups complementary to **4** also alter the reaction pathway? The transition states for both 6-exo- and 7-endo-epoxide openings are shown in Figure 2. There is an energetic preference of 2.6 kcal/mol for the 6-exo pathway. The 7-endo transition structure is later in terms of CO lengths and possesses more S_N1 character than the 6-exo structure, reflected in the charges at the carbon undergoing substitution (0.29 (endo) versus 0.16 (exo)).[13]

The preference for the exo pathway in the present case is not apparent in the OCC attacking angles (94° and 96°), in contrast to the 5-exo versus the 6-endo case.[7] Rather, the preference can be rationalized from the relative strain energies of the forming six- and seven-membered rings. The pyran product is 6.9 kcal/mol more stable than the oxepan, and some of this strain energy difference is manifested already in the six- and seven-membered transition states.[14]

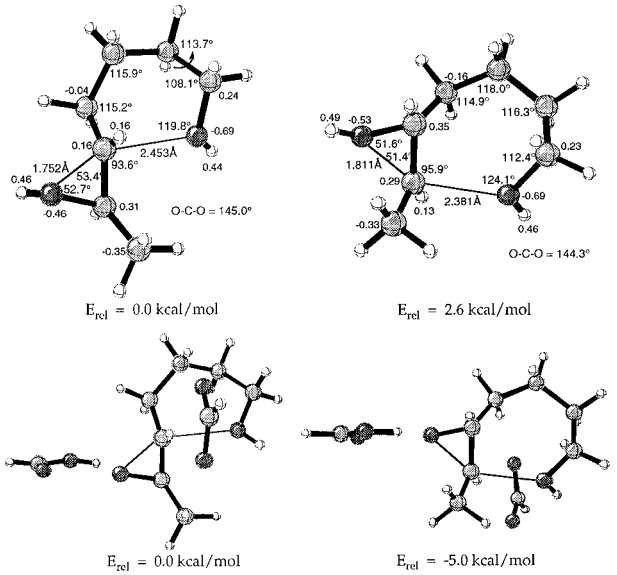
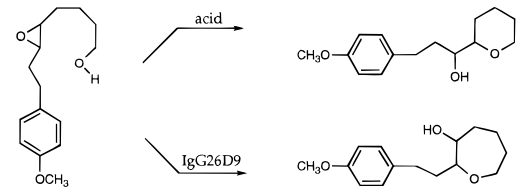


Figure 2. Transition states for the acid-catalyzed 6-exo and 7-endo cyclizations of *trans*-5,6-epoxyheptan-1-ol and the corresponding structures interacting with formic acid/formate theozyme. CHELPG charges are shown in small print.

Scheme 4



Calculations on the interaction of the formic acid/formate theozyme with the 6-exo and 7-endo transition states were carried out. As shown in Figure 2, the theozyme stabilizes both transition states, but the theozyme stabilizes the 7-endo transition state by 5.0 kcal/mol more than the 6-exo. We predict that antibody IgG26D9 should selectively stabilize the 7-endo opening of *trans*-5,6-epoxyheptan-1-ol to produce exclusively the oxepan product.

On the basis of this prediction, Janda, Shevlin, and Lerner studied the reaction shown in Scheme 4. Reactions performed under acid-catalyzed conditions confirmed previous experimental results,[12] giving a pyran/oxepan product ratio of 98:2. When the same reaction is carried out in the presence of antibody IgG26D9, the product distribution reverses to favor oxepan formation in >98% yield, with an enantiomeric excess of 78%. These results were recently published.[15]

Conclusion. The procedure reported here uses quantum mechanics to predict plausible orientations of groups binding to a hapten and to test the catalytic potential of the "theozyme".

Acknowledgment. We are grateful to the National Science Foundation for financial support of this research and to Richard A. Lerner and Kim D. Janda for helpful discussions.

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(13) The greater S_N1 character for the endo opening was found to be true for the cis case also, as well as the cis and trans 4-exo- vs 5-endo-epoxide openings.

(14) Cycloheptane is 6 kcal/mol more strained than cyclohexane: Engler, E. M.; Andose, J. D.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1973**, *95*, 8005.

(15) Janda, K. D.; Shevlin, C. G.; Lerner, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 2659.