Theozymes for Intramolecular Ring Cyclization Reactions

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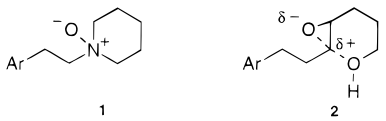
Abstract: A complex of the five-membered transition structure for intramolecular cyclization of 4,5-epoxyhexan-1-ol with the "Houk" theozyme which is 3.5 kcal/mol lower in energy than the complex previously reported as a model for the antibody IgG26D9 catalyzed intramolecular cyclization reverses the preference of that theozyme to favor furan formation. This shows the reported theozyme complex was not a global minima and therefore not a satisfactory model for the antibody reaction. We report a new theozyme favoring pyran formation consistent with the antibody result and the recently published crystal structure of the antibody Fab 5C8 with hapten.

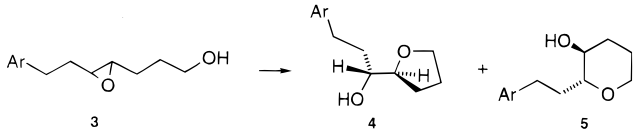
Introduction

Lerner and Janda[1] demonstrated that the catalytic antibody IgG26D9, elicited from a tertiary amine oxide antigen (hapten) **1**, catalyzes the intramolecular rearrangement of one of the enantiomers **3** stereospecifically to the tetrahydropyran **5**. This product is the result of intramolecular nucleophilic cyclization with inversion of configuration and is formed in preference to tetrahydrofuran **4** (Figure 1), the chemically favored reaction.[2] The tetrahydropyran **5** is considered to arise because the transition structure to its formation, **2**, has similarities to the



1 2

hapten **1**. We had previously reported[3] that the Lewis acid catalyzed rearrangement of an analogue of **3**, namely **6**, resulted in formation of the tetrahydrofuran ring **7**, a 5-exo-tet process, in preference to the 6-endo-tet tetrahydropyran product **8**[4] (Figure 2). Ab initio calculations[5,6] at the HF/6-31G* level of the transition structures **9** and **10** from protonated **6**[7] to the tetrahydrofuran **7** and tetrahydropyran **8** (Figure 2) are consistent



3 4 5

Figure 1. Intramolecular cyclization of **3** to **5**.

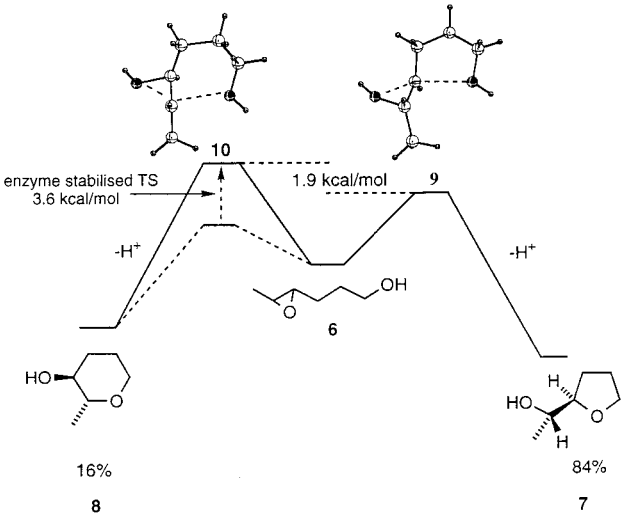


Figure 2. Schematic reaction coordinate diagram of acid-catalyzed rearrangement of epoxide.

with these experimental results since the transition structure to the former product **7** is 1.9 kcal/mol lower.[7−10] Reaction of **3** with antibody IgG26D9 to give pyran product **5** (>99%) is considered to result from antibody transition structure stabilization estimated to be ca. 3.6 kcal/mol.[5]

Houk[11] recently reported a theoretical model for the antibody transition state catalysis based on optimizing electrostatic stabilization of the *N*-oxide hapten dipole with an acid and a

(1) Janda, K. D.; Shelvin, C. G.; Lerner, R. A. *Science* **1993**, *259*, 490−493. Janda, K. D.; Shelvin, C. G.; Lerner, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 2659−2660.

(2) No tetrahydrofuran could be detected in the product.

(3) Coxon, J. M.; Hartshorn, M. P.; Swallow, W. H. *Aust. J. Chem.* **1973**, *26*, 2521−2526.

(4) This preference was subsequently encapsulated in the rules for ring closure enunciated by: Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734. See also: Norman, R.; Coxon, J. M. *Principles of Organic Synthesis*; Blackie Chapman and Hall: London and John Wiley and Sons: New York. 1993; p 678. Baldwin, J. E.; Lusch, M. J. *Tetrahedron* **1982**, *19*, 2939.

(5) Na, J.; Houk, K. N.; Shelvin, C. G.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 8453−8454. "Formation of the 5-*exo*-product is predicted to be favored by about 1.8 kcal/mol in aqueous solution. This should produce a 96:4 5-exo:6-endo ratio. To favor the 6-endo product by a similar amount, the catalytic antibody must lower the 6-endo activation energy 3.6 kcal/mol more than it lowers the 5-exo activation energy."

(6) *Chem. Eng. News* **1996**, *Sept* 30th, 35. "Using the theozyme technique, Houk has explained and experimentally verified why the reactions of a hydroxy epoxide catalysed by an antibody yields a tetrahydropyran rather than the tetrahydrofuran largely produced with acid or base catalysis."

(7) We had previously calculated transition structures for furan and pyran formation where the proton was syn to the methyl in epoxide **6** and **9** and **10**. There is negligible difference in energy and framework geometry. Coxon, J. M.; Thorpe, A. J. *J. Org. Chem.* **1999**, *64*, 5530−5541.

(8) We thank Professor Ken Houk and Dr. Jim Na for supplying their coordinates.

(9) Coxon, J. M.; Maclagan, R. G. A. R.; Rauk, A.; Thorpe, A. J.; Whalen, D. *J. Am. Chem. Soc.* **1997**, *119*, 4712−4718. Coxon, J. M.; Morokuma, K.; Thorpe, A. J.; Whalen, D. *J. Org. Chem.* **1998**, *63*, 3875−3883.

(10) The preferential formation of furan **7** via **9** reflects a more favorable co-linear trajectory of the approaching alkyl hydroxyl and the lesser strain necessary to form the five-membered ring. See also reference 7.

(11) Na, J.; Houk, K. N. *J. Am. Soc.* **1996**, *118*, 9204−9205.

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base, produced by positioning a molecule of methanol (or formic acid) to stabilize the electronegative oxygen of the hapten and a formate ion near the electropositive nitrogen. Constraints were placed on the positions of the acid and base stabilizing groups relative to each other. Removing the hapten created a theoretical catalytic cavity of the enzyme. It is this model for which the term "*theozyme*" is coined.[6,11] The 5- and 6-membered transition structures **9** and **10** for formation of furan and pyran, with the epoxide proton removed, were positioned by trial and error within this fixed theozyme cavity. The internal coordinates of the transition structures, with the epoxide proton deleted, were fixed, with the exception of the hydroxyl OH bond length which was allowed to vary. The complex was optimized specifically allowing the epoxide oxygen···H···OMe hydrogen bond lengths to optimize along with the orientation of the transition structure with respect to the formate anion.[12]

The calculations were reported[11] to show the *methanol/formate model* to stabilize the six-membered transition structure **10** over the five-membered transition structure **9** by 1.2 kcal/mol at the HF/6-31G* level. The six-membered transition structure was considered to be more stabilized relative to the five-membered transition structure since in the former the developing carbocation center was closer to the formate anion. If these complexes are global minima then the relative energy of the complexes gives support for the hypothesis that electrostatic stabilization from the antibody IgG26D9 occurs to a greater extent when bound to the six-membered transition structure **10** than to the five-membered transition structure **9**. We now report a study that shows that this was not a global minimum and comment on the enzyme pocket and its importance in establishing regiochemistry.[13]

Computational Methods

Initial conformations of the methanol/formate stabilized *N*-oxide hapten were determined through extended grid calculations at the AM1 level. Ab initio calculations at the HF/6-31G* level using the Gaussian94 program[14] were performed on the lowest energy hapten stabilized conformations. Five- and six-membered transition structures optimized at the HF/6-31G* level[8] were used in conjunction with the various methanol/formate models.[8] The transition structures, with the epoxide proton removed, and with constraints applied to all internal bond lengths, angles, and torsions (except the alkyl oxygen-proton bond length), were optimized with respect to methanol and formate ion, whose positions relative to each other were also constrained.

Results

Our results question the validity of the specific methanol−formate theozyme model reported[11] since we find a complex of the five-membered transition structure with the published theozyme which is 3.5 kcal/mol lower in energy than the published complex thereby favoring furan formation in parallel to an acid-catalyzed reaction in solution.[15] We report a theozyme for which the 6-membered transition structure is more stabilized than the 5-membered transition structure consistent with the antibody result.

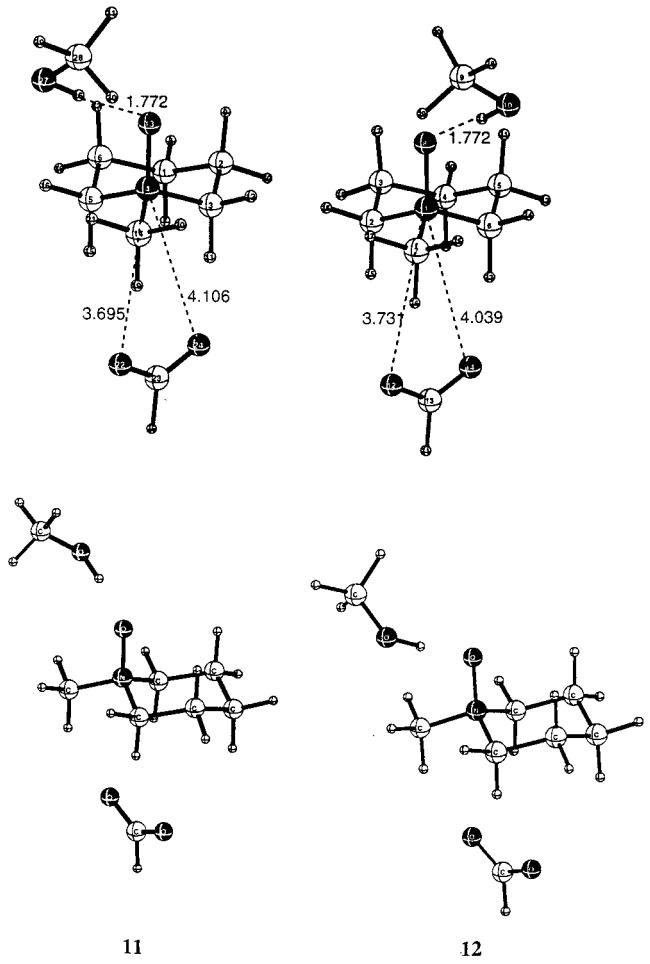

Figure 3. Hapten model bound to the theozymes as generated by Houk[8] (**11**) and the present work (**12**). Two views of each complex are given.

In attempting to reproduce the structure of the published theozyme, and in the absence of published coordinates, we produced a structure of the hapten stabilized by methanol and formate anion **12** which has coincidently the same energy as the Houk theozyme/hapten complex **11** but with a different orientation of methanol relative to the formate ion (Figure 3).

Knowing that our theozyme was different from that already published[11,8] we examined the positioning of the 5- and 6-membered transition structures within the cavities of both theozymes. In attempting to reproduce the published complexes we located a complex of the published theozyme[8] with the 5-membered transition structure, **15**, 3.5 kcal/mol lower in energy than the reported analogous 5-membered transition structure complex **14** (Figure 4) and 2.3 kcal/mol lower than the 6-membered transition structure complex **13**.[16] This result excludes the published methanol/formate theozyme as a model supporting the catalytic antibody experiment.[17] Closer inspection

(12) It was envisaged that hydrogen bonding between the methanol proton and the epoxide oxygen and between the hydroxyl proton of the deprotonated transition structures **9** and **10** and the formate anion is the important feature of the catalytic stabilization. In addition the carbocation would be stabilized by the formate anion.

(13) Gruber, K.; Zhou, B.; Houk, K. N.; Lerner, R. A.; Shevlin, C. G.; Wilson, I. A. *Biochemistry* **1999**, *38*, 7062−7074.

(14) Gaussian 94, Revision D.2. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1995.

(15) Houk noted that "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 energy occurs. Both six-membered complexes are energy-minimized structures."[11]

(16) We recognise the important contribution by Houk in support of electrostatic catalytic stabilization from a theozyme, and believe an alternate positioning of the methanol/formate ion is required to support the experimental result.

(17) The reported model is not a global minima complex.

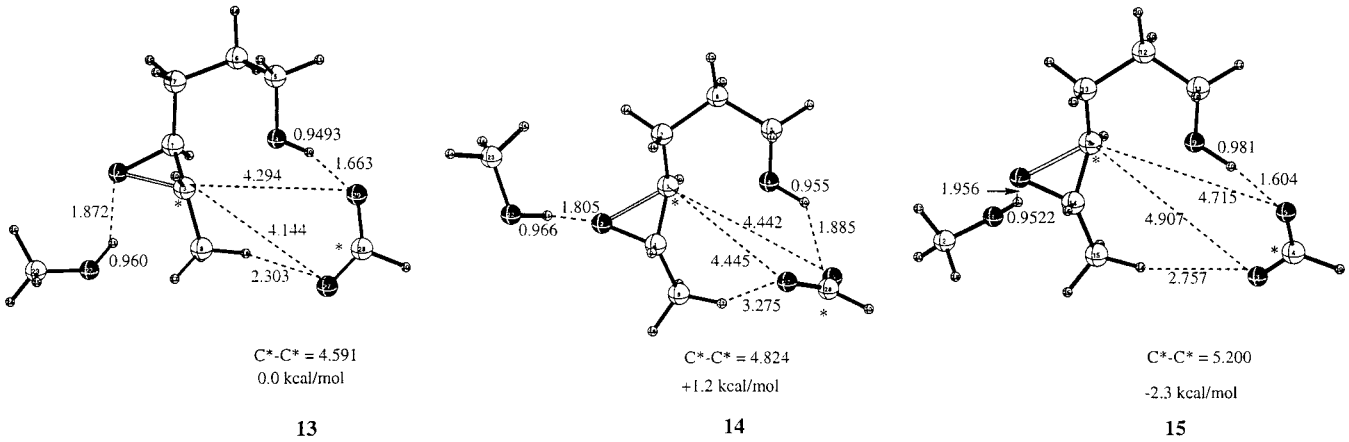

Figure 4. Complexes of 5- and 6-membered transition structures with the Houk theozyme.

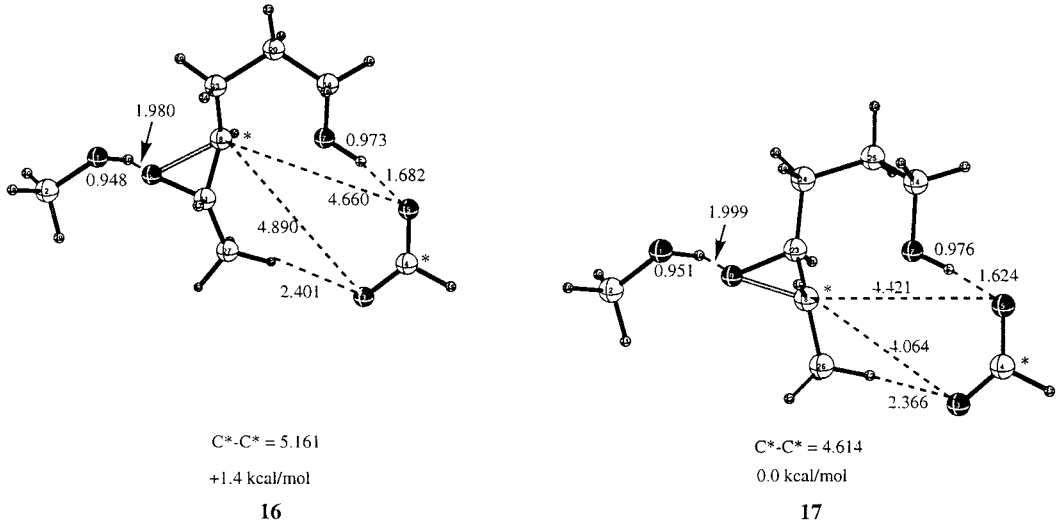

Figure 5. Complexes **16** and **17** of 5- and 6-membered transition structures stabilized with theozyme.

of the five-membered transition structure complex with the published theozyme **14** shows the methanol methyl and transition structure alkyl chain to be sterically crowded. This crowding is not present in the lower energy 5-membered transition structure theozyme complex **15**. In addition the hydrogen bond distance of the formate and substrate hydroxyl is reduced in **15** relative to **14**.

The stabilization of the new lower energy 5-membered transition structure complex **15** relative to the 6-membered transition structure complex **13** is not due to a more favorable electrostatic stabilization of the carbocation with the formate ion (see Figure 4) since the carbocation center is positioned further from the formate ion in the former complex. The methanol hydrogen bond length to the epoxide oxygen is longer in **15** than in **13**; however, the hydrogen bond between the formate anion and the alkyl hydroxy proton of the transition structure in the former is shorter than in **13** by a similar amount.

In our search for the global minima of the complexes of the 5- and 6-membered transition structures with our theozyme we found lowest energy complexes where the 6-membered transition structure **17** was lower in energy by 1.4 kcal/mol to the 5-membered transition structure **16** (Figure 5). Both complexes **16** and **17** are lower in energy than the corresponding complexes **14** and **13**. For the complexes **16** and **17**, the transition structures are inserted between the methanol/formate complex in a similar relative position (see Figure 5) in contrast to **14** and **13** where this is not the case. The greater stabilization of **17** over **16** is manifested by the closer proximity of the carbocation of the six-membered transition structure to the formate anion and by a shorter bond length between the formate anion and the alkyl hydroxy proton.

Conclusion

The results of this study support the experimental antibody results of Lerner and Janda[1] and show the need for caution in predicting the structure of theozymes since conformational space needs to be fully explored. The study in itself does not rule out the need for more than two substituents to characterize a theozyme to define an appropriate transition state complex. However, in combination with the recently published X-ray crystal structure of the antibody 5C8 and hapten, the study provides evidence that the proton and anion stabilization in a pocket allows the transition structures to exist in an optimum orientation with the proton and anion. The pocket appears to facilitate rather than dictate.

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