Design of the hapten for the induction of antibodies catalyzing aldol reaction

Kazuo Teraishi,† Minoru Saito, Ikuo Fujii, and Haruki Nakamura

Protein Engineering Research Institute, Osaka, Japan

†Tonen Corp., Corp. R & D Lab., Nishi-Tsurugaoka, Ohimachi, Iruma-gun, Saitama, Japan

The transition states of the aldol reaction and their analogues are reported here for the design of the haptens generating catalytic antibodies. The structural and the electrostatic properties were calculated by an ab initio *molecular orbital method and were compared using a graphic software. Two transition states that lead to the corresponding stereo isomers of the aldol products were characterized. Also, the suitable transition state analogues were found. It is suggested that the stereo selectivity can be controlled using the catalytic antibodies elicited against the haptens designed here.*

Keywords: ab initio *molecular orbital, structure, charge, catalytic antibody, hapten, aldol*

INTRODUCTION

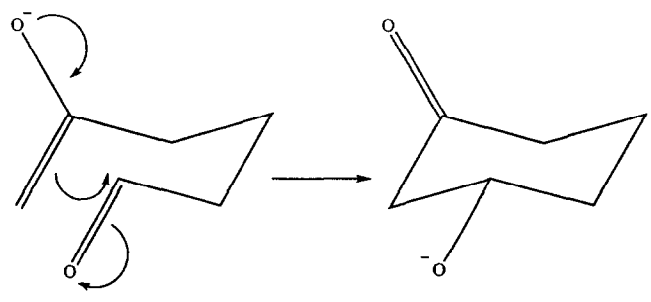
In our previous work,[1] we have demonstrated the similarity between the tetrahedral intermediates and phosphorous-containing analogues in the ester and amide hydrolysis by means of *ab initio* molecular orbital calculations. Here, we report the application of our methodology to the aldol reaction, proposing the transition states and their analogues: the latter being possibly useful haptens for the induction of antibody-catalysts.

Aldol condensation is one of the most important reactions which create a new covalent bond between two carbon atoms. The reaction mechanism and the stereo selectivity have been studied for many years, both experimentally and computationally.[2–5] The stereo selectivity of the reaction is decisive according to the transition state, which is controlled by the catalyst being employed. In the base-catalyzed aldol condensation, for example, the favor of the transition state (TS) with the enolate double bond oriented antiperiplanar to the carbonyl group (T.S.1, shown below) gives the corresponding aldol stereo selectivity.[6] The catalytic antibody has a potential to be a catalyst that yields the product with desired stereo chemistry, because if the structure of the transition state on a reaction path leading to a desired product is known and its analogue can be found, it is possible to raise a catalytic antibody that catalyzes the reaction via only that path using the analogue as a hapten. Especially, the important point is the possibility to catalyze the reaction pathway which is disfavored under the conventional catalyst.[7] In our institute, some antibodies were raised against the haptens that were designed to imitate the transition state structure, and showed indeed the catalytic activity with the expected specificity.[8,9]

In this study, the unimolecular cyclization reaction (Figure 1) is studied, where the enolate ion (1) may be prepared from the keton by the base catalyst. For this reaction, two transition states corresponding to the different reaction path and their analogues are calculated.

Design of transition state analogues

Two transition states (T.S.1 and T.S.2) are calculated (Figure 2). For the reactants possessing substituents at C^1, the products through T.S.1 and T.S.2 are distinguishable. Therefore, it is worth investigating the analogues of T.S.2, as well as T.S.1, from the viewpoint of the stereo selectivity control by the catalytic antibody. For each TS, three kinds of analogues are designed (Figure 3). In this figure, *R* denotes the linker of the antigen with carrier proteins, and was expressed by methyl in the calculation. The first kind of analogue (A.11 for T.S.1, A.12 for T.S.2) is a six-membered ring containing a phosphorous. Phosphorous was used because a



1 2

Figure 1. Mechanism of the aldol reaction studied here.

Color Plates for this article are on page 292.

Address reprint requests to Dr. Nakamura at the Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka, 565, Japan.
Received 22 February 1994; accepted 23 March 1994

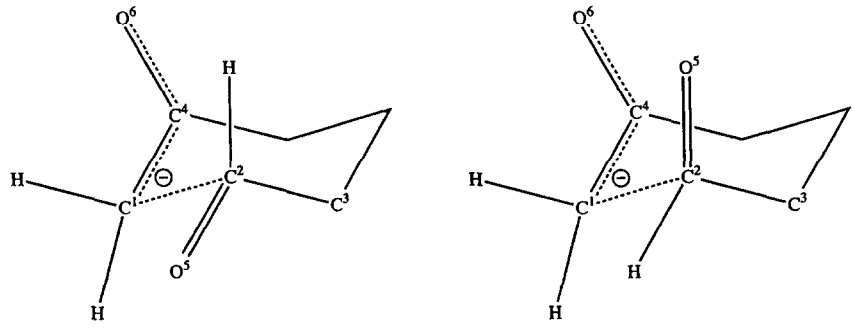


Figure 2. Two transition state structures leading to the different stereo products.

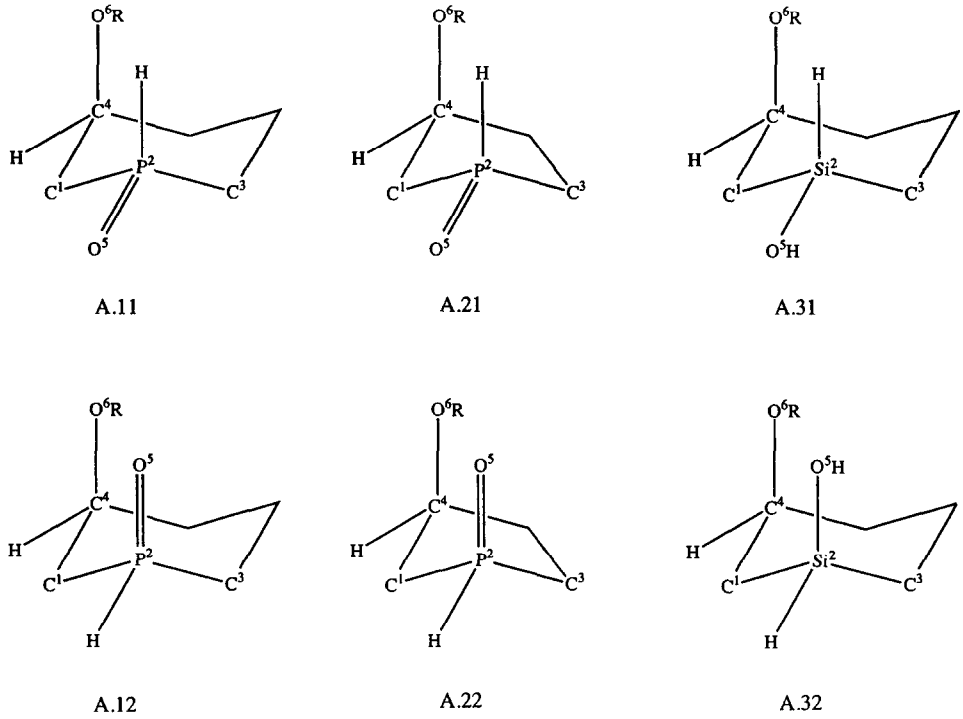


Figure 3. Three kinds of analogues for T.S.1 (A.11–A.31) and T.S.2 (A.12–A.32). R *is the linker of the antigen.*

P—C bond is longer than a covalent C—C bond, and should be a suitable analogue for the forming C—C bond in the transition state. The second kind (A.21 for T.S.1, A.22 for T.S.2) is a five-membered ring containing a phosphorous. These compounds are of particular interest because the five-membered ring is strained and thus might resemble the distorted structure of transition state. The third kind (A.31 for T.S.1, A.32 for T.S.2) is a six-membered ring containing a silicon. These compounds have an Si—C bond, which is even longer than a P—C bond. Silicon has the character of a metal and bears a positive charge, which would imitate the charge distribution around the electrophilic carbon.

Calculations

All the geometry optimizations and electrostatic potential (ESP) and electron density calculations were performed by an *ab initio* molecular orbital (MO) method at the MP2/6-31 + G* level. Geometries are optimized by Berny method[10] with no constraints on the variables. Hessian matrices are positive definite for the analogues, and have only one negative eigenvalue for the transition states. The largest element in the eigenvector whose eigenvalue is negative corresponds to the distance between two carbons that are about to form a bond. Atomic charges are least-squares fit to reproduce the ESP around the molecule.[11] Gaussian 90,[10] running on a Fujitsu VP-2600 at Protein Engineering Research Institute, was used for all MO calculations.

The structures and the charges were compared by taking a root mean square deviation (rmsd). All heavy (non-hydrogen) atoms were concerned in the comparison between A.11, A.12, A.31, and A.32 and the transition states, while five atoms involved in the reaction (two oxygens, two carbons, and the phosphorous or its counterpart) were taken

into account for the comparison between A.21 and A.22 and the transition states. For the structural comparison, first the atoms used for the comparison were superimposed and then the rmsd was calculated.

The electron density around the molecule was calculated, and the isosurface was illustrated using Application Visualization System (AVS). The ESP on the surface was indicated by color. Because the transition states have a negative charge, the ESP becomes negative entirely if the surface is far from the nuclei. Therefore we chose 5×10^{-2} electrons/Bohr^3 isosurface for transition states and 2×10^{-2} for analogues.

RESULTS AND DISCUSSION

From the structural data (Table 1), although P—C and Si—C bonds of analogues are longer than the covalent C—C bond (C^2–C^3), they are shorter than the forming C—C bond (C^1–C^2) in the transition states. The cancellation of these deviations results in a good agreement of the overall shapes (Table 2). The positive atomic charge of the electrophilic carbon (C^2) and the negative charge of the nucleophilic carbon (C^1) are expressed well by the charge distribution of P or Si and the carbon attached to them. As expected, phosphorous and silicon are generally good substitutes to imitate a forming C—C bond both structurally and electronically.

The comparison showed that A.11 and A.12 are the most similar to T.S.1 and T.S.2, respectively (Table 2). A.31 and A.32 are slightly less similar to T.S.1 and T.S.2. than A.11 and A.12. The similarity of A.21 and A.22 are poorer than the other analogues, especially with regard to the atomic charges. The most analogous A.11 and A.12 will be discussed in detail below.

The similarity can be seen instantly from the isosurface pictures (color plates), where besides the methyl group, the shape and the color (ESP) of the analogues resemble their respective transition states. However, for the quantitative discussion, the values in Table 1 should be compared with the results of ester hydrolysis,[1] for which phosphonate is

Table 1. Structural data of two transition states and the lowest energy conformers of three analogues. *X* is C for T.S.1 and T.S.2; P for A.11, A.21, A.12, and A.22; and Si for A.31 and A.32, respectively.

		T.S.1	T.S.2	A.11	A.21	A.31	A.12	A.22	A.32
distance (Å)	C^1X^2	2.352	2.449	1.822	1.834	1.880	1.828	1.840	1.885
	X^2C^3	1.524	1.520	1.818	1.839	1.870	1.820	1.830	1.878
	C^1C^4	1.397	1.402	1.525	1.521	1.526	1.526	1.526	1.526
	X^2O^5	1.251	1.244	1.511	1.511	1.690	1.507	1.508	1.680
	C^4O^6	1.279	1.273	1.432	1.432	1.436	1.425	1.427	1.444
angle (degree)	$C^1X^2C^3$	96.29	92.84	101.79	95.16	104.07	101.35	95.24	103.10
	$C^4C^1X^2$	90.16	88.41	113.27	104.40	109.91	113.17	105.28	109.06
	$O^5X^2C^1$	109.02	111.46	114.95	117.34	111.14	118.15	119.23	109.73
	$O^6C^4C^1$	126.27	126.46	105.10	104.58	104.80	105.35	105.03	105.31
torsion (degree)	$C^4C^1X^2C^3$	60.5	65.1	47.1	17.7	45.7	48.0	10.3	48.9
	$O^5X^2C^1C^4$	−174.9	−60.1	173.6	142.5	160.5	−80.4	−117.8	−71.4
	$O^6C^4C^1X^2$	89.4	94.4	65.3	76.7	63.4	66.5	82.8	61.7
charge	C^1	−0.92	−0.96	−0.52	−0.35	−0.66	−0.48	−0.30	−0.91
	X^2	0.69	0.57	0.93	0.83	1.10	0.88	0.76	1.19
	C^4	0.46	0.53	0.29	0.20	0.41	0.29	0.15	0.47
	O^5	−0.71	−0.60	−0.72	−0.69	−0.88	−0.67	−0.66	−0.85
	O^6	−0.71	−0.67	−0.37	−0.38	−0.41	−0.34	−0.33	−0.40

Table 2. Structural and atomic charge comparison between two transition states and the three analogues.

	T.S.1			T.S.2	
	structure (Å)	charge (a.u.)		structure (Å)	charge (a.u.)
A.11[a]	0.23	0.22	A.12[a]	0.28	0.27
A.21[b]	0.28	0.32	A.22[b]	0.37	0.39
A.31[a]	0.27	0.23	A.32[a]	0.31	0.28

[a] rmsd concerning only heavy (non-hydrogen) atoms.
[b] rmsd concerning five atoms involved in the reaction (2 oxygens, 2 carbons and the phosphorous).

known to work well as a hapten. Structural deviation of A.11 from T.S.1 is almost the same as phosphonate from ester hydrolysis intermediate (0.23 v.s. 0.20 Å), but the charge difference is about twice (0.22 v.s. 0.11 a.u.). Structural and charge differences between A.12 and T.S.2 are yet larger than the differences between T.S.1 and A.11. Although the deviations are slightly larger than in the case of ester, we can conclude that A.11 is a good analogue for T.S.1, while A.12 imitates T.S.2 quite well. This result suggests the possibility to control the stereo selectivity of aldol reaction by choosing the appropriate analogue as a hapten.

The analogues may flip to the conformers. For example, from A.11, O^6R becomes equatorial and O^5 becomes axial. Physically, A.11 and its conformer coexist with a certain ratio in solution. Therefore, although the antibodies which recognize the undesired conformer might be induced, the ones which recognize A.11 shall also be induced.

Conclusion

A calculation was made for the structures of two transition states of the aldol reaction associated with the different pathways, and the analogues that imitate respective transition states. Quantitative comparison by means of an *ab initio* molecular orbital method revealed that the analogues A.11 and A.12 are respectively analogous to T.S.1 and T.S.2, indicating the possibility of inducing a catalytic antibody that catalyzes the only pathway that leads to a desired product. The synthesis of these compounds is currently being studied in our laboratory.

ACKNOWLEDGMENTS

We acknowledge Yoshiharu Iwabuchi in our institute for comments on the compounds explored here.

REFERENCES

1 Teraishi, K., Saito, M., Fujii, I., and Nakamura, H. *Tet. Lett.* 1992, **33,** 7153–7156
2 Li, Y., Paddon-Row, M.N., and Houk, K.N. *J. Am. Chem. Soc.* 1988, **110,** 3684–3686
3 Li, Y., Paddon-Row, M.N., and Houk, K.N. *J. Org. Chem.* 1990, **55,** 481–493
4 Denmark, S.E. and Lee, W. *Tet. Lett.* 1992, **33,** 7729–7732
5 Goodman, J.M., Kahn, S.D., and Paterson I. *J. Org. Chem.* 1990, **55,** 3295–3303
6 Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry.* Pergamon Press, Toronto, 1983, Chapter 8, pp 302–308
7 Janda, K.D., Shelvlin, C.G., and Lerner, R.A. *Science.* 1993, **259,** 490–493
8 Miyashita, H., Karaki, Y., Kikuchi, M., and Fujii, I. *Proc. Natl. Acad. Sci. USA,* 1993, **90,** 5337–5340
9 Iwabuchi, Y., Miyashita, H., Tanimura, R., Kinoshita, K., Kikuchi, M., and Fujii, I. *J. Am. Chem. Soc.* 1994, **116,** 771–772
10 Frisch, M.J., Head-Gordon, M., Trucks, G.W., Foresman, J.B., Schlegel, H.B., Raghavachari, K., Robb, M.A., Binkley, J.S., Gonzalez, C., Defrees, D.J., Fox, D.J., Whiteside, R.A., Seege, R., Melius, C.F., Baker, J., Martin, R.L., Kahn, L.R., Stewart, J.J.P., Topiol, S., and Pople, J.A., *Gaussian 90.* Gaussian, Inc., Pittsburgh, PA 15213, 1990
11 Singh, U.C. and Kollman, P.A. *J. Comp. Chem.* 1984, **5,** 129–145