

On the Concept of Orbital Steering in Catalytic Reactions

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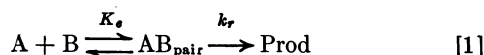
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ABSTRACT Angular displacement from linear overlap of but a few degrees in the transition state of the enzyme-substrate complex has been postulated to be of great kinetic significance ("orbital steering"). The concept of orbital steering is shown to have evolved from the orientation parameters of an equation previously proposed to evaluate the kinetic importance of propinquity. This equation is shown to be naive. Arguments provided against the concept of orbital steering include: (a) force constants predicted from orbital steering are about 100 times those experimentally determined from displacement of nuclei in a direction normal to the axis of a covalent bond (for example, at room temperature vibrational bending amplitudes of $+5^\circ$ or more are common); (b) because of the lessened directionality of orbitals containing nonbonded electron pairs, the force constants in transition states should be even smaller than in the case of a covalent bond; and (c) molecular orbital calculations predict shallow total energy minima for orbital alignment. The experimental rate data offered as a basis for the concept of orbital steering are shown to find rationalization in the previously observed dependence of ΔS^\ddagger on kinetic order and the energy requirements for the freezing-out of single bonds in the transition state leading to the formation of medium-size ring compounds from extended ground states. It is concluded that if orbital steering does exist, experimental and theoretical evidence to support this concept have yet to be presented.

A task of the physical organic chemist has been one of providing useful models for enzymatic processes. Any proposed model must be consistent with what we know to be true in terms of the fundamental theories and principles of basic physics and chemistry. In this paper we shall present a discussion of the implications of a recently proposed model, termed orbital steering (1), whose purpose is to explain the large enhancement of the rates of a particular set of intramolecular reactions over their intermolecular counterparts. We will in addition present an alternative interpretation of the experimental observations in question, based on thermodynamics and reaction rate theory.

DISCUSSION

The contribution of the proper approximation of reacting groups in the productive enzyme-substrate complex to the efficiency of enzyme catalysis has been widely discussed. A very popular treatment (2) holds that the contribution of approximation cannot be great. The simple physical model supporting this view follows: under the *pseudo*-first order conditions of $[A] \gg [B]$ and the assumption that A and B are of size comparable to water molecules

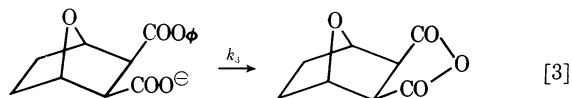
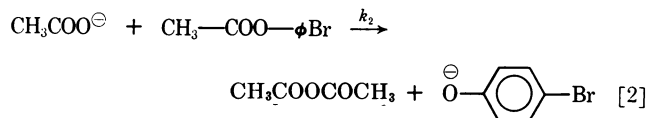


$$K_e = \frac{55.5}{\text{number of nearest neighbor molecules to B}} = \frac{[AB]_{\text{pair}}}{[A][B]}$$

$$v = (55.5/n) k_r [A][B]$$

From Eq. [1] it follows that if A and B were approximated, as in an intramolecular reaction, the rate constant would only be $55.5/n$ M greater than that for the bimolecular reaction proceeding *via* the AB_{pair} . At the maximum, where the atom undergoing reaction can have only one nearest neighbor in solution (solvent or B), approximation should then only afford a 55.5-fold rate constant enhancement. The basic assumption of Eq. [1] is that the equilibrium constant for the complexation of A and B is completely independent of the nature of the reactants; that is, the equilibrium constants for all reactions of this type are universally equal to $55.5/n$. We know, however, that equilibrium constants typically vary over many powers of ten. In essence, the above assumption ignores any attractive or repulsive forces between the reacting A and B molecules.

The kinetics of intramolecular reactions have been reviewed by one of the authors recently (3, 4) and it suffices to simply state that rate ratios ($k_{\text{intra}}/k_{\text{intermolecular}}$) of the order of 10^3 – 10^8 M are common. The most investigated system involves nucleophilic catalysis, *via* intermediate anhydride formation, of carboxyl-anion-catalyzed ester hydrolysis. The rate ratio for reactions 2 and 3 is $\sim 10^8$ M in H_2O and $\sim 10^9$ M in 1.0 M H_2O in dimethyl sulfoxide (5). According to ref. 1,



rate ratios such as the one cited above, in excess of those predicted by Eq. [1], can be rationalized by the inclusion of large orientation factors ($1/\theta$), with the production of Eq. [4]:

$$v = (55.5/n)(1/\theta)k_r[A][B] \quad [4]$$

For Eq. [4] to account for the observed rate ratio of k_3/k_2 of approximately 10^8 M, θ would have to be about 10^{-7} – 10^{-6} .

It is worthwhile first to examine the implications of a θ factor which is as small as this in terms of the structures of

the reacting molecules. We can then examine the nature of the energy surface that describes the interaction of the reacting groups as a function of the angles and distances implied by the corresponding θ factor. If we consider as an approximation to a reacting group a sphere, which has on it a small reactive portion representing only θ of the total surface of the sphere (Fig. 1), then it is possible to estimate the size of the angle, 2α , and hence obtain a measure of the criticality of orientation implied by a particular θ factor. If the reacting spot is taken to be a circle with radius r and R is the radius of the sphere, the ratio of the area of the surface that leads to reaction to the total surface area available is given by

$$\theta \approx \pi r^2 / 4\pi R^2 \quad [5]$$

Then

$$\alpha = r/R \approx 2\theta^{1/2} \text{ radians} \quad [6]$$

Converting to degrees gives the approximate result

$$\alpha \approx 10^2 \theta^{1/2} \text{ degrees} \quad [7]$$

Explanation of the observed rate ratio of k_3/k_2 would require α to be about 0.1 degree. Further, if we assume R to be on the order of van der Waals radii, for example 2.5 Å, then the diameter of the reactive portion of the surface is no greater than 0.01 Å.

In summary, the above calculation implies that the rate of a reaction would change by a factor of θ in going from the most favorable to a less favorable orientation and that the displacements involved amount to only tenths of degrees and hundredths of angstroms.

At this point in our discussion, it is appropriate to consider rate changes in terms of the changes in the activation energies involved. A rate change of 10^6 involves a corresponding change of about 7 kcal/mol in the activation energy of the reaction. If orbital steering is to be operative, then this change in activation energy would have to occur with atomic displacements of the same order of magnitude as those discussed above. Of interest, then, is the nature of the multidimensional energy surface that describes the interaction of the enzyme and the reacting groups. It is well known that the overall energy changes accompanying a chemical reaction can be very large; however, our interest is in the magnitude of the energy changes associated with only very small changes in the reaction coordinate. If these changes are large then one could expect large changes in activation energy and hence rate of reaction as a function of small differences in orientation. Therefore, we are interested in the relative smoothness of the energy surface near or along the path of the reaction coordinate. Although little is known about the detailed nature of these energy surfaces in complex molecules, a great deal is known about the nature of the chemical bond and in particular the behavior of the energy as a function of the relative positions and orientations of the atoms involved in the bond. Hence in order to estimate the character of the energy surface along the reaction coordinate, we shall consider the nature of the forces and energies associated with simple molecular systems. An appropriate example involves the torsional oscillation of $\text{H}_2\text{C}=\text{CH}_2$, which provides information about the energy of the C-C π -bond as a function of the amount of overlap of the carbon p orbitals. The potential energy as a function of the angle, β , between the two CH_2 groups and hence the carbon p orbitals

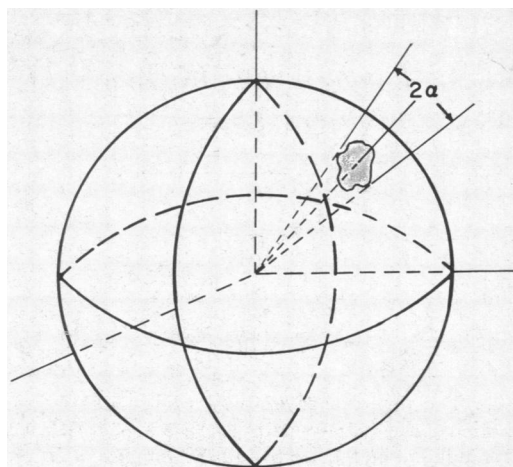


FIG. 1. A spherical approximation to a reactive group having only a small reactive portion on its surface, denoted by the darkened area. The magnitude of the angle, 2α , is an indication of the criticality of the relative orientation of the group in order for reaction to be possible.

is given by

$$V(\beta) = V_2/2(1 - \cos 2\beta) \quad [8]$$

where V_2 is about 25 kcal/mol (6). The derivative of the energy with respect to the torsional angle at its equilibrium configuration is zero; however, the maximum value of the derivative which occurs at $\pm 45^\circ$ (and $\pm 135^\circ$) is still small and amounts to only about 450 cal/degree. Further, it is important to note that this energy function is determined with the molecule in its most stable configuration; as the distance between interacting groups or orbitals increases we would expect a marked overall decrease in the interaction energy. Hence, even for this very rigid system, changes in the relative orientation of the interacting groups of $10\text{--}15^\circ$ are required to account for an energy change of 7 kcal/mol.

The mean vibrational amplitudes as determined from spectroscopic and electron diffraction measurements on simple molecules provide another source of information regarding how readily molecules distort and the associated energy changes. At 298°K, where the thermal energy available is less than 0.6 kcal/mol, they range from 0.03–0.16 Å, although the values 0.05–0.09 Å are more representative (7, 8). This then implies that as a consequence of the zero-point or higher vibrations in the molecule, there are variations in the atomic positions of the same order of magnitude. The important conclusion to be drawn from these examples is that the energy functions describing the interactions between atoms or groups of atoms found in molecules are not rapidly varying functions with respect to small changes in angles and displacements. In fact, the force constants implied by the orbital steering model are about 100 times larger than those that are observed. An alternative way of expressing this conclusion is that if a very small change in the relative orientation of orbitals in the transition state could account for magnitudes of variation in rate constant, then one would anticipate that angle strain would be very large for displacement of nuclei in a direction normal to the axis of a covalent bond; indeed, the force constants are not great for this type of motion, and thermal agitation at room temperature provides for vibra-

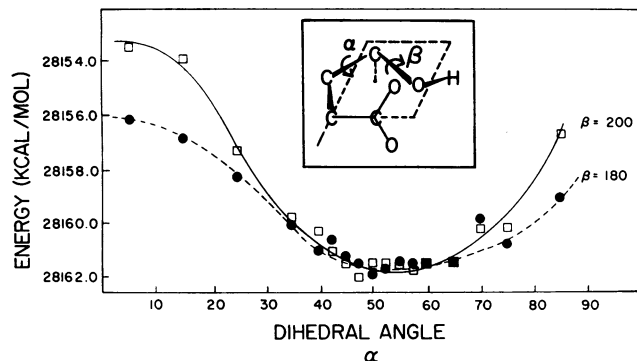


FIG. 2. The dependence of total energy on the rotational conformation of the hydroxymethylene group of 4-hydroxybutyric acid. The angles, α and β , are the dihedral angles in degrees made by the C_2-C_3 and C_4-O bonds and the C_3-C_4 and $O-H$ bonds respectively (see inset). $\alpha \equiv \beta \equiv 0^\circ$ in the eclipsed rotamer.

tional bending amplitudes of $\pm 5-10^\circ$ or more (8). An even greater displacement should be allowed in the transition state. Thus, electrons in a lone-pair orbital occupy rather large and diffuse clamshell-shaped orbitals. Only when bonded does the orbital become sausage-shaped, with the electrons more restricted to the space between the nuclei (9). In the transition state, overlapping orbitals should have a rather bulbous nature and if the transition state is reached about half-way along the reaction coordinate, the dependence of ΔF^\ddagger upon a few degrees of displacement normal to the axis of the incipient bond should be trivial.

So far we have only been considering small molecules; however, if we extend the above arguments to very large systems having many internal degrees of freedom so that changes in conformation can occur in a cooperative fashion, one would conclude that the energy changes associated with small changes in orientation would be virtually negligible. In summary, simple vibrational and torsional amplitudes at $RT \approx 0.6$ kcal/mol are many times greater than the small displacements implied by the orbital steering model. Indeed, as the temperature is raised and the available thermal energy is distributed among the vibrational modes, with corresponding increase in vibrational amplitudes, one would expect that the ease with which exact orbital alignment could be achieved would as a consequence decrease as a function of temperature. Therefore, one might inquire whether orbital steering would require that the reaction rate decrease as the temperature is increased.

The development of a semiempirical (self-consistent field)-(linear combination of atomic orbital)-(molecular orbital) approach for the evaluation of preferred conformations by Pople and Segal (10) has prompted us to examine the conformational surfaces of 4-hydroxybutyric acid, the simplest molecule in which orbital steering has been postulated. The coordinates of the nuclei were calculated using dihedral angles and bond lengths which were average values published for similar molecules (11). Bond angles were assumed to be tetrahedral, except for the carboxyl carbon, where an angle of 120° was assumed. The conformation that was assumed to approximate the transition state, and the parameters defining the rotations examined, are shown in Fig. 2.

If the orbital steering hypothesis is correct, the angular dependence of such a phenomenon would produce a very steep potential well which will be observable only over a very nar-

row range of dihedral angles. Thus, when the C_1-O internuclear distances are reasonably short, a steep decrease in total energy should be observed as the critical orientation is approached.

The C_1-O internuclear distance varied between 1.923 and 2.070 Å for the rotation of the hydroxymethylene group. The variation in energy for rotation of the hydroxymethylene group is illustrated in Fig. 2 for two typical examples. Similar curves for rotation of the hydroxyl group are presented in Fig. 3. It is noteworthy that the calculated energies form a very broad local minimum. Furthermore, this local minimum corresponds to the conformational minimum one would predict on the basis of bond positions. In the absence of any bonding interactions between the carboxyl and hydroxyl groups, the barrier to rotation of the hydroxymethylene group should not be affected by the orientation of the hydroxyl group, except for a vertical displacement of the various curves.

When the carboxyl group and hydroxyl group are aligned so that the orbitals on the oxygen and carbon are along the internuclear axis, a net decrease of 1.7 kcal/mol per 10° rotation is observed. Since the calculated barriers to rotation for the hydroxymethylene and hydroxyl groups are much larger than the experimental values,* this net decrease for each 10° rotation of the carboxyl group will have a negligible effect on aligning the reactant orbitals. When the internuclear distance is approximately 2.0 Å, partial bond formation has occurred, and one may conclude that at this distance along the reaction coordinate for the formation of lactone, the total energy is a smooth function of orbital overlap. These calculations provide no compelling evidence to suggest that the reacting orbitals must be aligned along the internuclear axis. At positions along the reaction coordinate that correspond to little bond formation, it appears that the energy necessary for a bond-making interaction between orbitals that are 10° out of alignment is approximately the same as the energy involved for normal vibrations at ambient temperature. Since the transition state must resemble in structure some combination of the properties of the initial and final states, we conclude that lack of orbital alignment amounting to as much as 10° in the transition state should be of little kinetic significance. It is our contention that if orbital steering has any physical significance, the experi-

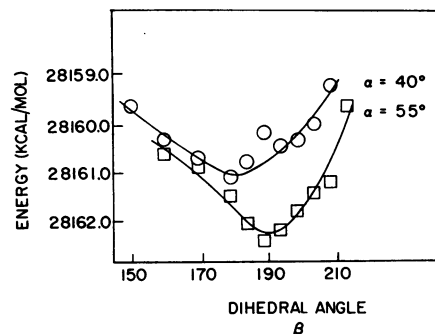

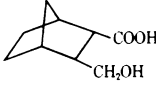
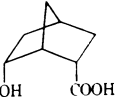


FIG. 3. The dependence of total energy on the rotational conformation of the hydroxyl group of 4-hydroxybutyric acid. The angles α and β are those defined in Fig. 2.

* For example, a 7-10 kcal/mol barrier to rotation was calculated for the hydroxymethylene group, whereas the experimental value is 0.77 kcal/mol in CH_3CH_2OH .

TABLE 1. *Relative rates of dehydration reactions*

	Observed (ref. 1)	Calcd.
I AcOH + EtOH	1	1
II 	0.79×10^2	8.5×10^2
III 	6.6×10^3	1.31×10^5
IV 	1.03×10^6	2.1×10^7

mental and theoretical data supporting such a phenomenon have yet to be presented.

Orbital steering thus appears highly unlikely as an explanation for intramolecular rate enhancements. We would like to reiterate an alternative interpretation. In Table 1 we present the experimental relative rates given in ref. 1 as support for the concept of orbital steering along with calculated values based on a simple model involving changes in ΔF^\ddagger as a function of conformation of the reacting species and the increase in $T\Delta S^\ddagger$ resulting from a decrease in kinetic order. The essence of this model is as follows. From data for a series of 21 spontaneous, second- and third-order displacement reactions upon phenyl and thiol esters, it has been found (3, 4) that $-1/4$ (experimentally determined $T\Delta S^\ddagger$) \approx (the change in kinetic order), and consequently one would expect an increase in rate in going from I to II in Table 1 of about 10^3 , simply as a result of the raising of the entropy of activation. A second effect is the "freezing out" of the rotation about single bonds in going from II to III to IV. In each case there is one fewer internal rotation to be frozen out than in the previous case in order to achieve a cyclic transition state. If this amounts to about a 3 kcal/mol decrease in ΔF^\ddagger for each internal rotation that is already frozen out and hence need not be frozen out in the transition state, then there would be a corresponding increase in rate of about 160 in each case in going from II to III to IV, as shown in the second column of Table 1. It is difficult, if not impossible, to justify on purely theoretical grounds the exact magnitude $\Delta\Delta F^\ddagger$ for this series of reactions; however, barriers to internal rotation about unhindered single bonds typically lie in the range of 1–4 kcal/mol (for hindered bonds, as great as 8

kcal/mol or more) and this, coupled with a consecutive increase in ΔS^\ddagger toward zero for the series, could easily lead to an observed $\Delta\Delta F^\ddagger$ of about 3 kcal/mol (12, 13). One might note that the model depicted by Table 1 bears a striking resemblance to one offered in 1960, which was at that time interpreted to indicate "the tremendous enhancement in rate that an enzyme could achieve by fixing the reacting species in a steric conformation closely resembling that of the transition state for the reaction" (14).

CONCLUSIONS

The simple analysis of the implications of the proposed orbital steering model leads us to conclude that it is not consistent with what is known about molecular force fields and reaction surfaces. Further, it also appears that the experimental data upon which the model is based can be satisfactorily interpreted in terms of the usual concepts of physical and organic chemistry.

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