Theoretical Aspects of Orbital Steering

(enzyme/substrate orientation/catalytic efficiency/rate of reaction/entropy)

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ABSTRACT A calculation based on transition-state theory leads to the conclusion that rate accelerations of 10^3–10^5 could be achieved in an optimally oriented reaction relative to a similar randomly oriented bimolecular reaction. This factor is obtained by the use of partition functions of simplified systems and is based on contributions to rotational and vibrational entropy from reasonable transition states. A simple harmonic oscillator calculation leads to a similar conclusion for series of intramolecular reactions. Although the uncertainty in theoretical calculations of this type is considerable, the results add support to the conclusion based on experimental studies that orientation factors can play a very significant role in the catalytic power of enzymes.

It has recently been suggested that one factor responsible for the large rate accelerations observed in enzymatic reactions is the ability of the enzyme to orient the reacting atoms in an optimal manner relative to the random collisions of normal chemical reactions (1). Evidence from enzymatic experiments consistent with this suggestion has been found in studies on thiolsubtilisin in our laboratory (2), on elastase by Thompson and Blout (3), on chymotrypsin by Henderson (4), and on the chymotrypsin–chymotrypsinogen conversion by Kraut and coworkers (5). The possible relevance of this model to heterogeneous catalysis has been noted by Heinemann (6). Experimental studies in a controlled series of intramolecular esterification reactions support this hypothesis (1, 7, 8). On the other hand, Bruice *et al.* (9) have made theoretical calculations which suggest that the contributions of orbital steering cannot be very large.

Theoretical calculations of the potential magnitude of various contributions to orientation seemed desirable both to ascertain what factors would be reasonable on the basis of transition-state structures and for the design of future experiments. Orbital steering is defined in operational terms to describe the situation in which reactive atoms, at least one of which contains spherical asymmetry in its valence orbitals, are constrained to react along selected pathways. Such constraints can be imposed by the binding of substrates at the active site of an enzyme or by the superstructure of the molecule in an intramolecular reaction. If the constraints steer the reacting molecules along an optimal pathway, an orientation factor greater than 1 should arise; contributions to the magnitude of this factor could come from bending vibrations, stretching vibrations, nonbonded interactions, and nonproductive complexing in the model systems.

In a previous paper, the magnitudes of contributions from bending vibrations and the distribution of approach orientations were considered with the use of a modified collision-theory approach (10). In this paper contributions of rotational entropy, bending, and stretching vibrations are considered from the standpoint of transition-state theory.

PROXIMITY AND ORIENTATION

The calculation of orientation factors relative to a bimolecular reaction depends on the relationship between orientation and proximity illustrated in Fig. 1. The assumptions made in the original derivation of the proximity correction (11) were: (a) that molecules (or reactive atoms) A and B are the size of water molecules, (b) that there is no net attraction or repulsion between A, B, and/or solvent, and (c) that a molecule with no orientational preferences can react with any of its n nearest neighbor molecules. A molecule, A, which has an orientational preference such that it can react at $1/\theta_A$ of its solid surface, has a probability of $n(B)/55\theta_A$ of being in a reactive relationship with molecule B, which has no orientational preference. The rate acceleration expected, therefore, in a perfectly oriented and juxtaposed AB pair (as on an enzyme surface), compared to a random collision process when $(B) = 1$ M, would be $(55/n)\theta_A$. The rate acceleration for two molecules, both of which have orientational requirements, would be $(55/n)\theta_A\theta_B$.[*] As discussed earlier, for real molecules larger than water, A and B refer to the reactive groups, e.g., an OH of glucose, a phosphorus of ATP, etc., which are essentially the size of water molecules.

This calculation has been criticized by Bruice and coworkers on the grounds that (a) real molecules may have a net attraction or repulsion (9), (b) that k_{intra}/k_{inter} for many reactions is actually far greater than $55/n$, and hence the proximity correction is incorrect (9, 12), and (c) that a k_{intra}/k_{inter} ratio of 10^8 leads to angles $(1/\theta_A)$ much smaller (0.1°) than reasonable values for bending vibrational amplitudes (9).

In regard to (a), the purpose of the proximity derivation is to obtain the idealized entropic correction. The assumptions of ideality were clearly stated (11) and methods for correcting for deviations of real molecules from idealized behavior have been described qualitatively (11) and quantitatively (10). This procedure of deriving the equation for ideal behavior and correcting for deviations in real molecules is standard practice in similar derivations such as ideal-gas laws, Debye–Hückel theory, perfect-solution theory, etc.

In regard to objection (b), it is our assumption that an experimentally observed k_{intra}/k_{inter} ratio involves many factors including proximity, orientation, ring strain, etc. The proximity derivation was devised to understand the fraction of the observed ratio contributed by proximity; the fact that many observed k_{intra}/k_{inter} ratios are greater than the $55/n$ factor

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[*] As discussed previously (11), factors much larger than this are obtained for the proximity effect under physiological conditions, where substrates are at low concentrations. The $55/n$ factor arises in the comparison of the saturated enzyme with 1 M reactants.
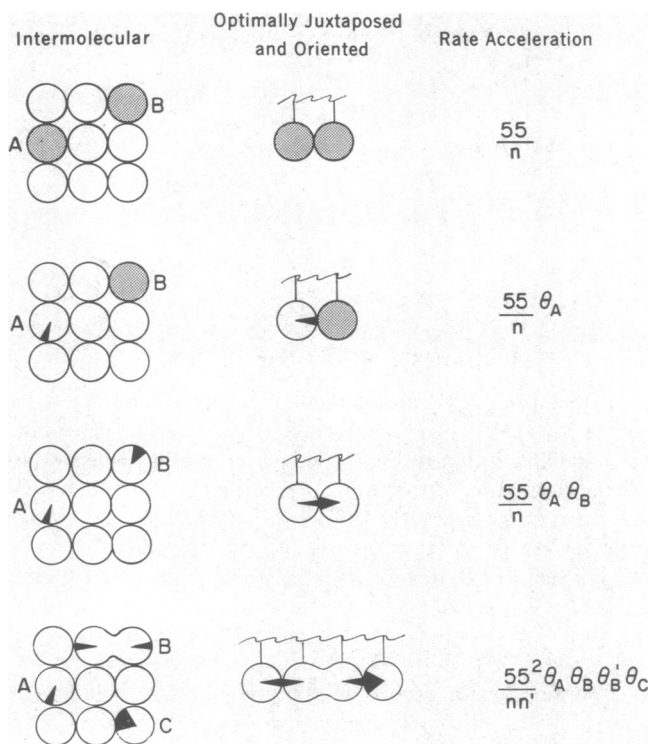
FIG. 1. Kinetic effects of proximity and orientation. The *circles* represent spherical molecules or functional groups about the size of water molecules. Groups reactive over their entire surface are *shaded;* the reactive surface area of other groups is represented in two dimensions by *pie-shaped wedges.* The θ factors may be considered schematically as the total surface area of the sphere divided by its reactive area, and the n are corrections for the number of nearest neighbors. Note that intramolecular reactions in which only one group can have orientational preferences are unlikely in practice and that higher-order reactions may involve more than one orientation parameter per group.

FIG. 2. General features of the MeOH–HCOOH transition state. Structural parameters are given in Table 1.

indicates in our view that other factors beside proximity contribute significantly to the observed rate ratio, not that the proximity ratio is incorrect.

In regard to objection (c), the small angle of 0.1° resulted from assumptions (9) that only one of the two reacting groups had an orientational requirement and that the observed $k_{\text{intra}}/k_{\text{inter}}$ ratio resulted solely from orientation and proximity. In the carefully controlled series of reactions reported by us (1), an orientation factor of 10^4 was obtained *after* correction for proximity and torsional strain. A factor of 10^4 only requires angles of 10° for two reacting atoms in which $\theta_A = \theta_B$, compared to a value of 5–10° observed for ordinary bending vibrations (9). Suitable corrections would have to be made on the reaction selected by Bruice *et al.* (9) in order to ascertain the factor identified with the angular requirement. Thus, angles significantly smaller than 5–10° may be required as a result of future work, but they are not required by the existing data.

It should be noted that the factors obtained in the following calculations do not depend on the assumption of a proximity factor. The comparison of theory and experiment when intramolecular and intermolecular reactions are compared will, of course, depend on one's assumptions in regard to the proximity factor. However, the transition-state calculation and the comparison of one intramolecular reaction with another do not depend on any assumption in regard to the translational entropy term of the bimolecular reaction.
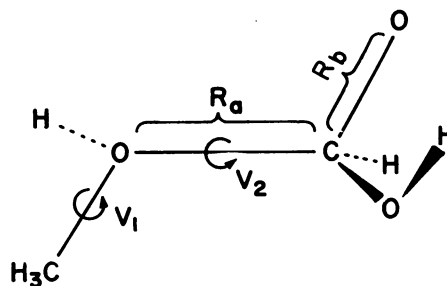
Reuben has suggested (13) that "duration of proximity" or "substrate anchoring" may yield rate enhancements as high as 10^6–10^9 in enzymatic catalysis, based on a derivation that uses increased residence times on an enzyme surface compared to the lifetime of collisions in solution. This derivation, however, compares one encounter between two molecules in solution with one encounter at an active site, whereas a molecule in solution actually may undergo a very large number of encounters during the residence time of a reference molecule on the enzyme. Clearly, a rate ratio requires that the probability of reaction of AB pairs on the enzyme be compared with the total probability for all encounters of AB pairs in solution over the same time interval. For systems of many molecules, the resulting expression is susceptible to averaging by use of the fundamental hypotheses of statistical mechanics (14). If we assume that the probability of reaction in any one encounter is proportional to the lifetime of that encounter (the assumption used to derive "substrate anchoring"), but consider the total number of encounters, the relative rate is simply the proximity correction $55/n$ we have described above.

TRANSITION-STATE APPROACH TO ORIENTATION FACTORS

The loss of translational freedom of two reacting molecules is accommodated in the proximity factors. In transition-state theory, therefore, the formation of a transition state from two nearest-neighbor molecules with orientational requirements requires the conversion of the rotational freedom (entropy) of the two molecules about their own centers, plus that of the pair itself into the freedom of a single transition state, plus freedom of internal motion in this transition state. Atoms that have no orientational requirements (e.g., Br^-, $H\cdot$) possess no rotational entropy; further, the stringency of the orientational requirement for a bimolecular reaction ("the size of the reactive area") is related to the amount of internal freedom available to the transition state. In contrast, substrates oriented by an enzyme or properly oriented functional groups in an intramolecular reaction have lost most of their freedom of motion with respect to each other; they have little left to lose by becoming bound in a transition state.

These qualitative effects, when expressed in quantitative terms, correspond to some of the major terms of the steric factor for a bimolecular gas-phase reaction, i.e., the factor p in the rate expression $k = pZe^{-E_0{}^\circ/RT}$, and can be estimated by transition state theory. The method of evaluating the partition functions of p has been outlined by Benson (15). The result of such an analysis is Eq. 1 for the reaction of A and

$$ p = \frac{q_{AB}{}^{\ddagger}{}_{(\text{rot})}}{q_{AB(\text{rot})}q_{A(\text{rot})}q_{B(\text{rot})}} \cdot \frac{q'_{AB}{}^{\ddagger}{}_{(\text{vib})}}{q_{A(\text{vib})}q_{B(\text{vib})}} \cdot \frac{q_{AB}{}^{\ddagger}{}_{(\text{int rot})}}{q_{A(\text{int rot})}q_{B(\text{int rot})}} \qquad (1) $$

B molecules to form $AB^{\ddagger}$. The *q*'s stand for statistical-me-
chanical partition functions and the subscripts rot, int rot, and
vib refer to rotational, internal rotational, and vibrational
modes of motion.† The term $q_{AB(rot)}$ merits some comment;
it is defined (15) as a two-dimensional partition function for
the collision complex of *A* and *B*, considered as a diatomic
molecule, and represents, in a rough way, the overall rotational
freedom of a nearest-neighbor pair considered as a single
entity.

This theoretical equation can be applied to a real reaction
provided reasonable assumptions for the structure of the
transition state can be made. The best approach to such a
calculation seemed to be to choose reactions in which data for
the molecules in the ground state were known and then to
make reasonable assumptions for changes in force constants,
bond lengths, etc. in the transition state. The first step of the
reaction of methyl alcohol with formic acid to form methyl
formate, the exchange reaction of Br^- with methyl bromide,
and the addition of hydroxide ion to CO_2 were chosen because
of the simplicity of the molecules and the large amount of
physical data available. Three different transition states were
selected for the first reaction to indicate the sensitivity of the
factors to the assumptions of transition-state structure. The
general features of the assumed structure are shown in Fig. 2.

The results are given in Table 1. It is seen that the steric
factors range from 10^{-3} to 10^{-5} for the esterification reaction
and depend on the "tightness" of the transition state. Lower
factors (about 10^{-2}) are obtained for the reaction in which one
of the molecules has no orientational requirement (Br^-) or
slight orientational requirement (OH^-). By the arguments
above, the p factors in these reactions should be the reciprocals
of the orientation requirements, thus giving a $\theta_A\theta_B$ factor of
10^3–10^5 for the first reaction and 10^2 for the second, and a
θ_B factor of about 10^2 for the third. The corresponding entropy
terms for intramolecular ring-closure reactions have been
discussed by Benson (16), and are in accord with the assump-
tion that the steric factor can strongly retard a bimolecular
reaction relative to an oriented intramolecular counterpart.

ANGULAR DEPENDENCE IN A SIMPLE INTRAMOLECULAR SYSTEM

The arguments presented so far have concentrated on the
question of rate ratios between properly oriented intramolecu-
lar reactions and bimolecular reactions. Another consequence
of orbital steering is that two otherwise similar intramolecular
(or enzymatic) reactions in which the ground-state alignment
is slightly altered should proceed at quite different rates. In
terms of a simple transition-state theory, this situation is
equivalent to two reactions for which pre-exponential terms
(partition functions) and ground-state strain are similar or
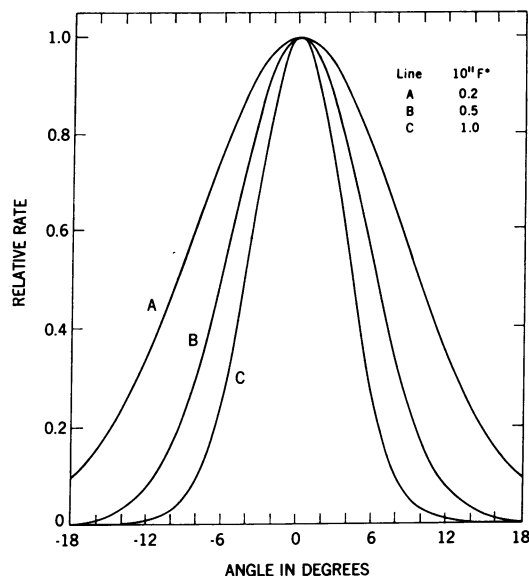corrected for. Under such conditions, a ground state con-

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† The qualitative argument given here that the partition function
ratio represented by the steric factor is largely orientational in
nature can be expressed mathematically in simple cases. This
relationship follows from the fact that the collision integrals of
ref. 10 can also be regarded as statistical mechanical configuration
integrals, which are in turn related to classical or quantum-
mechanical partition functions (18). The coordinate system de-
vised by Herschbach *et al.* is especially useful for obtaining these
relationships (19). Likewise, the original picture of orientation as
involving spheres with reactive areas (1, 11) can be regarded as
representing a simplified set of configuration integrals. In ex-
tremely simple models, we have been able to show that the three
treatments lead to essentially equivalent results.

FIG. 3. Relative rate as a function of angle of approach. The
function shown is $\exp[-V^*/kT]$, in which V^* represents a
change in potential energy of the transition state as a function
of angular distortion; in the harmonic-oscillator approximation
with two bending modes distorted, $V^* = F^*\phi^2$. F^* is a bending
force constant in erg/rad^2; 10^{-11} corresponds roughly to an
O—C—O bend at $400\ cm^{-1}$.

strained to an improper orientation will simply lead to a tran-
sition state containing angle strain. The estimation of the
effect of transition-state strain on rate is quite simple in
theory. In the idealized case, in which the force constants for
valence-bond bending, F^*, that represent the new bond in the
transition state are considered much smaller than ground-
state force constants for bending modes that maintain the
orientation (very roughly descriptive of the initial step of an
intramolecular esterification), transition-state deformation
will be represented predominantly by bending of this weak
bond between the reacting atoms. By use of the harmonic-
oscillator approximation and valence-bond coordinates such
that the deformation of either group is represented by two
perpendicular (orthogonal) coordinates, deformation from the
optimal angle by an amount ϕ in any one coordinate will raise
the potential energy of the transition state by an amount
$V^* = F^*\phi^2/2$. Since this is a change in potential energy, it
will change the rate by a factor of $\exp[(-F^*\phi^2/2kT)]$ relative
to an unstrained transition state.

Various types of misorientation can be imagined that would
result in deformation of anywhere from one to four modes in
the transition state and, of course, will result in additive terms
in the potential energy. The function for the case in which
only one coordinate of each group is distorted (arising from a
simple lateral displacement of one group to change the angle
of approach by ϕ) is plotted in Fig. 3 for three arbitrary but
reasonable values of the force constant (in erg/rad^2). This
derivation is too highly idealized for an observed reaction, since
such a derivation must allow for minimization of distortion
throughout the whole molecule. The qualitative conclusion,
however, can be used as a rough measure and shows that
rather modest changes in orientation can lead to large rate
effects. The steepness of the relative-rate curve is not unduly
sensitive to the value of the force constant (or to the number
of modes involved). Although this treatment cannot be ex-
tended to large angles with the harmonic-oscillator approxi-

TABLE 1. *Calculation of partition functions and steric factors at 25°C*[a]

	Ground states				Transition states (see Fig. 2)		
Reaction:	MeOH[b]	+	HCOOH[c]	→	Loose[d]	Medium[d]	Tight[d]
q_{rot}	3.175×10^3		8.908×10^3		98×10^3	98×10^3	82×10^3
$q_{int\ rot}$	1.431		1		~60	~60	~10
q_{vib}[e]	1.019		1.117		57(55)	4.1(4.0)	2.4(2.4)
$q_{AB(rot)}$[f]	...		...		1.6×10^3	1.6×10^3	1.2×10^3
P	...		...		4.4×10^{-3}	3.2×10^{-4}	3.6×10^{-5}

Reaction:	OH[−][g]	+	CO_2[h]	→	HCO_3[−][i]
q_{rot}	11.0		265.6		15.3×10^3
q_{vib}[e]	1		1.087		1.186(1.17)
$q_{AB(rot)}$[f]	...		...		489
P	...		...		1.2×10^{-2}

Reaction:	Br[−][j]	+	MeBr	→	$[BrCH_3Br]^-$
q_{rot}	1		2.334×10^3		39.0×10^3
q_{vib}[e]	1		1.077		(6.49)
$q_{AB(rot)}$[f]	...		...		9.14×10^3
P	...		...		1.1×10^{-2}

Other critical properties of MeOH·HCOOH transition states.

All structures have tetrahedral angles and normal bond lengths, except as indicated.

Tight transition state (equivalent to reaction intermediate): frequencies <1000 cm^{-1}: 636(1), 400(5).

$R_a = R_b = 1.43$ Å. $V_1 = V_2 = 2.7$ kcal/mole.

$I_{1(red)} \simeq 5.2 \times 10^{-40}$ g cm^2.

$I_{2(red)} \simeq 2.4 \times 10^{-39}$ g cm^2.

Medium transition state: frequencies <1000 cm^{-1}: 636(1), 500(1), 280(4).

$V_1 = 1.1$ kcal/mole, $V_2 = 0$.
$R_a = 1.8$ Å, $R_b = 1.33$ Å.

Loose transition state: frequencies <1000 cm^{-1}: 636(1), 500(1), 100(4).

$V_1 = 1.1$ kcal/mole, $V_2 = 0$.
$R_a = 1.8$ Å, $R_b = 1.33$ Å.

[a] The assumptions and approximations used are those applied successfully to inorganic reactions by Herschbach, D. R., H. S. Johnston, K. S. Pitzer, and R. E. Powell, *J. Chem. Phys.*, **25**, 736 (1956).

[b] Obtained from data of Ivash, E. V., J. C. M. Li, and K. S. Pitzer, *J. Chem. Phys.*, **23**, 1814 (1955).

[c] Obtained from data of Green, J. H. S., *J. Chem. Soc.*, 2241 (1961).

[d] Structure and vibrational frequencies estimated on the basis of S. W. Benson, "Thermochemical Kinetics," (John Wiley & Sons, Inc., New York, N.Y., 1968), Tables A.13 and A.14. Vibrations were taken as those of MeOH and HCOOH, except C=O stretch and O=C—O bend of HCOOH, plus added degrees of freedom. The reduced moments of inertia (I_{red}) were obtained as in [a]. Differences in rotamer stability were ignored.

[e] The vibration corresponding to the reaction coordinate has been omitted from values in parentheses.

[f] Partition function for a hypothetical diatomic molecule with a bond length equal to the distance between the centers of mass of the two reacting groups at the transition state.

[g] Obtained from an estimated bond length of Acquista, N., and S. Abramowitz, *J. Chem. Phys.*, **51**, 2911 (1969) (based on microwave data for RbOH and CsOH).

[h] Obtained from data of Wentink, T., Jr., *J. Chem. Phys.*, **30**, 105 (1959). See Gordon, J. S., *J. Chem. Eng. Data*, **6**, 390 (1961).

[i] Obtained from data of Bernitt, D. L., K. O. Hartman, and I. C. Hisatsune, *J. Chem. Phys.*, **42**, 3553 (1965). We have been unable to resolve a discrepancy between our calculated molar entropy ($S°_{298.16} = 61.9$ e.u.) and that calculated by Bernitt *et al.* (61.3 e.u.).

[j] All data obtained from gas-phase entropies calculated by de la Mare, P. B. D., L. Fowden, E. D. Hughes, C. K. Ingold, and J. D. H. Mackie, *J. Chem. Soc.*, 3200 (1955).

mation, the orientation effect at large angles explains the well-known and often very large effect of conformation on reactivity (17).

DISCUSSION

The results of the transition-state calculations indicate that steric factors of 10^{-2} can be obtained from the reaction between two atoms, one of which has spherically symmetrical orbitals and the other of which has orbital orientational requirements ($\theta_A = 1, \theta_B > 1$). Factors of 10^{-3}–10^{-5} are found for the reaction of molecules both of which have preferential orbital orientations ($\theta_A > 1, \theta_B > 1$). The calculations evaluate only factors from rotational, vibrational, and internal rotational entropy. As may be seen, these factors depend on a number of

assumptions in the system and on the particular bonds that are being formed or broken in the transition state. However, the results probably give a correct order of magnitude estimate for many reactions that involve nonlinear substrates and fairly strong bonding in the transition state, including reactions of biological interest.

The magnitude of orbital steering factors will depend on specific molecular events in the enzymatic or intramolecular reaction. If we assume that the enzyme holds substrates and transition states so that all rotational and most transition state vibrational freedoms are eliminated, the orientation factors ($\theta_A\theta_B$) will be the reciprocal of the steric factor, i.e., in the 10^3 to 10^5 range for esterification. If the enzyme eliminates rotation, but leaves transition-state vibration unaffected, the

factors will be multiplied by the vibrational partition functions to give factors of 10^4–10^5. The assumptions for intramolecular reactions will depend greatly on the particular molecule studied, but the results will presumably lie in the same range for highly confined reactions.

These calculations suggest: (*a*) that experimentally observed orbital steering factors in the range 10^3–10^5 could be explained on the basis of bending and stretching vibrations and rotational entropy, and (*b*) that factors much larger than this will require other contributions. (One possible source of other orientational effects would be nonproductive binding in the model reaction, which might contribute to both the exponential and pre-exponential terms.) This calculation supports our previous conclusion that orientation factors of 10^4 per reaction pair could be contributed by an enzyme that optimally orients two reactants that do not have spherically symmetrical orbital electrons. In some simple reactions, this orientation factor is of the same magnitude as the theoretical steric factor, *p*. However, since the measured steric factor *p* in solution can have many components, and orbital steering in enzymatic reactions may well involve both exponential and pre-exponential terms, extreme caution must be exercised in identifying the theoretical steric factor with the empirically observed *p* of a chemical reaction.

It should be emphasized that the above calculations follow the conventional assumptions that gas-phase formulas can be applied in solution with a reasonable degree of accuracy. Although this seems acceptable for rough evaluations, the dangers in going from the gas phase to a protic solvent such as water are very real. Furthermore, we do not know precisely what degrees of freedom are excluded on an enzyme or what constraints occur in an intramolecular compound. Finally, orientational phenomena in more complex types of reactions may not prove as simply separable theoretically between the pre-exponential and activation energy terms as the cases discussed here. For instance, a complete treatment of intramolecular reactions may not allow the cancellation of some partition functions as assumed in this rough calculation, and effects of zero-point energies, dipoles, and solvent should be considered in some cases. Nevertheless, it is possible to identify some components of orientation effects with well-known terms in transition-state theory and, conversely, to use some of these terms as indicators of the quantitative constituents of orbital steering effects in enzymatic reactions. The final determination of orientational rate factors will have to be experimental in view of the limitations of quantum and statistical mechanics in complex liquid systems. The fact that two theoretical approaches allow factors as large as 10^5 per reacting pair adds support to the conclusion that orientational effects of the reacting atoms can contribute very significant factors to enzymatic and intramolecular reactions.

NOTE ADDED IN PROOF

Page and Jencks have recently suggested that the proximity factor is far greater than $55/n$ and used measured values of the cyclopentadiene dimerization to obtain "effective molarities" of 10^8 [Page, M. I., and W. P. Jencks, *Proc. Nat. Acad. Sci. USA*, **68**, 1678 (1971)]. However, orientational effects must be disentangled from their overall entropy change for a reaction such as cyclopentadiene dimerization, and we feel that the bulk of their calculated rate factor is in fact orientational. To illustrate this, we have performed an exactly parallel calculation on bromine atom re-

combination, a $2A \rightarrow B$ reaction involving similar translational entropy changes, but having no orientational preference. We have calculated the function $(G^\circ - H_0^\circ)$ (described by Jencks) for the reaction $2Br \cdot \rightleftarrows Br_2$, using published data (*JANAF Thermochemical Tables*, The Dow Chemical Company, Midland, Mich., December 31, 1961). The data have been corrected to a 1 M standard state at $298^\circ K$ and the ground-state degeneracy of $Br \cdot$ ($q_e = 4$) has been removed in order to allow direct comparison with Jencks' data. The gas phase result is $\Delta G^\circ = 3.267$ kcal/mol $+ \Delta H_0^\circ$. The entropic effect discussed by Jencks is the first term of this equation and gives $K = 4.02 \times 10^{-3}$, or a maximum possible rate acceleration of $1/K = 2.49 \times 10^2$ M. The gas–liquid approximation of 3 entropy units used for cyclopentadiene by Jencks has been applied to the bromine reaction, giving $\Delta G^\circ = 2.37$ kcal/mol $+ \Delta H_0^\circ$, $K = 1.82 \times 10^{-2}$, and $1/K = 55.0$ M. This number is not strictly comparable with our proximity correction since bromine recombination involves a greater degree of restriction than nearest-neighbor pair formation. It should, therefore, if anything be greater than the proximity correction. Nevertheless, the two processes are basically similar for bromine atoms and the rough agreement between the value of $1/K$ obtained above and $55/n$ reinforces our previous conclusions about the rate acceleration obtainable from proximity alone. These values of $1/K$ may be compared with values of about 10^8 M given by Page and Jencks for cyclopentadiene dimerization or similar reactions; the difference of about 10^6 M in our view becomes in large part another example of the influence of orientation on a reaction.

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