"ORBITAL STEERING", ENTROPY, AND RATE ACCELERATIONS

W. P. Jencks[1] and M. I. Page[2]

[1] Graduate Department of Biochemistry,
Brandeis University, Waltham, Massachusetts 02154

and

[2] Department of Chemistry, The Polytechnic,
Huddersfield HD1 3DH

Received February 25,1974

Summary

Some important conceptual and quantitative differences are described between the "orbital steering" and entropy descriptions of the rate accelerations in intramolecular and enzymic reactions that may be brought about by geometric constraints other than distortion. The treatments differ by a factor of 10^3 - 10^4 in the maximum rate acceleration that may be obtained from these constraints. The estimation of a "proximity factor" without taking adequate account of the translational and overall rotational entropy terms gives a misleading value for this factor. The conclusion is reaffirmed that increasing the probability of reaction by restricting the free translational and rotational movement of reacting groups can play a large role in the catalytic power of enzymes.

Dafforn and Koshland (D and K) have recently compared "orbital steering" and entropy as ways of describing the rate accelerations caused by certain geometric restrictions in intramolecular and enzymic reactions and have concluded that the statistical mechanical treatments [i.e. entropy] are an adjunct but not a satisfactory substitute for the concepts of proximity and orbital steering (1). They further pointed out that the two approaches should give similar results if properly applied and concluded, from an analysis of cyclopentadiene dimerization, that this is indeed the case (1). We reluctantly add still another paper to the literature on this subject in order to present an extension of this comparison (Table I) that leads us to different conclusions.

The comparisons of Table I show that there are important

TABLE I

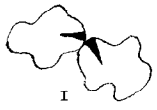
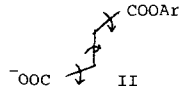
"Orbital Steering"	Entropy
1. Most of the rate increase in unstrained intramolecular (and some enzymic) reactions compared to corresponding bimolecular reactions in solution is attributed to an orientation factor, with an angular preference far greater than previously estimated. This orientational preference is exhibited <u>after</u> reacting molecules are brought into proximity with their reactive atoms in contact (I) and is measured by the product of the orientation factors θ_A and θ_B (1,2).  I	This rate increase is attributed to the improbability of two molecules in dilute solution losing their freedom of translation and rotation in three dimensions upon forming a transition state or product. This probability is greatly increased in an intramolecular or enzymic reaction when the reacting groups are brought together so that translational and rotational motions are constrained. The increase in probability is measured by a difference in entropy (3). Although an exact separation of translational and rotational entropy terms is not possible in solution, the contributions of both terms are comparable and a large part of the entropy loss has <u>already</u> taken place when the reacting atoms are in contact (I).
2. The maximum rate acceleration from <u>orientation</u> has been estimated, from the decrease in the rotational partition functions upon conversion of two molecules to a single transition state, to be about 10^4 for typical molecules (4). However, this calculation is inconsistent with the above definition of "orbital steering," because the two molecules in I have <u>already</u> lost their freedom to rotate independently.	The maximum rate acceleration from the loss of 20-25 units of (overall) rotational entropy,* which is equivalent to the decrease in rotational partition functions, is about 10^4 - 10^5 for typical molecules (3).
3. The spatial (as opposed to orientational) requirements for a reaction are incorporated into a "<u>proximity factor</u>" for the formation of I that is estimated to be about $55/n_n \cong 10$ M, equivalent to 4.5 e.u. (1). The loss of translational freedom of two reacting molecules is accommodated in the proximity factor (4), as is also the loss of free (overall) rotation of polyatomic, nonlinear molecules (I).	The spatial requirements are accommodated in the loss of <u>translational entropy</u> of the reacting molecules. This loss is typically 30 e.u. in the gas phase and 5 ± 5 e.u. less in solution, which corresponds to a maximum factor of 10^4 - 10^7 M (3). A spatial requirement closer to 10^7 M than to 10 M has also been estimated from distance distribution functions (6).
4. The entropy of reaction of two bromine atoms to form Br_2 (-13 e.u. (5)) is taken as a model for the proximity effect because Br atoms have no orientational requirement for reaction (1,4).	The spatial requirements and the loss of translational entropy in the bromine reaction are the same as for other reactions (-31 e.u., corresponding to a factor of 10^7 M), but are offset by an atypical <u>gain</u> of rotational entropy upon formation of the Br_2 molecule (we might call this "orbital <u>anti</u>-steering") (5).
5. The maximum <u>total</u> advantage in an intramolecular or enzymic reaction from "orbital steering" and "proximity" is approximately $10^4 \times 10$ M = 10^5 M (4).	A conservative estimate of the maximum total advantage that may arise from the restriction of translational and rotational motions in an intramolecular or enzymic reaction in solution is about 10^8 M (3).
6. It is emphasized that the "orientation" of orbital steering is not the gross orientation of substrates and catalytic groups on the enzyme surface or the juxtapositioning of reacting atoms (2).	It is precisely these factors that are responsible for the major part of the rate acceleration from entropic factors in intramolecular and enzymic reactions. Most of the entropy of a rotation is lost with a relatively mild orientational restriction (e.g. an 80% loss upon conversion of a free internal rotation, corresponding to 7 e.u., to a vibrational frequency of 300 cm^{-1} which, for carbon atoms, corresponds to an angle of approximately 30°). The juxtapositioning of reacting atoms requires the loss of 3 degrees of translational freedom, corresponding to ~30 e.u. We believe that one of the most important functions of an enzyme is to increase the probability for the formation of the transition state simply by bringing about the snug juxtapositioning of reacting atoms, with a resulting restriction of low frequency stretching and other motions that replace translation (3,7).

TABLE I Continued

7. Experimental support for "orbital steering" factors of 10^4 was adduced from comparisons of the rate constants for a series of acid-catalyzed esterification and lactonization reactions (8). However, orbital steering is defined as the rate acceleration that may be obtained from orientation factors after the reacting atoms are in contact (I). Consequently, these results provide no quantitative support for "orbital steering," because the reacting atoms in the compounds examined are not initially in contact.	The intramolecular reaction of succinate half esters II is faster by a factor of 10^5 M than  the corresponding intermolecular reaction (3,9). A conservative estimate, which ignores the differences in strain energy (3,10), increases this factor to 10^8 M if the three internal rotations of II are frozen into favorable positions and a bicyclic compound with these rotations frozen exhibits a rate increase of 10^8 M (3,9). Most of the rate increase of 10^5 M results from increasing the probability of reaction by preventing independent translational motions and from a mild restriction on the rotation of the reacting groups. It clearly does not result from an orientational restriction of juxtaposed reacting atoms and, hence, cannot be explained by the "proximity" - "orbital steering" approach.
8. The basis for the conclusion that "orbital steering" and entropy calculations give similar results is the assignment of an orientation factor of 10^6 - 10^7 to the cyclopentadiene reaction. This factor is stated to be "not unreasonable" compared to a factor of 10^4 for esterification, but no calculations are given (1, Figure 2, footnote f).	(i) No more entropy can be lost upon freezing a rotation than was present in the rotation initially. The complete loss of the rotational entropy of the reacting cyclopentadiene molecules in the transition state corresponds to 24 e.u. or a factor of 10^5 (3). The published values of the rotational partition functions and entropies for esterification (4) are closely similar to those for cyclopentadiene dimerization (3). (ii) Once the reactive atoms are in contact (as required by the definition of "orbital steering") all rotations are already lost. The complete freezing of remaining bending motions (e.g. 4 rocking modes each of 300 cm^{-1}) would give a loss of 5.6 e.u. and a rate factor of 20. (iii) If only two of the four reacting atoms are initially in contact (as shown in Figure 2 of D and K, (1)), the complete freezing of one free internal rotation (moment of inertia 11 x 10^{-39} g cm^2) and 4 bending motions as above could give a loss of 14 e.u. and a rate factor of 10^3, not 10^6 - 10^7.
9. Support for the hypothesis that orbital steering can introduce large rate effects in intramolecular and enzymic reactions is adduced from the observation that certain structural changes in cyclic reaction systems cause rate decreases (11).	It is important to keep in mind that the magnitude of rate decreases in constrained systems cannot be equated with the rate increase that may be obtained by optimal constraint of an unconstrained system. For example, the rate of a reaction that occurs on only one side of a group can be decreased by $>10^6$ by constraining a previously unconstrained reactant to the unreactive side (III), but can be increased by only a factor of 2 by constraining it to the reactive side (IV).  III IV
10. It is suggested that if each θ factor is 10^3 - 10^5, a combination of two substrates and two catalytic groups could produce a factor of 10^9 - 10^{15}, just what is needed to bridge the gap in enzymic and nonenzymic rates (2).	There is no doubt that additional catalytic groups in the active site of an enzyme can cause rate accelerations from entropic effects (7). However, the rate factor must be related to comparable nonenzymic reactions, which are 4th order rather than 2nd order in this case (12). Since the rate constants for these hypothetical 4th order reactions are not reported, the comparison is incomplete. It should be noted that the addition of a molecule of catalyst to a solution reaction ordinarily requires the loss of 3 degrees of translational and of rotational freedom; consequently, 4th and 5th order reactions are rare.

*Entropy is expressed in units of calories mol^{-1} $°K^{-1}$, abbreviated as e.u. Standard states are taken as 1 M at 25° throughout.

differences between the "orbital steering" and entropy approaches, with respect to both their definitions and their quantitative description of reaction rates. The most important differences are in (i) the maximum total rate acceleration that can be expected from these effects in an unstrained intramolecular or enzymic reaction (ca. 10^5 vs 10^8 M) and (ii) the attribution of these effects principally to a high degree of orientational restriction that occurs after the reacting atoms are juxtaposed in "orbital steering" and to a snug juxtapositioning of the reacting atoms accompanied by a relatively mild orientational restriction according to the entropy calculations. The entropy calculations are not novel and are in agreement with experiment (3,13). If orbital steering is identified with rotational entropy the differences appear in the assignment of a factor of up to ~10 M (4.5 e.u.) to "proximity" (4) and a factor of up to 10^7 M (30 e.u.) to translational entropy (3). Other identifications do not account for the missing 25 e.u. We note further that the application and "instinctive understanding" (1) of entropy and probability requirements for a reaction are no more difficult than the correct application and understanding of "proximity" and "orbital steering."

We conclude from the comparisons summarized in Table I that the "proximity" and "orbital steering" treatments, when carefully defined and rigorously applied, may be an adjunct, but are not a satisfactory substitute for the concept of entropy or the methods of statistical mechanics. We would be willing to accept a definition of "orbital steering" based on rotational and perhaps even vibrational partition functions of the reactants and transition state (4) (these terms are equivalent to S_{rot} and S_{vib}), but believe that it can only lead to confusion to suggest "orbital steering" effects that are larger than the total loss of rotational

entropy in a reaction and to include the loss of overall rotational freedom in the definition of "proximity."

The effects of geometric restrictions on the rates of intramolecular and enzyme catalyzed reactions have been described over the years by the terms entropy loss (3,10,14), approximation, orientation, anchimeric assistance (15), propinquity, rotamer distribution (9), proximity, orbital steering (1,4), stereopopulation control (16), distance distribution function (6), togetherness (7), other terms that are not appropriate for the open literature, and FARCE (Freezing At Reactive Centers of Enzymes (17)). This exponential scholastic proliferation is becoming burdensome to students and could soon fill the presently available journals. We believe that it would be desirable to reverse this growth by dropping some of the more colorful and imprecisely defined of these terms and accordingly offer to initiate this process by withdrawing the term "togetherness."

Acknowledgment. We are grateful to the National Science Foundation (GB 4648) and the National Institute of Child Health and Human Development of the National Institutes of Health (HD 01247) for financial support. Contribution No. 933 from the Graduate Department of Biochemistry, Brandeis University.

REFERENCES

1. Dafforn, A. and Koshland, Jr., D.E. (1973) Biochem. Biophys. Res. Commun. 52, 779.
2. Storm, D.R. and Koshland, Jr., D.E. (1970) Proc. Nat. Acad. Sci. U.S. 66, 445.
3. Page, M.I. and Jencks, W.P. (1971) Proc. Nat. Acad. Sci. U.S. 68, 1678.
4. Dafforn, A. and Koshland, Jr., D.E. (1971) Proc. Nat. Acad. Sci. U.S. 68, 2463.
5. Page, M.I. (1972) Biochem. Biophys. Res. Commun. 49, 940.
6. DeLisi, C. and Crothers, D.M. (1973) Biopolymers 12, 1689.

7. Jencks, W.P. and Page, M.I. (1972) Proc. 8th FEBS Meeting, Amsterdam 29, 45.
8. Storm, D.R. and Koshland, Jr., D.E. (1972) J. Amer. Chem. Soc. 94, 5805.
9. Bruice, T.C. and Pandit, U.K. (1960) J. Amer. Chem. Soc. 82, 5858; (1960) Proc. Nat. Acad. Sci. U.S. 46, 402; Bender, M.L. and Neveau, M.C. (1958) J. Amer. Chem. Soc. 80, 5388; Gaetjens, E. and Morawetz, H. (1960) J. Amer. Chem. Soc. 82, 5328; Bruice, T.C. and Turner, A. (1970) J. Amer. Chem. Soc. 92, 3422; Bruice, T.C., in The Enzymes, Vol. II, ed. P.D. Boyer, H. Lardy and K. Myrbäck (Academic Press, New York, N.Y., 1970), 3rd ed., p. 217.
10. Page, M.I. (1973) Chem. Soc. Revs. 2, 295.
11. Storm, D.R. and Koshland, Jr., D.E. (1972) J. Amer. Chem. Soc. 94, 5815.
12. Jencks, W.P. (1969) Catalysis in Chemistry and Enzymology, McGraw-Hill Book Co., New York, N.Y., p. 19.
13. Kistiakowsky, G.B. and Lacher, J.R. (1936) J. Amer. Chem. Soc. 58, 123; Wassermann, A. (1941) Proc. Roy. Soc. A178, 370; O'Neal, H.E. and Benson, S.W. (1970) Internat. J. Chem. Kinetics 2, 423.
14. Westheimer, F.H. (1962) Advan. Enzymol. 24, 455.
15. Winstein, S., Lindegren, C.R., Marshall, H., and Ingraham, L.L. (1953) J. Amer. Chem. Soc. 75, 147.
16. Milstien, S. and Cohen, L.A. (1970) Proc. Nat. Acad. Sci. U.S. 67, 1143.
17. Nowak, T. and Mildvan, A.S. (1972) Biochemistry 11, 2813.