PROXIMITY, ENTROPY AND ORBITAL STEERING

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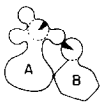
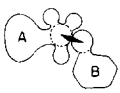
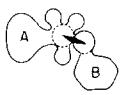
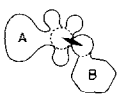
SUMMARY: Analysis of the factor for proximity and orientation is given for cyclopentadiene dimerization and for the general case of more complex biological molecules. Using either a probability or a statistical mechanical approach, the same ratios are obtained. The proximity effect factor is found to be about $55/n_n$ per reacting pair of molecules which show no net attraction or repulsion. The use of translational entropy without inclusion of a rotational entropy term gives a misleading value for this proximity factor. The calculations support the argument that orientation factors can play a large role in the catalytic power of enzymes.

The means by which enzymes achieve their remarkable catalytic power is the subject of considerable interest in an era of increasing sophistication in knowledge of enzyme mechanisms and protein structure. Some recent suggestions of ours (1-4) have become the source of considerable controversy (5-8). Much of the controversy seems to have arisen from the use of different physical models and the difficult problem of calculating the population of intermediate states by different theoretical procedures. A new look at the various approaches has allowed us to make parallel calculations which may clarify the situation.

In Figure 1 are shown three classes of molecular complexes which are of interest in comparing enzymatic and non-enzymatic reactions. Class I is the set of molecular complexes in which the two substrates are in contact at some portion of their surface area. Class II is a smaller subset in which only the reactive atoms, e.g., the phosphorus atom of ATP and the 6-OH atom of glucose in the hexokinase reaction, are in contact. Class III is the even smaller subset in which the reactive atoms are juxtaposed and in optimal orbital orientation. Two different ways of calculating the number of molecules in each of these classes and hence of the factors contributed by proximity and orien-

Class of Molecules	Types of Molecular Pairs in Class Designated			Relative Concentration of Class *
	Unfavorable Orientation Unfavorable Proximity	Favorable Proximity Unfavorable Orientation	Favorable Proximity and Orientation	
I. Molecules A and B in contact anywhere on surface				$(\Sigma_A)(\Sigma_B) f_{AB}(A)(B)$
II. Reactive atoms in contact				$(\Sigma_A)(\Sigma_B) f_{AB}\left(\frac{\Sigma'_A}{\Sigma_A}\right)\left(\frac{\Sigma'_B}{\Sigma_B}\right)(A)(B)$
III. Reactive atoms in contact and properly oriented				$(\Sigma_A)(\Sigma_B) f_{AB}\left(\frac{\Sigma'_A}{\Sigma_A}\right)\left(\frac{\Sigma'_B}{\Sigma_B}\right)\frac{1}{\theta_A \theta_B}(A)(B)$

* Where Σ represents surface area of molecule and Σ' surface area of reacting atom, f_{AB} is a shape factor to allow for specific sterically impossible positions, and θ is an orbital orientation factor. Absolute concentrations require a further constant to obtain units of moles/liter.

Figure 1. Calculation of types of molecular pairs in solution in which there is no net attraction and no net repulsion between the reacting molecules. Types of molecular pairs within a class are illustrative.

tation in an enzymatic reaction have been used by us and others. The first of these is the probability approach, which essentially assumes an instantaneous picture of the molecules in solution and counts the number of molecules in each category (9). The second is a statistical mechanical calculation using translational, rotational, and vibrational entropy (2,6). There is no doubt of the value of such calculations but the implication has been made (7) that low numbers calculated for the proximity effect are in conflict with the large numbers calculated for loss of translational entropy in forming a complex. It seemed to us that the two methods should give similar results if properly applied. Hence we have analyzed the general case and the specific one of cyclopentadiene dimerization from both viewpoints.

In Figure 1 the expected concentrations of each class in an aqueous solution where molecules A and B have no net affinity or repulsion (as would

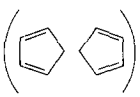
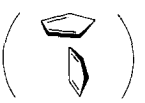
Class	Types of Molecular Pairs in Class Designated			Concentration of Class from Probability	Concentration from Statistical Mechanics
	Unfavorable Orientation Unfavorable Proximity	Favorable Proximity Unfavorable Orientation[a]	Favorable Proximity and Orientation[a]		
I				3.5 (A)(B)[b]	1.6 (A)(B)[c]
II				0.1 (A)(B)[d]	0.26 (A)(B)[e]
III				$\frac{0.1}{\theta_A \theta_B}$ (A)(B)[f]	$\sim 10^{-8}$ (A)(B)[g]

Figure 2. Calculation of concentration of different types of molecular pairs for the case of cyclopentadiene. The figure applies the generalizations of Figure 1 to the specific case of cyclopentadiene polymerization. (a) The optimal orientation is taken as that in which the two fold symmetry axes of the cyclopentadienes are perpendicular, allowing maximum orbital overlap for formation of endo-dicyclopentadiene. (b) Ref. 10, eq. XV.2.16, with r_{AB} = 9 Å, W_{ABS} = 0. (c) Based on $K = [q_{AB(tr)} q_{AB(rot)} q_{AB(vib)}]/[q_{A(tr)}]^2$ with r_{AB} = 9 Å, ω_{vib} = 33 cm^{-1}, and a 3 e.u. gas-liquid correction. (d) Based on Ref. 9, n_n = 5 so 5/55 ≈ 0.1. (e) Estimated on basis of 2 of 5 atoms or 40% of surface area reactive. This is equivalent to integration of a phase integral with limits defined by proximity (cf. H. Eyring, D. Henderson, B. J. Stover, and E. M. Eyring, "Statistical Mechanics and Dynamics", John Wiley & Sons, Inc., New York, 1964, pp. 58-59). (f) The coefficient for this category involves the reciprocal of the proximity factor and the reciprocal of the orientation factor ($1/\theta_A\theta_B$). The latter has not been determined independently for cyclopentadiene dimerization but a factor of 10^6-10^7 is not unreasonable for such a highly restricted reaction, particularly in view of the value of 10^4 for esterification (1,2). (g) Estimated from Ref. 6, assuming that the orientational requirements of the transition state are similar to those of the product.

be reasonable for ATP and glucose) are shown. The number of molecules in contact (Class I) would be a function of the concentrations of A and B, the surface areas of A and B (Σ_A, Σ_B) and a shape factor (f_{AB}) to make a correction for those portions of the surface area which could not be juxtaposed due to steric obstructions. Class II would contain the same factors, except for an additional term which represents the fraction of the surface occupied by the reacting atoms (Σ'_A/Σ_A, Σ'_B/Σ_B). It is quite clear that between these terms the surface areas of the molecules cancel out. As has been described elsewhere

(9), this leads to the factor $55/n_n$ (where n_n is the number of nearest neighbors) for the proximity effect for two reacting molecules with reactive atoms approximately the size of water molecules ($n_n \approx 5$). Because this is a rather universal number, and because it is the number which is theoretically most significant in interpreting enzymatic reaction velocities relative to the non-enzymatic analogues, the proximity effect was defined in terms of the molecules of Class II, i.e., the concentration effect achieved by the enzyme in juxtaposing reacting atoms. It has been widely accepted in the literature and in textbooks, and no substantial theoretical or practical argument has been advanced to change this definition to include all the molecular species of Class I, or to confine it to molecular species of Class III. Although the orientation factors θ_A and θ_B were used in the past (9) to express the ratio of molecules of Class III to molecules of Class II, this was frequently confused with the gross orientation included within the proximity effect, i.e., ratio of Class II to Class I. Hence the term orbital steering was used (1,2) to emphasize that the θ factors referred to acceleration achieved by the orientation of the molecules after reacting atoms were juxtaposed.

If molecules are small and the structural and spectroscopic data available, the calculation of the molecules in each category can be made with reasonable accuracy; this has been done for cyclopentadiene dimerization (cf. Figure 2). Some parts of the statistical mechanical calculations have been previously described (2,6) and further details of the calculations are given in the legend to Figure 2. They show that the calculations give essentially the same order of magnitude whether derived from statistical mechanics or by probability. Thus, the probability calculations give 0.1 ($n_n/55$) and the statistical mechanics 0.26 for the coefficient of Class II molecule concentration. In a previous publication (2), rather than make a theoretical assignment of the fraction of the molecules of cyclopentadiene which were oriented versus those merely juxtaposed, we used the device of comparing cyclopentadiene dimerization to bromine recombination since both involved reaction between two

molecules of essentially the same size, but the bromine reaction had no orientation requirement. The figure for the proximity effect obtained for that reaction would give a coefficient of greater than 0.02-0.1 in good order of magnitude agreement with the values (0.1 and 0.26) calculated by either statistical mechanics or probability in Figure 2. Thus, the statistical mechanical and probability calculations give the same answers when all the data are available.*

How can one reconcile the small factor for the proximity effect calculated here and the large loss of translational entropy in forming a molecular pair? The answer is seen to be quite simple. The loss of translational entropy for the formation of any one pair, e.g., either (a) or (b) or (c), from the separate molecules A and B is the same. However, the formation of any loose complex results in the appearance of new and large rotational terms describing the tumbling of the complex through the solution as shown in Figure 3 (9). These terms have the same form and magnitude as the rotational terms in the collision number of gas phase kinetic theory and the rotational term in bromine atom recombination.** Their omission in a calculation of the proximity effect gives a loose complex with an incorrect number of degrees of freedom. In simplified terms, the rotational or vibrational entropy terms are the statistical mechanical equivalent of considering all the possible molecular pairs of the Class I, II or III subsets. Equating the proximity factor with the loss in translational entropy underestimates the number of states available in

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* Another way of expressing the statistical mechanics of orientation is the steric factor as described previously (2). The set of numbers shown in Figure 2, footnote c, for proximity appear in the collision number of gas phase kinetics. The product of the two gives the complete preexponential factor of transition state theory.

** Page states that the bromine calculation is fortuitously low because the rotational term increases. In fact the term increases because Br has no orientational requirements. As shown here the parallel proximity calculation for cyclopentadiene gives values in rough agreement with the Br calculation. Such an increase in total rotational entropy must occur whenever the partners in a loose complex retain their freedom to rotate about their own centers of mass.

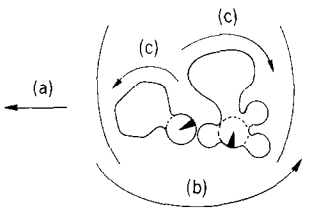

Figure 3. Degrees of freedom in a molecular pair as a loose complex.

Class II and leads to a misleadingly large number for the proximity effect. The disagreement therefore is not in the assignment of the total factor for proximity plus orientation, but in the definition of proximity in such a way as to exclude orientational components.

These calculations also illustrate why the statistical mechanical treatments are an adjunct but not a satisfactory substitute for the concepts of proximity and orbital steering. Small compact molecules like cyclopentadiene can be analyzed into translational, rotational, and vibrational functions from spectroscopic or thermodynamic data, but statistical mechanical calculations for ATP, glucose or other biological molecules are quite impossible with the data now at hand. It is relatively easy, however, to calculate the proximity factor for these molecules and less easy, but possible, to design experiments to quantitate the orientation factor. Hence, the latter analysis allows one to understand enzyme action in terms of processes which biochemists and organic chemists instinctively understand, i.e., a concentration effect (proximity) and a factor concerned with the more detailed orientation (orbital steering).

From the formulae shown together with previous analyses, the role of the enzyme can be delineated. The proximity factor can in some cases be very small, e.g., hydrolytic reactions in which H_2O is already 55 M. In other cases it can be very large, e.g., when the two substrates repel each other electrostatically in the absence of enzyme or when the substrate concentrations are very low under physiological conditions. In special cases, the geometry of the reactants will necessitate orientation in order to achieve proximity in which case separation

of the factors will be difficult. In the general case however, a factor of about 10 per reacting pair is reasonable to compare the saturated enzyme with the non-enzymatic reaction at 1 M concentrations of reactants.

The orientation factors per reacting pair will also depend on the particular reaction. Some reactions, e.g., the Br recombination may have low orientational requirements; therefore the enzyme can do little to accelerate the reaction by optimal orientation. In other cases, however, the factors may be very high, e.g., 10^4 per reacting pair for esterification reactions and ~ 10^6 per reacting pair for cyclopentadiene dimerization. In these cases the enzyme catalytic power may be in large part dependent on the exploitation of these orientation effects. The potential magnitude of this number was the key point of our previous papers and the calculations reported here add further support to the correctness of that assignment.

REFERENCES

1. Storm, D. R., and Koshland, D. E., Jr., Proc. Nat. Acad. Sci. U.S. 66, 445 (1970).
2. Dafforn, A., and Koshland, D. E., Jr., Proc. Nat. Acad. Sci. U.S. 68, 2463 (1971).
3. Storm, D. R., and Koshland, D. E., Jr., J. Amer. Chem. Soc. 94, 5815 (1972).
4. Dafforn, G. A., and Koshland, D. E., Jr., Bioorg. Chem. 1, 129 (1971).
5. Bruice, T. C., Brown, A., and Harris, D. O., Proc. Nat. Acad. Sci. U.S. 68, 658 (1971).
6. Page, M. I., and Jencks, W. P., Proc. Nat. Acad. Sci. U.S. 68, 1678 (1971)
7. Page, M. I., Biochem. Biophys. Res. Commun., 49, 940 (1972).
8. Capon, B., J. Chem. Soc. (B), 1207 (1971).
9. Koshland, D. E., Jr., J. Theor. Biol. 2, 85 (1962).
10. Benson, S. W., The Foundations of Chemical Kinetics, McGraw-Hill Book Co., Inc., New York, 1960, pp. 276-281.