Entropic Contributions to Rate Accelerations in Enzymic and Intramolecular Reactions and the Chelate Effect

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ABSTRACT It is pointed out that translational and (overall) rotational motions provide the important entropic driving force for enzymic and intramolecular rate accelerations and the chelate effect; internal rotations and unusually severe orientational requirements are generally of secondary importance. The loss of translational and (overall) rotational entropy for 2 \rightarrow 1 reactions in solution is ordinarily on the order of 45 entropy units (e.u.) (standard state 1 M, 25°C); the translational entropy is much larger than 8 e.u. (corresponding to 55 M). Low-frequency motions in products and transition states, about 17 e.u. for cyclopentadiene dimerization, partially compensate for this loss, but “effective concentrations” on the order of 10^4 M may be accounted for without the introduction of new chemical concepts or terms.

There are several reasons for believing that the specific binding process per se plays an important role in reducing the free energy of activation of reactions catalyzed by enzymes. We would like to know the nature and magnitude of the maximum increase in rate that may be brought about by bringing together two properly oriented reactants in the active site of an enzyme without invoking strain or desolvation. The rate acceleration in a monomolecular enzymic or intramolecular reaction compared to a bimolecular reaction may be expressed as an “effective molarity” of one reacting group or molecule relative to the other. It has been assumed by a number of investigators, including one of us, that this effective molarity is on the order of 1–55 M (up to 8 entropy units), corresponding to the probability of finding solute molecules next to each other, plus a relatively small additional factor reflecting orientational requirements of the transition state (1, 2). A similar approach, with “effective concentrations” of 55–110 M, has been proposed to explain the chelate effect (3). A recent renewal of interest in this subject has prompted the introduction of the terms “rotamer distribution” (4), “orbital steering” (5) and “stereopopulation control” (6) to describe the contribution of approximation and, in particular, orientation to intramolecular rate accelerations. The purpose of this communication is to point out that large “effective concentrations” may be accounted for by well-known chemical principles, without the introduction of new chemical concepts or terms. Although these principles have frequently been discussed with reference to the problems considered here (3, 7, 8), their application and quantitative significance for reactions in solution do not seem to be generally appreciated.

Large rate accelerations in intramolecular reactions of rigid, crowded molecules may be caused by steric strain or desolvation. Unequivocal evidence that theories giving rate accelerations on the order of 55 M from entropic factors are wrong or incomplete is provided by several intramolecular reactions, such as ring closures of succinate derivatives, in which the presence of one or more free rotations ensures that a reacting group can move out of an unfavorable conformation so that the fraction of the starting material in a high-energy, strained or desolvated form will be negligible. The comparison between intra- and intermolecular reactions may be based on the free energy of either the transition state, from rate measurements, or the product, from equilibrium measurements, relative to the starting material; the latter comparison has the advantage that the structure of the product is known. Thus, the equilibrium constant for succinic anhydride formation (Eq. 1) is 3 \times 10^6

\begin{align}
\text{COOH} \quad & \rightleftharpoons \quad \text{COOH} \\
\end{align}

more favorable than that for acetic anhydride formation from the free acids, which implies an “effective concentration” of 3 \times 10^4 M of the carboxylic acid groups of succinic acid relative to each other (2, 9), and the “effective concentration” of the neighboring carboxylate group that determines the rate of anhydride formation from substituted monophenyl succinate anions (Eq. 2) is about 10^4 M (4, 10, 11). Such physically impossible “effective concentrations” correspond to some 23 e.u., much larger than 8 e.u. Similarly, the chelate effect for ethylenediaminetetraacetate amounts to a factor of 10^{4.4} \rightarrow 10^{4.5} compared to two molecules of iminodiacetate, and effects of comparable magnitude have been reported for other chelation equilibria; these large effects were attributed by Schwarzenbach to a high activity coefficient of a chelating ligand group (12).

Reaching the transition state or (monomolecular) product of a bimolecular reaction reduces the number of independent
species in the system, with a consequent loss of three translational and up to three rotational degrees of freedom. Since this loss may be avoided if the reactants are bound to the active site of an enzyme, or by conversion to a first-order intramolecular reaction, the first step in analyzing this problem is to estimate the entropy change corresponding to the loss of these motions in a $2 \to 1$ reaction. Translational and rotational entropies can be evaluated with considerable confidence both theoretically and experimentally in the gas phase; some typical values are shown in Table 1. The problem is to evaluate these quantities for reactions in solution in the face of (a) the absence of a satisfactory theory of liquids, (b) the difficulty of separating translational and rotational motions in the liquid phase (13), (c) the entropy of transfer from the gas to the liquid phase, as indicated by Trouton's rule and its modifications (14, 15), which will influence the entropy of any $2 \to 1$ reaction, and (d) the difficulty of directly interpreting experimental entropy values in the liquid phase because of solvation effects. However, the sum of the translational and (overall) rotational entropies in a liquid phase reaction may be estimated as in Eq. 3.

\[
\Delta S = \Delta S_{\text{gas}} + \Delta S_{\text{soln}} + \Delta S_{\text{vap}}
\]

\[\Delta S_{\text{gas}} = -29 \text{ e.u.} \quad \Delta S_{\text{soln}} = -30 \text{ e.u.} \quad \Delta S_{\text{vap}} = -40 \text{ e.u.}
\]

The Diels–Alder dimerization of cyclopentadiene is taken as a concrete example. The losses of translational and rotational entropy upon forming the product (or transition state) of this reaction, calculated from standard formulas for the partition functions and using the moments of inertia estimated by Wassermann (19) are (see Table 1), $-31$ and $-21 \text{ e.u.}$, respectively, giving a total entropy loss of $-52 \text{ e.u.}$ in the gas phase and at a standard state of $1 \text{ M, } 400^\circ\text{K.}$ The difference between this value and the observed equilibrium $\Delta S$ values between $-31$ and $-39 \text{ e.u.}$ (20) is a consequence of an unexpectedly large residual entropy from low-frequency internal motions in the product; the internal entropy of cyclopentadiene is $17 \text{ e.u.}$ [calculated from the difference between the total (21) and the translational plus rotational entropies], which is $11 \text{ e.u.}$ larger than the internal entropy of the reactants (21). Entropy changes for reaching the transition state are almost identical to those for the overall equilibrium constant of this and other Diels–Alder reactions (18), and the internal entropy of the transition state of some 18 e.u., which could be accounted for by $5 \text{ low-frequency vibrations near } 100 \text{ cm}^{-1}$, is similar to that for the reaction product (22). The limiting translational plus rotational entropy change of about 50 e.u. is equivalent to a factor of $10^{11} \text{ M,}$ but this value corresponds to a smaller free energy change in the gas phase because of the (relatively small) correction for the translational and rotational enthalpy function at $298^\circ\text{K}$ (Table 1).

The total entropy change in solution may be estimated from the entropies of transfer of the reactants to the liquid phase. Although the standard entropy of vaporization of many liquids at 1 atm is close to 21 e.u. (14), approximately half of this quantity is simply the entropy of dilution from a pure liquid to a standard state of 1 atm, 10.8 e.u. for a 10 M liquid. After correction to a common standard state of 1 M and to $25^\circ\text{C,}$ based on the empirical relationship between the boiling point of a liquid and its entropy of vaporization at any temperature (15), the entropy of vaporization of cyclopentadiene is 9 e.u. and that of the higher-boiling endo-dicyclopentadiene is 15 e.u., so that the net change in the entropy of the reaction in the liquid compared to the gas phase is only $18 - 15 = 3 \text{ e.u.}$ (Eq. 3), similar to the experimental value of 5 e.u. (23). This is in accord with the well-known experimental fact that equilibrium and activation entropies for Diels–Alder reactions of $-30$ to $-40 \text{ e.u.}$ are very similar in the gas and liquid phases (18, 23). We were initially surprised by this similarity, in view of the widespread assumption that there is a severe restriction to translational and rotational motion upon condensation to the liquid phase, but the reasons for it are apparent—once the entropy of dilution effect has been allowed for, much (or all) of the remaining expected difference of approximately 10 e.u. between the entropies of vaporization of the reactant and products will ordinarily be compensated by the larger entropy of vaporization of the product (or transition state) because of its higher boiling point. It is probable that for most solutes the loss of rotational entropy is small upon transfer to the liquid phase, and the observed entropy change may be regarded as primarily a loss of translational entropy (24). This is in agreement with the fact that there is no significant difference between the entropies of solution of noble gases (which have no rotational entropy) and of hydrocarbons of comparable size (25).

We conclude that losses of translational plus rotational entropy of 40–50 e.u. are to be expected for many bimolecular reactions in solution, in view of the small dependence of these quantities on molecular size (Table 1), and that this loss will be compensated to a variable extent by low-frequency motions in the product or transition state. Thus, the entropic barrier that must be overcome for a relatively simple reaction having residual freedom of internal motions corresponding to about 15 e.u., based on a standard state of 1 M, is some $-35 \text{ e.u.}$

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‡ This effect may also be expressed in terms of the free volume in the liquid phase (16). The factor of approximately 150 estimated for a free volume of 0.5 ml and a molar volume of 75 ml is equivalent to the 10 e.u. (a factor of 150 at $25^\circ\text{C}$) estimated directly from Trouton's rule for reactants and products with similar heats of vaporization (see below).

† A further difficulty arises from the fact that thermodynamic activation parameters for gas-phase reactions usually refer to constant volume, whereas those for solutions ordinarily refer to constant pressure. It has been suggested that comparisons should be made at constant volume (17, 18), but this requires the use of liquid-phase data obtained under unusual conditions of high pressure. We believe it is both simpler and more meaningful to carry out comparisons at constant pressure, for which $\Delta H$ for a $2 \to 1$ reaction in the gas phase differs ideally only by RT from that at constant volume. Since this correction is small relative to other uncertainties, we have neglected it for present purposes.

§ This statement must not be taken too literally in view of the previously mentioned difficulty of separating the entropic components of liquids (13); a more rigorous statement is that upon solution or condensation of a nonpolar molecule of moderate size, there is little loss of the entropy that is attributable to rotation in the gas phase, although this entropy may be partially converted to other modes in the liquid.

¶ Estimated from the strain energy of cyclopentane, 7.3 kcal/mol $\div 5 = 1.4$ kcal/mol (48).
TABLE 1.  Typical entropy and free-energy contributions from translations, rotations, and vibrations at 298°K

| Motion                                    | S° (cal deg⁻¹ mol⁻¹) | H°⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻笥
|                            | G°⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻ţi

| Three degrees of translational freedom for molecular weights 20–200, standard state 1 M  
|                            | 29–36 | 1.48  | -7.2 to -9.1 |

| Three degrees of rotational freedom  
|                            | Moments of inertia  
| Water                       | 5.8 X 10⁻¹²  
| n-Propane                   | 10.5 X 10⁻¹²  
| endo-Dicyclopentadiene      | 27.2 X 10⁻¹³  
| Internal rotation           | 3.5 X 10⁻¹³  
| Vibrations                  | ω, cm⁻¹  
| 1000                        | 0.1 0.03 0.0  
| 800                         | 0.2 0.05 -0.01  
| 400                         | 1.0 0.20 -0.10  
| 200                         | 2.2 0.35 -0.31  
| 100                         | 3.4 0.46 -0.56  

* Calculated from standard equations for translation (Sackur–Tetrode equation), rotation (rigid rotator), and vibration (assuming a harmonic oscillator) in the gas phase; see for example, Pitzer, K. S., and L. Brewer, Thermodynamics (McGraw-Hill Book Co., Inc., New York, 1961).

+ Product of three principal moments of inertia, g² cm⁴.
+ Symmetry corrected.
+ Typical value; this quantity is a function of the barrier to rotation and the partition function.

This corresponds to a rate acceleration of 10⁴ M that should be obtained by binding to an enzyme active site or conversion to an intramolecular reaction. The entropy of activation for the thermal disrotatory ring closure reaction of Eq. 4,

![Diels-Alder reaction](4)

which might be regarded as an intramolecular analog of the Diels–Alder reaction, is -4.7 e.u. in cyclohexane solution (26). The difference between this value and that for bimolecular Diels–Alder reactions, such as cyclopentadiene dimerization (ΔS² = -33 e.u.), corresponds to a factor of 10⁴ M at 25°C favoring the intramolecular reaction.

How, then, may these large entropic contributions of -35 to -50 e.u. be reconciled with earlier, smaller estimates for reaction rates and equilibria, including chelation reactions, and with the many observed activation and equilibrium entropies in the range of -8 to -20 e.u. for reactions of medium-size molecules? Either the smaller or the larger values must be "normal" and the other must be "abnormal" or in error; previously we had believed that the smaller range represented the standard values. Several factors (in addition to experimental error) may contribute to the difference:

1. Observed equilibrium entropies of many readily reversible association reactions to form hydrogen-bonded or charge-transfer complexes are about -10 to -20 e.u. (27), as expected from naive earlier estimates of the approximation and orientation effects. However, in a typical complex of this sort, (Eq. 5), three degrees of rotational and translational freedom,

$$A + B \rightleftharpoons [A \cdots B] \rightleftharpoons AB \tag{5}$$

"cage" weak bond
"tight" transition state or product

(corresponding to some 45 e.u., have been converted into a low-frequency stretching vibration, an internal rotation, and four low-frequency bending modes which must contribute up to 30 e.u. of residual entropy to the loose complex. An unhindered internal rotation will contribute roughly 7 e.u.; low-frequency stretching, deformation, and torsional motions involving the hydrogen or charge-transfer bond, in the range of 50–300 cm⁻¹ (28) account for the remainder of the difference. Similarly, the low-frequency vibrations of metal–ligand bonds and other motions (29) make a significant contribution to the internal entropy of chelate complexes, and a comparable situation exists for the freezing of an organic compound into a crystal lattice—the entropy of fusion of only some -10 e.u. that is observed for many organic molecules (30, 31) may be regarded as a result of the replacement of translational and rotational degrees of freedom by low-frequency motions within the lattice (32). For example, it has been estimated that in crystalline hexamethylenetetramine the "translational" frequency of 73 cm⁻¹ and torsional frequency of 45 cm⁻¹ contribute 14.2 and 15.1 e.u., respectively, to the entropy at 298°C (32).

2. Differences in the solvation of polar and hydrophobic groups of reactants, transition states, and products are likely to make large and unpredictable contributions to observed equilibrium and activation entropies, especially in aqueous solution. Thus, the equilibrium entropies for reaction 6 range from -5.4 to -28 e.u. for the addition of semicarbazide, hydrogen cyanide, amides, water, and hydrogen peroxide to

$$XH + \text{C-OH} \rightleftharpoons X-OH \tag{6}$$

the carbonyl group (33, 34). The reduction of ketones by morpholine-borane, with ΔS² = -40 e.u., provides a good example of a reaction with a large negative entropy that is almost solvent-independent and therefore is probably not seriously perturbed by solvation effects (35).

INTERNAL ROTATIONS

We believe that the contributions of internal rotations or high degrees of orientational restriction to entropies of activation and reaction or to rate accelerations in systems in which these rotations are initially frozen are generally small. It has previously been pointed out that very severe orientational requirements would require force constants in a transition state larger than those for a covalent bond (36), and it may be noted further that the fact that some intramolecular reactions are slower than others because of unfavorable geometry and steric effects does not necessarily imply a high orientational requirement for either intermolecular or intramolecular reactions. No more entropy can be lost upon freezing a rotation.
than was present in the rotation initially. Entropies of internal rotation in the gas phase are known experimentally and are in good agreement with theory (37); they will not be larger in solution. Some representative examples, based on a series of ring closures, are shown in Table 2. The entropy changes are primarily a consequence of losses of internal rotation that may be partially compensated by low-frequency motions in the cyclic products; contributions from changes in overall rotational entropy, symmetry, other internal vibrations, and losses of two hydrogen atoms upon cyclization are relatively small and will be neglected for present purposes. The entropy loss per internal rotation for saturated hydrocarbons of up to 8 carbon atoms is 2.7–4.3 e.u.; after correction for low-frequency motions in the C4, C6, C8, and C9 rings, the entropy loss, ΔSrot, is 3.7–4.9 e.u. per internal rotation. Entropies of activation for ring closure reactions are similar or smaller (Table 2), so that we may take 4.5 e.u. as a representative value for the entropy that may be lost upon freezing an internal rotation, in the absence of compensating factors. This value, which is in good agreement with the calculated value (37), is less than the maximum that would be expected for free rotation because of the entropy loss resulting from the barrier to free rotation in saturated hydrocarbons (38). One important consequence of this barrier is that the loss of rotational entropy upon ring closure of a system containing a double bond is not significantly different from that of a saturated system which initially has one more internal rotation (Table 2). This is a consequence of the reduced barrier to rotation adjacent to the double bond and of changes in the symmetry axis in the acyclic compound (39) and brings into question the common assumption that ring-closure reactions of unsaturated systems are favorable entropically compared to those of saturated systems.

It should be noted that the entropy of an internal rotation is almost completely lost upon the imposition of a relatively small 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), and no significant further loss occurs with very high degrees of orientational restriction. Even the complete freezing of a free internal rotation with the loss of 7 e.u. corresponds to a factor of 34 which, based on 360° rotation in one plane, gives a maximum degree of "orbital steering" of only 11° that can be attributed to loss of entropy. Finally, the loss of entropy upon freezing an internal rotation is partially compensated by a favorable enthalpy function change of about 0.5 kcal/mol, so that the increases in free energy from the loss of each internal rotation in the formation of 4-, 5-, and 6-membered rings are 0.99, 0.90, and 0.76 kcal/mol, respectively, corresponding to a rate factor of about 5 at 25°C. The relative importance of different entropic contributions is well illustrated by the dimerization of 2-propene to cyclohexane, for which the overall entropy loss of 50.1 e.u. (30) is composed of 48.7 e.u. from translational and overall rotational entropy (39) and only 1.4 e.u. from internal entropy, which includes the loss of two internal rotations (5.8 e.u.) and a compensating increase from internal vibrations (9.2 – 4.8 = 4.4 e.u.) (40).

This rate factor of 5 is considerably smaller than the factor of 230 which has been suggested for the freezing of each internal rotation and which led to the suggestion that favorable "rotamer distribution" is a factor of prime importance in intramolecular and enzymatic rate accelerations (4, 36). The value of 230 is based on comparisons of rates of ring closure of different-sized ring systems in acyl-transfer reactions, but is by no means general. For example, although the ring closure of phenyl succinates is about 200 times faster than that of phenyl glutarates, the relative rates of ring closure of other 5-membered ring systems, including other acyl-transfer reac-

<table>
<thead>
<tr>
<th>Transition state</th>
<th>ΔS° (cal deg⁻¹ mol⁻¹)</th>
<th>-ΔS°/ (no. int. rot.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₂CH₂NO₃ → C₂H₄ + HNO₂</td>
<td>-9</td>
<td>4.5</td>
</tr>
<tr>
<td>Elimination of HX from alkyl halides</td>
<td>+5 to -3</td>
<td>-5 to 3</td>
</tr>
</tbody>
</table>

**Table 2. Entropy changes accompanying cyclization at 298°K**

* Ref. 47.
tions (41), are essentially the same or even smaller compared to those of the corresponding 6-membered systems (42). These differences reflect the specific geometric and electronic requirements for the transition states of these different reactions, so that no generalization as to the magnitude of the contribution of “rotamer distribution” can be made from this kind of data. For example, the transition state for the phenyl glutarate reaction contains an sp² carbon atom in the acyl group, which is known to destabilize 6-membered rings (43), and the transition state for the phenyl succinate reaction may gain some 3–4 e.u. from the low-frequency puckering motion characteristic of 5-membered rings (44). The relatively slow rate of closure of 6-membered rings in certain S₂2 displacement reactions (45) presumably reflects the unfavorable geometry brought about by a 90° bond angle, which is also largely responsible for the relative instability of most 6-membered compared to 5-membered chelate rings (46). The largest total loss of entropy from internal rotations that is likely to be encountered is about 20 e.u. for the closure of an unsubstituted 6-membered ring, which Westheimer and Ingraham (8) have pointed out is enough to offset most of the contribution of translational entropy to the chelate effect (estimated from gas-phase values), but even this value should be reduced by the contribution of low-frequency motions involving the metal–ligand bond to the internal entropy of the chelate complex.

The observed “effective concentration” of about 10⁸ M in succinate reactions may be corrected for the loss of three internal rotations upon ring closure to provide an estimate of the maximal rate acceleration that may be expected for an enzymic acyl-transfer reaction as a consequence of optimal binding of substrates, without strain or desolvation. Allowing 0.9 kcal/mol for the rotation around the methylene–methylene bond and 1.8 kcal/mol for each free rotation about the methylene–carboxyl residue bond (47) to give a rate factor of 10⁴ and an additional factor of 10 for eclipsing of the methylene hydrogen atoms in the 5-membered succinate ring the total “effective molarity” is 10⁹ M, which may be compared to the value of 10¹⁰ M cited above. This supports the conclusion that the similar factor that has been directly observed for a rigid bicyclic analog of phenyl succinate may be accounted for without invoking strain or desolvation (4, 11). It further suggests that the observed entropy of activation of many acyltransfer reactions involving uncharged reactants, which is often on the order of −30 to −50 e.u. and is usually attributed to orientation of solvent (49), may be in large part a consequence of the loss of translational and rotational entropy in the relatively tight transition state that is made possible by the availability of orbitals from the unsaturated carbonyl group in such reactions.

In conclusion, we suggest that the contributions of translational and (overall) rotational entropy to reaction rates and equilibria in solution are larger than has generally been believed and support the view that enzymes can carry out a large fraction of their extraordinary rate accelerations by virtue of their ability to utilize substrate-binding forces to act as an “entropy trap” (50). It follows from this conclusion that low-energy conformational changes that are possible within the enzyme–substrate complex are disadvantageous with respect to the contribution of this entropy effect to the catalytic process, as they are also for the utilization of strain or desolvation.

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