A Contribution to the Theory of Enzyme Catalysis. The Potential Importance of Vibrational Activation Entropy

By **David B. Cook** and **James McKenna,*** Chemistry Department, The University, Sheffield S3 7HF

Because of the expected very large number of low-frequency vibrational modes in an enzyme–substrate complex, and the manner in which many of these may be altered during reaction, a substantial catalytic effect could be associated with vibrational activation entropy. Other suggested possibilities for catalysis based on vibrational effects are examined.

ENZYME catalysis is usually discussed explicitly or implicitly in terms of potential energy changes, although the well recognised entropic differentiation between first- and higher-order reactions has also been very properly emphasised [1a] in this context. We suggest here that the contribution of vibrational activation entropy may be of importance in reducing the free energy of activation of the reaction of the complexed substrate.

Enzymes are large complex molecules maintained in their reacting conformations by extensive weak bonding; furthermore, the substrate is itself attached mostly by similar weak bonds. These factors together constitute an effective recipe for an abundance of low frequency vibrational modes, some of which will inevitably be substantially altered during reaction.

In the Table we give for a series of harmonic oscillators the thermodynamic quantities $G^0 - U_0^0$, zero-point energy, ZPE, and the sum of these last two terms which is equivalent to $G^0 - \varepsilon$, ε being the static potential energy (chemical binding energy). These values have been calculated by computer from the standard formulae (1)—(3) where $x = h\nu/kT = 1{\cdot}4387\omega$ $(\text{cm}^{-1})/T$.

Thermodynamic quantities for harmonic oscillators at 300 K

ν/cm^{-1}	ZPE/ kcal mol^{-1}	$G^0 - U^0$/ kcal mol^{-1}	$G^0 - \varepsilon$/ kcal mol^{-1}
1	0·0014	−3·1851	−3·1837
5	0·0071	−2·2313	−2·2241
10	0·0143	−1·8251	−1·8108
25	0·0357	−1·3000	−1·2642
50	0·0715	−0·9214	−0·8499
100	0·1430	−0·5753	−0·4323
150	0·2145	−0·3980	−0·1835
200	0·2860	−0·2881	−0·0020
400	0·5720	−0·0946	0·4773
600	0·8581	−0·0345	0·8235
800	1·1441	−0·0129	1·1311
1000	1·4302	−0·0049	1·4252
1500	2·1453	−0·0004	2·1448
3000	4·2906	0·0000	4·2906

$$\text{ZPE} = \tfrac{1}{2}h\nu = \omega\ (\text{cm}^{-1})/699{\cdot}2\ \text{kcal mol}^{-1} \quad (1)$$

$$G^0 - U_0^0 = RT\ln(1 - e^{-x}) \quad (2)$$

$$= 0{\cdot}5962\ [\ln(1 - e^{-x})]\ \text{kcal mol}^{-1}\ \text{at 300 K} \quad (3)$$

Analytically, $G^0 - \varepsilon$ includes all other components of standard free energy except the potential energy, *viz.* zero-point energy, ZPE, thermal energy, TE, the pressure–volume term, pV, *changes* in which are unimportant in solution, and the temperature–entropy term $-TS^0$ [equation (4)]. Terms summing to standard

$$G^0 = \underbrace{\underbrace{\varepsilon + \text{ZPE}}_{U_0^0} + \text{TE}}_{U_T^0} + pV - TS^0 \quad (4)$$

$$H_T^0$$

internal energy U^0 or enthalpy H^0 are also indicated above.† We have not encountered the previous use of the quantity $G^0 - \varepsilon$ in evaluations of the effects of vibrational modes on equilibria or reactivity; such discussions are usually conducted in terms of either S^0 or $G^0 - U_0^0$ ($\equiv F^0 - E_0^0$). However, since the zero-point energy is inseparably associated with each oscillator, it seems best to include this term directly in the analysis, while the well-known partial cancellation of thermal energy (TE) and entropy ($-TS^0$) contributions to free energy are also automatically allowed for in the expression $G^0 - \varepsilon$. However, for very low frequencies this expression in fact reflects mainly the $-TS^0$ term, and at high frequencies (as seen from the Table) the zero-point energy.

It is clear from the Table that free energy changes associated with marked alterations in a number of very low frequency modes, for example, as between Michaelis complex and transition state for an enzyme-catalysed reaction may have a substantial component, vibrational activation entropy in the example, corresponding to a $\Sigma\Delta(G^0 - \varepsilon)$ term; this may be comparable with and perhaps even greater than the $\Sigma\Delta\varepsilon$ term, since we are concerned with alterations in very weak bonding. Obviously, then, the usual discussions of free energies of activation (or rate constants) of enzyme-catalysed processes implicitly or explicitly in terms of potential energy changes are in general likely to be unsatisfactorily incomplete. This point appears to have been overlooked in the past, most probably because vibrational activation entropies are relatively unimportant for reactions of normal, smaller more rigid molecules lacking any or many entropy-rich, very low frequency vibrational modes. Substantial positive activation entropies associated with vibrational changes are indeed sometimes

† This type of analysis, isolating potential energy (or potential energy changes) is of general applicability in physical organic chemistry, and will often be superior to the more customary analysis in terms of enthalpy and entropy (changes). Many commonly used concepts such as strain or resonance energy refer to potential energy contributions.

[1] (*a*) M. I. Page and W. P. Jencks, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1678; *Proc. 8th FEBS meeting, Amsterdam*, 1972, **29**, 45; (*b*) W. P. Jencks, *Chem. Rev.*, 1972, **72**, 705.

observed in the thermal decomposition of small molecules or in rearrangements accompanied by bond fission where there is no contrary contribution from solvation or freezing of internal rotations, but almost necessarily in such cases the favourable activation entropies are associated with the penalty of large activation enthalpies. We believe, however, that the kinetic advantage can, as indicated above, be obtained without such a sizeable penalty in the special circumstances involving changes in weak bonding applicable in enzyme catalysis, or, more generally, during a reaction of any weakly complexed substrate where appropriate alteration in a sufficiently large number of low frequency vibrational modes is expected. The additional possibility is evident for kinetic discrimination through this mechanism *against* a substrate electronically or sterically less satisfactorily complexed to a particular enzyme (or other large molecule), so that a useful element of selectivity is also included.

Alteration in bonding between Michaelis complex and transition state will of course have an effect on a group of related normal modes; in principle indeed, all the vibrational modes are affected to an extent determined by the strength of coupling between the complexing bonds, the reacting bonds, and the rest of the molecule, and we are currently investigating this aspect by appropriate model calculations. The general effect of coupling a group of oscillators is to spread out the derived frequency range at both ends, the lower resultant frequencies being, of course, of particular importance for the effect suggested. There seems to be the additional possibility of better overall coupling through the complexing bonds between low-frequency modes of the protein and substrate modes at the transition state rather than at the Michaelis complex stage, since partly broken or formed bonds will be much weaker than normal. Enzyme mechanisms, as generally depicted, are characterised by the synchronous or near-synchronous making or breaking of many bonds, at least five, for example,[1b] in typical general acid–base catalysed processes. These considerations on coupling seem to give useful hints in two further directions: first, in relation to a possible role in catalysis for parts of the enzyme molecule not forming the active centre (why are such large molecules involved?) and secondly on the control of enzyme activity by attachment of regulatory molecules to secondary sites.

We may note here that the type of coupling postulated above is unlikely to be of similar importance in smaller, more rigid molecules undergoing intramolecular reactions. Indeed, the most typical model for illustration of intramolecular reactivity, consisting of two reacting groups attached by strong covalent bonds to adjacent positions of a small, rather rigid ring system, might almost have been specifically designed to maintain 'group' vibratory motions in the exocyclic reactive parts.

Comparison of the postulated entropy effect with the usual solvation activation entropies is of interest; such solvation effects are sometimes, of course, very large. To a degree the enzyme may in fact be regarded as a surrogate solvent for its substrate, but there is the obvious (and here useful) difference that enzyme structure and conformation and perturbations thereof are likely to be clearly defined over a much greater volume than is the case for a solvation shell.

Nothing in the foregoing suggestions is contrary to the general proposition [2] of stronger affinity between enzyme and substrate in the transition state than in the Michaelis complex, provided that one does not evaluate such variation in affinity solely as a potential energy change, but rather as change in free energy. We certainly do not suggest that potential energy factors are unimportant in catalysis, and indeed these must be of prime importance in interpretations [3] based for example on variable fit or strain, on differential solvation, on acid–base catalysis, or on certain postulated [4] co-operative effects. We do wish to emphasise the possibility of an important accompanying contribution associated with the variable statistics of distribution of the vibrational energy of the system among readily accessible low-lying vibrational energy levels as it undergoes reaction.

These considerations suggest an examination of the temperature dependence of the reaction rate constants of complexed substrates initially in suitably chosen enzyme model systems. It is not expected of course that all derived experimental activation entropies, even in the best chosen systems, will be positive since there may be accompanying contributions of negative sign. However, the better substrate will have overall the more positive or less negative value to the extent that the above ideas hold good for specific comparisons. Relatively little relevant information is already available in the literature since the particular point basic to our argument has apparently not previously been examined, but in some instances [5] a better substrate has indeed been shown to have a more rate-favouring activation entropy than a poorer both in enzyme and in model studies. Other explanations, however, have been offered [5] in these cases, relating to changes in internal rotational entropy or in hydrophobic bonding, but it should be possible to isolate or eliminate these features with suitable systems which are also tailored to emphasise particularly the point under examination.

Internal rotations, being generally more entropy-rich than all but the lowest frequency vibrations, usually figure more prominently than the latter in most arguments concerning presumed internal entropy changes, but we believe (*cf.* ref. 1) that in many reactions of complexed substrates there will be relatively very few

[2] L. Pauling, *Nature*, 1948, **161**, 707; *cf.* R. Wolfenden, *Accounts Chem. Res.*, 1968, **1**, 321.

[3] For a general account see W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969.

[4] G. G. Hammes, *Accounts Chem. Res.*, 1968, **1**, 321.

[5] M. L. Bender, F. J. Kézdy, and C. R. Gunter, *J. Amer. Chem. Soc.*, 1964, **86**, 3714; C. A. Blyth and J. R. Knowles, *ibid.*, 1971, **93**, 3021.

(if any) internal rotational motions which may be frozen or unfrozen before the transition state is reached, corresponding to the relatively large number of fairly low frequency vibrations undoubtedly undergoing change. Hence we emphasise vibrational changes in this paper, but we note too that only an obvious slight extrapolation is required in our argument to cover both modes of internal motion.

The vibrational properties of enzyme–substrate complexes have previously been invoked [6] in a different manner as an explanation of a catalytic effect: the expected large vibrational amplitude along the reaction co-ordinate, it has been suggested, might imply a faster reaction. It is difficult to analyse this suggestion precisely on transition state theory, in which one formally derives a universal term kT/h for rate of passage along the reaction co-ordinate through the saddlepoint region. However, by qualitative consideration of the other parts of the potential energy reaction surface, larger vibrational amplitudes could perhaps at least permissively be associated with lower potential energies of activation.

Another related recent paper [7] is noteworthy in the use of an approach in part implicitly outside transition-state theory for discussion of (primarily) the expected rate constant differences between inter- and intramolecular reactions, the latter including enzyme-catalysed reactions. While there is little overlap in coverage therefore with the area we have surveyed above, the subject connection is close enough to lead us to examine here the basis of the authors' approach. Two points are made, that vibrational rather than translational energy is required to activate a reaction, and that during reaction sufficient time for adequate vibrational coupling to reacting bonds must be allowed, *i.e.*, sufficient time, it is suggested, for several vibrations. The second point is regarded as novel, and leads to the expectation of reduced reaction rates for inter- in comparison with intra-molecular reactions, since reacting groups in the latter case undergo ' softer ' collisions taking a longer time.

In connection with these views it is of interest to note (*a*) that the standard theory of translational → vibrational energy transfer shows that this is *increased* for complex molecules by ' harder ' collisions and also by increasing molecular complexity, a feature which also enhances vibrational → vibrational energy transfer in collisions, and (*b*) that the following *has* been recognised previously,[8] ' if energy possessed by various degrees of freedom *is* to overcome the activation barrier to a bimolecular reaction it must be able to appear in the place and form that is needed *during the lifetime of a " collision "* ' (original author's italics). At thermal velocities this may be estimated [9] at *ca.* 5×10^{-13} s, about the same order of magnitude as the period of a low frequency molecular vibration (or of several medium frequency vibrations). Moreover, metastable ' collision-complexes ' or ' quasi-bound states ' are already known [10] to be formed in molecular beam studies for several collisions which have been the subject of detailed scrutiny, even although these involve species of very low complexity as would be judged by an organic chemist. This phenomenon increases effective collision time and increases the tendency towards internal relaxation. At least in more complex systems, therefore, this relaxation could reasonably be expected to reach completion in bimolecular processes, giving the transition-state method of state counting in the limit.[10] Thus even for individual collisions the authors' view implying something short of vibrational relaxation between quite complex groups seems to be at best of borderline acceptability. In solution, however, we have vibrational coupling in a solvent cage between reactant molecules and between these and solvent molecules to an extent approximating to that expected for a single large molecule, and this process continues for a relatively long period before reaction (for a period of perhaps up to 100 collisions even in the limit of diffusion-controlled reactions). There seems no reason, therefore, to assume anything less than full vibrational equilibrium in discussions of either inter- or intramolecular reactions in solution.

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[6] L. Schäfer, S. J. Cyvin, and J. Brunvoll, *Tetrahedron*, 1971, **27**, 6177, *cf.* R. Lumry in ' The Enzymes,' eds. P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press, New York, 1959, 2nd edn., pp. 215 and 227.

[7] R. A. Firestone and B. G. Christensen, *Tetrahedron Letters*, 1973, 389.

[8] R. P. Wayne in ' Comprehensive Chemical Kinetics ' eds. C. H. Bamford and C. F. H. Tipper Elsevier, Amsterdam, 1969, vol. 2, pp. 207—208.

[9] S. W. Benson, ' The Foundations of Chemical Kinetics,' McGraw-Hill, New York, 1960, pp. 155—156.

[10] *Cf.* R. D. Levine, *Accounts Chem. Res.*, 1970, **3**, 273.