Proximity Effects in Bimolecular Reactions in Solutions

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As has been discussed by Koshland,[1] Westheimer,[2] Jencks,[3] Bruice[4] and others, part of the catalytic effect of enzymes may arise from the bringing together of substrates and catalytic functionalities of the enzyme when the substrate(s) bind at the active site. For a simple bimolecular process, part of the entropy of activation will relate to the removal of translational degrees of freedom of the reactants as they come together, yet if the two species can be approximated at a surface or in other ways, the overall activation free energy may be lowered because of the less negative "approach" component of $\Delta S\ddagger$.[5] We report here a remarkably large rate-enhancement of a bimolecular reaction

arising from hydrophobic interactions between the two reactants. The relevance of approximation by hydrophobic forces to enzyme-catalysed reactions is clear, since it is known that hydrophobic interactions are responsible in many cases for the binding of substrate to enzyme prior to and during catalysis.[6]

Thus, the aminolysis by a long- and a short-chain amine (n-decylamine and ethylamine) of the p-nitrophenyl esters of a long- and a short-chain acid (n-decanoic and acetic acids) was studied. Second-order rate-constants were obtained spectrophotometrically. Aminolyses by n-decylamine were performed at concentrations well below the critical

TABLE

Second-order rate constants ($M^{-1}sec.^{-1}$) *for hydrolysis and aminolysis of* p-*nitrophenyl esters*[a,b]

	k_{OH^-}	$k_{ethylamine}$[c]	$k_{decylamine}$[c]	$k_{decylamine}/k_{ethylamine}$
p-Nitrophenyl acetate	9·45[d]	5·74[e]	39·1[f]	6·8
p-Nitrophenyl decanoate . .	1·19[g]	0·19[h]	133[i]	700
$k_{decanoate}/k_{acetate}$	0·13	0·033	3·4	100

[a] 35°; 0·99% (v/v) acetone–water; pH varied vetween 8·8 and 10·7.

[b] Buffers: 0·01 M to 0·15 M carbonate–bicarbonate. Since in different reactions either catalysis or inhibition by buffer was observed (*cf.*, ref. 9), all rate constants were obtained from extrapolated rates at zero buffer concentration.

[c] pK^a-values as quoted in ref. 10.

[d] ester: $5·13 \times 10^{-5}$ M.

[e] ester: 3·14 to $25·3 \times 10^{-6}$ M; amine: 1·81 to $14·3 \times 10^{-4}$ M.

[f] ester: 3·14 to $25·3 \times 10^{-6}$ M; amine: 4·50 to $36·0 \times 10^{-5}$ M.

[g] ester: $4·53 \times 10^{-6}$ M.

[h] ester: 6·0 to 48×10^{-6} M; amine: 3·71 to $29·7 \times 10^{-5}$ M.

[i] ester: $4·53 \times 10^{-6}$ M; amine: $4·08 \times 10^{-5}$ M.

micelle concentration (40 mM[7]) to avoid enhanced rates of hydroxide-ion-promoted hydrolysis of ester in a partially cationic micelle of n-decylammonium ions.[8] All rate-constants (except, of course, that for long-chain ester with long-chain amine) were shown to be invariant with amine concentration over the concentration ranges quoted in the Table.

In principle, approximation of two reactants can be brought about by covalent bonding (*i.e.*, intramolecular reactions) or by such intermolecular forces as charge-transfer interactions, electrostatic attractions, hydrogen bonds, or hydrophobic forces. Examples of the effect on reaction rates of proximity[4,11] and orientation[12] of neighbouring groups in the same molecule are common, but effects on bimolecular reactions have been less well studied. Small[13] or negative[14] accelerations have been observed in some systems as a result of charge-transfer or π-complex formation and similarly small effects have been observed for reactants which may interact electrostatically[15] or by London forces[16] (though for polymeric molecules, larger effects have been reported by Letsinger and his co-workers[17]). Work in frozen solutions[18] and micelles[8,19] has thrown up some interesting data relevant to the question of approximation, but to our knowledge there is no report of a large rate enhancement in a simple bimolecular reaction between two non-polymeric molecules.

From the Table the following points are apparent. (1) The $k_{decanoate}/k_{acetate}$ ratios for the nucleophiles OH⁻ and ethylamine are less than one, which is presumably the result of steric hindrance (though an effect of the microscopic dielectric constant cannot be ruled out). (2) Despite the expected steric hindrance arising from the coiling up in water of n-decylamine, the $k_{decylamine}/k_{ethylamine}$ ratio for p-nitrophenyl acetate is about 7. This may be attributed to approximation by hydrophobic forces between the p-nitrophenyl group and

the long-chain amine. (3) The $k_{decylamine}/k_{ethylamine}$ ratio for p-nitrophenyl decanoate is 700! Possible explanations for this large ratio are: (a) That it represents an increased rate of *hydrolysis* either by OH⁻ attacking the long-chain ester in a mixed cationic micelle[8] or "micromicelle"[20], or by general catalysis of OH⁻ attack by neighbouring decylammonium ions; (b) That it is due to an enhanced rate of *aminolysis* by the amine in a mixed micelle or "micromicelle". (The term "micromicelle" is used to describe groups of two to, say, six approximated molecules.)

The possibility that either hydrolysis or amino-lysis is occurring in a fully-formed micelle is eliminated by the amine concentration being about three orders of magnitude below the critical micelle concentration, and the ester concentration is four orders of magnitude below it. The enhanced rate must therefore arise because the long-chain ester is approximated to the long-chain amine, in 1 : 1, 2 : 1, 1 : 2 etc. complexes. (By analogy with one-substrate enzyme-catalysed reactions, the observation of simple second-order kinetics over at least four half-lives implies that the dissociation constant of the AB complex in the simple 1 : 1 case of A + B ⇌ AB → products, is much larger than [A] or [B].)

In order to estimate the contribution from (a) hydrolysis and (b) aminolysis to the rate enhancement, ethylamine ($4·04 \times 10^{-2}$M) and decylamine ($6·41 \times 10^{-5}$M) were allowed to compete for p-nitrophenyl decanoate (4×10^{-6}M). Product analysis of the ratio of the ethylamide to the decylamide by g.l.c., and comparison of this with the ratio expected from the rate constants in the Table (calculated on the basis that the hydrolysis rate is unchanged in the presence of amine) showed that approximately 47% of the rate enhancement is due to aminolysis, and the remainder arises from enhanced hydrolysis.

About half of the large rate enhancement seen in the reaction of long-chain ester with long-chain amine is therefore due to approximation of these reactants in solution. Preliminary experiments indicate that the 100-fold enhancement is much reduced when the reactions are performed in 50% v/v aqueous 1,4-dioxan, a solvent in which hydrophobic interactions are expected to be markedly decreased.[21]

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