Catalysis through Induced Intramolecularity: What Can Be Learned by Mimicking Enzymes with Carbonyl Compounds that Covalently Bind Substrates?

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Protein engineering and the use of catalytic antibodies have given substance to the possibility of making artificial enzymes, whereas chemical design has provided functional, though not yet useful, enzyme mimics. This article is devoted to a class of mimics that uses covalent binding to substrates at a site different from the reaction center and that exhibits turnover. These molecular devices are capable of converting the bimolecular reaction of a substrate into a fast intramolecular one and may be defined by the concept of catalysis by induced intramolecularity. As examples of such systems, several reactions of bifunctional substrates that involve catalysis by carbonyl compounds are brought together in this review. Their ability to catalyze difficult reactions such as the hydrolysis of unactivated amides by mimicking enzyme–substrate complementarity is the result of a uniform stabilization of bound species, including both the transition state and also encounter complexes, which is the specific feature of these systems. They allow quantitative analysis of differences between intramolecular and bimolecular processes and support the explanation based on the entropic disadvantage of bimolecular reactions that can be compensated by binding energy as in enzymes. Three-dimensional energy diagrams are proposed as valuable tools to describe these systems, as well as enzymatic mechanisms and other catalytic mechanisms that take advantage of induced intramolecularity. Unexpectedly, these diagrams are appropriate to a wide field of applications, such as the description of intramolecular and bimolecular reactions, resulting in a dynamic description of the notion of "tight" binding. In addition, the high efficiency of catalysts that covalently bind substrates raises the question of what their contribution may have been in early enzyme evolution. Their potential association with primitive enzymes as catalytic cofactors is considered.

Introduction

Catalysis by enzymes[1] is characterized by Michaelis−Menten kinetics, corresponding to reversible, non-covalent binding of a substrate (S) to an enzyme (E) (Scheme 1). This reaction is followed by reversible or irreversible chemical transformations, leading either directly or through bound intermediates (E·I), to the enzyme−product adduct (E·P). Regeneration of the catalyst and release of the product are achieved in a reversible final step. The high efficiency of enzymatic catalysis, ranging from 10^6 to 10^{17} in terms of k_{cat}/k_{non} [2,3] has prompted chemists to devise mimics in which catalysis is brought about by reversible binding to the substrate. Fast reactions arise from catalytic functional groups correctly positioned to react inside the

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Robert Pascal, born in 1952, studied physical chemistry at the University of Montpellier 2. In 1980 he obtained a Doctorat d'Etat with a thesis on catalytic improvements of Strecker synthesis under the supervision of Jacques Taillades and Auguste Commeyras. He joined the CNRS in 1979 and continued investigations in the field of amino acid chemistry until 1988. With an interest in the interplay between chemistry and life sciences, he moved on to peptide chemistry and solid-phase synthesis at the Centre de Recherche de Biochimie Macromoléculaire. Since 1997, he has been working in the group of Patrick Jouin at the Centre de Pharmacologie-Endocrinologie in Montpellier. His fields of interest are organic reactivity, peptide chemistry, solid-phase synthesis, prebiotic chemistry, and enzyme mimetics.

MICROREVIEWS: This feature introduces the readers to the author's research through a concise overview of the selected topic. Reference to important work from others in the field is included.

$$E + S \rightleftharpoons E{\cdot}S \rightleftharpoons (E{\cdot}I \rightleftharpoons) E{\cdot}P \rightleftharpoons E + P$$

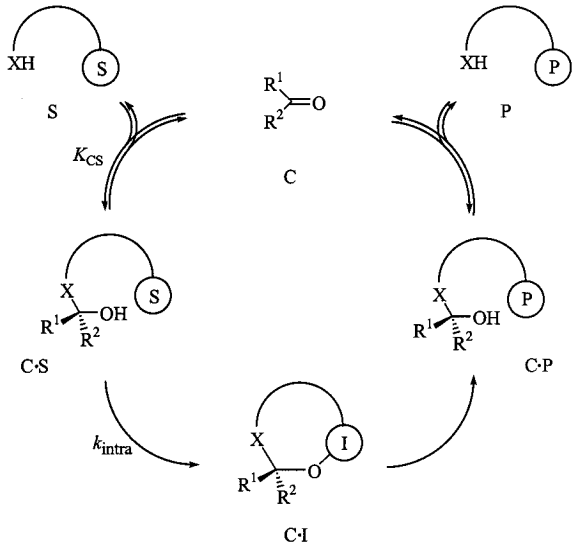
Scheme 1. Enzymatic catalysis; E: enzyme; S: substrate; I: chemical intermediate; P: product

adduct. Different categories of mimics have been described, involving − for example − molecular recognition by cyclodextrins or other supramolecular hosts and complexes with metal ions.[4−11]

Several mechanisms for hydrolytic or solvolytic reactions involving unusual but highly efficient catalysis by aldehydes, ketones, and even carbon dioxide have been reported.[12−30] They share features sufficiently similar that these mimics can be regarded as a distinct category. These mechanisms (Scheme 2) involve the formation of adduct C·S through the reversible addition of a nucleophilic group of the substrate S to the catalyst C. Participation of the newly formed neighboring hydroxy group in the chemical transformation of the reacting group within the adduct C·S is responsible for the overall catalysis. This catalytic scheme, in which catalysis is brought about by induced intramolecularity,[31] reproduces specific features of enzyme reactions, as was discussed as early as 1969.[32] Because reversible binding (although through a covalent bond) of these catalysts is followed by intramolecular catalysis,[33−36] carbonyl compounds can be viewed as very simple enzyme mimics. However, recent reviews on enzyme mimics have neither included these examples nor discussed the particular and informative features associated with this category of catalysts. The only related system to have been discussed is intramolecular catalysis by neighboring carbonyl groups taking place through the reversible formation of hydrates or, more generally, adducts with nucleophiles.[37,38] Moreover, the original discussion[32] of these systems was based on an inaccurate understanding of the advantages of intramolecularity. It was later revised to incorporate the notion that bringing reactants together in the proper position for reaction results in very large losses of entropy in bimolecular reactions.[34−36] Binding energy can therefore be utilized by enzymes to pay for this entropy loss and to increase the rate by a factor of up to 10^8 for 1 M reactants.[39] Although the importance of this revision has not always been recognized, it was considered "a milestone in enzymology of comparable importance to the role of quantum mechanics in chemistry".[40] In this article, information concerning several examples of catalysis by carbonyl compounds is reviewed, and the activity of these catalysts is discussed in the light of these modern views about intramolecular reactions and catalysis by enzymes.

Catalysis through Carbonyl Compound−Substrate Adducts

As catalysis is the result of the equilibrium giving adduct C·S and then the chemical transformation of the reacting group, both steps are essential for the catalytic process. The stability of adduct C·S is strongly influenced by the nature of the heteroatom X. Alcohols, thiols, or amines are well



Scheme 2. Intramolecular reactions of carbonyl compound adducts

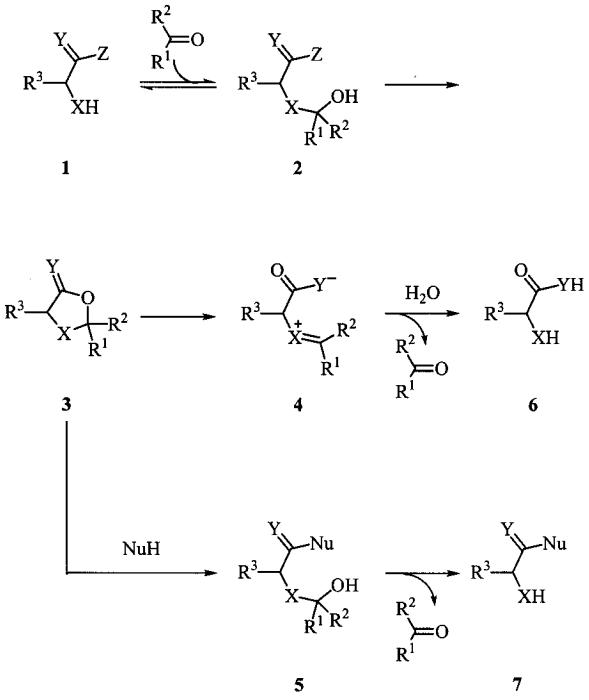
known to give rise to hemiacetals, hemithioacetals, or carbinolamines, respectively. Oxygen nucleophiles, however, have a much lower tendency (by several orders of magnitude) than sulfur or nitrogen nucleophiles to react with the carbonyl group.[41−44] Reactions are fast and do not generally require catalysis. However, reactive carbonyl compounds are to some extent hydrated[45] in water and can undergo reversible or irreversible side-reactions,[41−44,46] which must be taken into account when comparisons are made.

All the following examples involve the formation of five-membered rings, due to the presence of a heteroatom substituent X at the α-position with respect to the reacting group (Scheme 3). This situation favors high effective molarities (EM = k_{intra}/k_{inter}).[33]

A fast turnover of the carbonyl compound, needed in order to be a truly catalytic reaction, results from a fast reaction of cyclic intermediate **3** with nucleophiles (via carbinolamine **5**). The catalyst can alternatively be regenerated by elimination through intermediate **4**, an easy process with carbonyl compound derivatives, as already pointed out in a case of catalysis by an aldehyde hydrate.[47] Interestingly, aldehyde hydrates, resulting from post-translational modifications, have been shown to play the role of nucleophiles in the active site of sulfatases.[48] Elimination of sulfate from a covalent addition intermediate also results in a fast regeneration of the enzyme.

Hydrolysis and Methanolysis of Esters

Hydrolysis of amino acid esters **1a** and thioesters **1b** was the first reaction shown to involve induced intramolecularity through carbonyl compound adducts.[12−17] The rates of hydrolysis of glycine, phenylalanine, and leucine *p*-nitrophenyl esters are fast in the presence of aromatic aldehydes at neutral pH (4.8−7.6).[14−15] The proposed mechanism involves an oxazolidin-5-one intermediate (**3a**; R^1 = Ar,

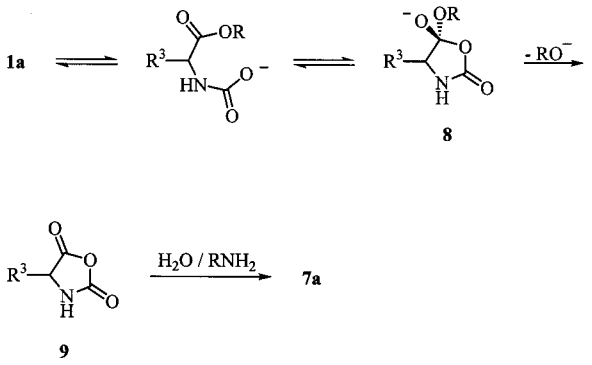


a: X = NH, Y = O, Z = OR
b: X = NH, Y = O, Z = SR
c: X = O, Y = O, Z = OAr
d: X = NH, YZ = N, Y = NH
e: X = N-Ac, YZ = N, Y = NH
f: X = N-Me, Y = O, Z = NR_2
g: X = S, Y = O, Z = NR_2

Scheme 3. Reactions of bifunctional compounds subject to carbonyl compound catalysis

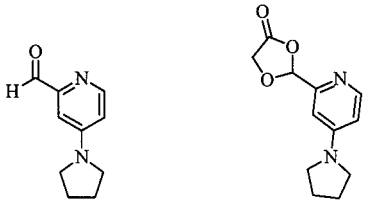
R^2 = H), the hydrolysis of which must be fast in order to account for the observed efficient catalysis.

Carbon dioxide catalysis of α-amino acid *p*-nitrophenyl ester hydrolysis has also been observed[12,13,15–17] and attributed to a similar mechanism through *N*-carboxy anhydrides **9** (Scheme 4). Since *N*-carboxy anhydrides are highly susceptible to polymerization, oligopeptides are produced as well as free amino acids. Unlike in amino acid ester self-condensation, dioxopiperazines are not obtained, because of the intermediacy of *N*-carboxy anhydrides **9**. The implication of bicarbonate-catalyzed polymerization of amino acid esters in the formation of polypeptides on the primitive earth has been discussed.[16,17] However, catalysis seems to be limited to activated esters (*p*-nitrophenyl esters) or thioesters. Methyl esters, for instance, do not undergo bicarbonate-catalyzed polymerization in aqueous solution.[17] This is an example of what Menger called the *p*-nitrophenyl ester syndrome: impressive rate accelerations observed for these substrates often vanish when less reactive esters are used.[49] In fact, the breakdown of tetrahedral intermediate **8** leading to *N*-carboxy anhydride is strongly influenced by the leaving group capability of the alcohol component of the ester. Poor leaving groups such as $Me-O^-$ are much less easily expelled than the carbamate ion from tetrahedral intermediate **8**, which reverts to the reactants.



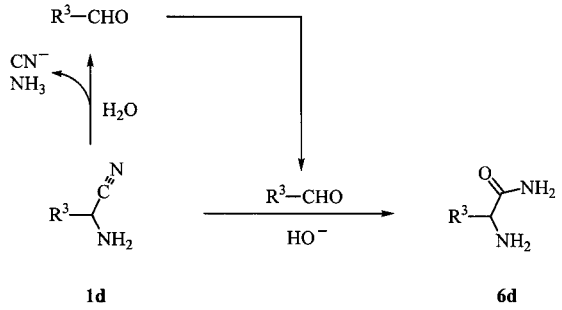
Scheme 4. Carbon dioxide promoted reaction of α-amino acid esters

An elaborated catalyst (**10**) for the methanolysis of α-hydroxy esters (**1c**) has been devised.[27–29] Although the stability of hemiacetals **2c** is limited in comparison with that of carbinolamines, efficient catalysis has been observed. The 4-aminopyridine moiety introduced into the catalyst is necessary for catalysis to be observed. Though it had been devised as an intramolecular acyl transfer agent derived from 4-(dimethylamino)pyridine, this moiety does actually act as a general base. The dioxolanone intermediate **11** was shown to accumulate and its reaction with methanol is the rate-limiting step of the overall process.



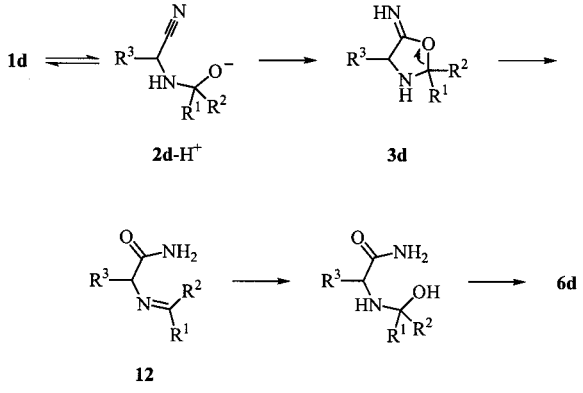
Hydration of Nitriles

The alkaline hydration of amino nitriles (**1d**) into α-amino amides is fast in comparison with that of other nitriles. Surprisingly, carbonyl compound catalysis has been shown to be responsible for this spontaneous reaction, and a mechanism related to autocatalysis actually takes place (Scheme 5).[18–20] The occurrence of this pathway is due to the reversion of α-amino nitriles into aldehydes or ketones, capable of promoting the conversion of α-amino nitriles into α-amino amides. Generally, aldehyde precursors of natural amino acids are needed in low proportion for hydration to be complete, but these precursors are not stable under the alkaline conditions of the reaction and overall yields are far from quantitative. The addition of a stable and simple ketone such as acetone bypasses the spontaneous process and yields are substantially increased.[19,21–23,50,51] Recyclable as well as enantioselective catalysts have also been introduced.[52,53] The reactions are third order (first order



Scheme 5. Pseudo-autocatalytic pathway for the spontaneous hydration of α-amino nitriles

each in substrate, catalyst, and OH^- concentration), which is consistent with a rate-determining cyclization of carbinolamine anion **2d**-H^+ (Scheme 6). With 2-aminopropionitrile, the order of magnitude of the effective molarity was estimated to be ca. 10^8 M, and $\Delta H^{\neq}$ values of 12, 4, and -2 kJ·mol^{-1} and $\Delta S^{\neq}$ values of -230, -185, and -140 J·K^{-1}·mol^{-1} have been measured for acetone, acetaldehyde, and formaldehyde, respectively.[20] These strongly negative values for entropy are consistent with a tight transition state formed by the association of catalyst and substrate. The almost zero value for enthalpy can be accounted for by the summation of a negative contribution for carbinolamine formation and a positive one for the formation of the cyclic transition state.[54] Because of a fast elimination of the *O*-carbamoyl group, the postulated 5-iminooxazolidine intermediate **3d** rearranges into the observed imine **12** (Scheme 6).

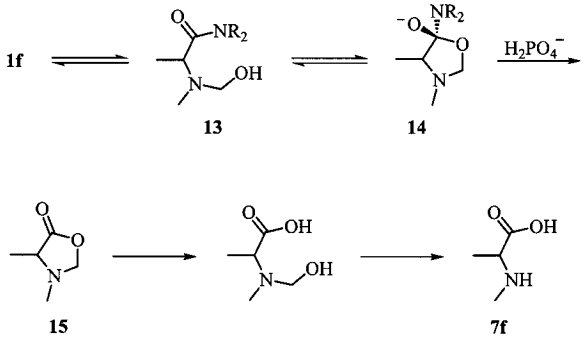


Scheme 6. Aldehyde- and ketone-promoted hydration of α-amino nitriles

Starting from acylamino nitriles **1e** and formaldehyde, the adduct is still formed, though acid−base catalysis is required.[24−25] As a result of acylation, cyclic intermediate **3e** (Y = NCH_2OH or NH, $R^1 = R^2 = H$) is stabilized and can be isolated. Its breakdown takes place either by elimination through acyliminium compound **4e** (Y = NCH_2OH or NH, $R^1 = R^2 = H$), as mentioned above for the unsubstituted compound, or by nucleophilic attack via **5e**, depending on the conditions and substituents.

Amide Hydrolysis

Since the amide group is stabilized by resonance, its hydrolysis usually requires severe conditions or efficient catalysts. Consequently, only a limited number of enzyme mimics capable of hydrolyzing unactivated amide bonds under mild pH conditions are available.[55−57] In every case, metal ions are involved. Formaldehyde catalysis is an exception to this rule, giving rise to a serine protease mimic that has the unique property of being functional at neutral pH. Thus, *N*-methylalanine amides **1f** (Scheme 3) undergo hydrolysis in neutral phosphate buffers (pH = 6−8) in the presence of formaldehyde (Scheme 7).[26] Both nucleophilic and acid−base catalyses are involved in the reaction. Hydrolysis of the postulated oxazolidin-5-one intermediate is fast. Similarly, amides of thioglycolic acid **1g** (R^3 = H) are hydrolyzed in alkaline solution by cyclic ketone catalysis.[30]



Scheme 7. Formaldehyde-catalyzed hydrolysis of *N*-methylalanine amides in phosphate buffers

Related Processes

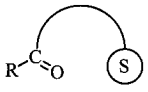
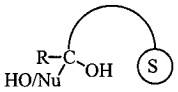
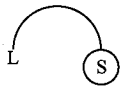
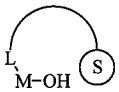
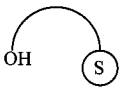
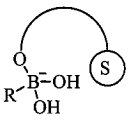
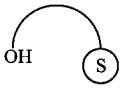
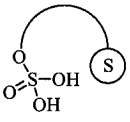
Other Mechanisms Involving Covalent Binding of the Catalyst

As mentioned above, nucleophilic catalysis by neighboring carbonyl groups[37,38] belongs to the category of catalytic mechanisms involving induced intramolecularity, although the actual catalyst in this case is water or another nucleophile (Table 1). Catalysis by mechanisms involving the reversible covalent binding of the catalyst followed by an intramolecular reaction is not limited to carbonyl compounds (Table 1). Hydrolytic mechanisms based on metal hydroxides that share these features have also been reported.[32,55−60] It has also been proposed that boric and boronic acids adducts with substrate hydroxy groups bring about catalysis by cyclization of covalent adducts.[32,61−65] It has even been proposed that sulfuric acid promotes cyanohydrin hydrolysis in such a way.[66]

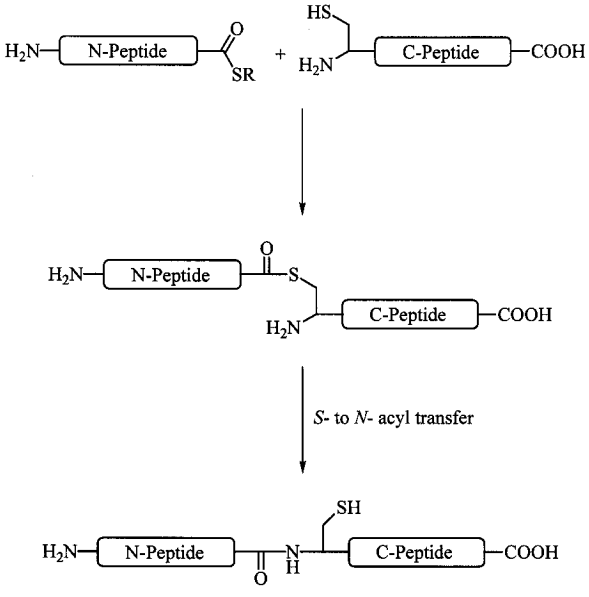
Related Stoichiometric Mechanisms

Intramolecular reactions of adducts formed by binding at a site different from the reaction site are also a useful

Table 1. Examples of intramolecular reactions of adducts of bifunctional substrates with various catalysts

Substrate	Catalyst	Adduct
	H_2O / NuH	
	M^+	
	$R-B(OH)_2$	
	H_2SO_4	

synthetic tool with which to effect selective organic transformations.[67] A noteworthy example of their applications is the chemical ligation of unprotected peptide segments, which during the past decade has resulted in a breakthrough in the field of chemical protein synthesis.[68−71] In this process, the selectivity of peptide bond formation arises from an intramolecular acyl transfer to the *N*-terminus of a peptide adduct (Scheme 8). In relation to the main subject of this review it should be noted that several examples of chemical ligation strategies use carbonyl compound adducts.[68][71]



Scheme 8. Chemoselective peptide ligation of unprotected peptide *C*-terminal thioesters with *N*-terminal cysteine peptides

Induced Intramolecularity

Intramolecular Reactions

Intramolecular reactions between nucleophilic and electrophilic centers (i.e., cyclizations) can be very fast.[33] Comparison with the corresponding bimolecular reaction is generally made by using the effective molarity (EM = k_{intra}/k_{inter})[33] of the catalytic group, which has the dimension of a concentration because the rate constant of a unimolecular reaction (in s^{-1}) is being compared with that of a bimolecular one (in $s^{-1}\cdot M^{-1}$). EMs correspond to nominal concentrations of reagent that would be needed to make the bimolecular reaction of a substrate as fast as the intramolecular one. EM values of 10^5 to 10^8 M are often observed, but they can reach values above 10^{13} M in extreme cases.[33] Because of this efficiency, intramolecular reactions are the only simple chemical systems that can rival enzymes.[11,34−36] Bimolecular reactions require the bringing together of two molecules, resulting in a concomitant loss of degrees of freedom; they are thus entropically disadvantaged because they are improbable.[34−36] Factors of up to 10^8 M have been estimated for this entropic contribution to EMs in chemical systems.[34−36] EM values consistent with this maximal contribution have repeatedly been observed but are difficult to dissociate from the contribution of strain that can be released on going from a linear reactant to a cyclic transition state.

Intramolecular Reactions of Covalent Adducts

Determination of EMs in the catalytic systems reviewed here is possible in only a few cases, because values of the product $k_{intra}\cdot K_{CS}$ rather than the cyclization rate constant k_{intra} are available (Scheme 2). However, an EM value of ca. 10^8 M was estimated for the cyclization of the carbinolamine anion **2d**-H^+ (Scheme 6), formed by the addition of alanine nitrile (**1d**) and isobutyraldehyde, in comparison with the bimolecular reaction of hydroxide ion with the nitrile group.[20]

A more meaningful comparison can be attempted in the case of formaldehyde catalysis of the hydrolysis of the unactivated amide *N*-methylalanine morpholide [**1f**; R_2 = $(-CH_2CH_2)_2O$] (Scheme 7). A rate constant (k_{cat} = 2.7 × 10^{-3} s^{-1}) has been determined for the conversion of formaldehyde adduct into product (50 °C, pH = 7, 0.75 M phosphate buffer, 2 M ionic strength).[26] The pseudo-first-order rate constant for the uncatalyzed hydrolysis of a peptide bond (k_{non} = 8 × 10^{-10} s^{-1} for Ac−Gly−Gly−NHMe at 50 °C and pH = 7 in water) can be calculated from published data.[72] Therefore, neglecting the likely effect of phosphate buffer[73] on the rate of the uncatalyzed reaction and the fact that substrates are different, the rate enhancement provided by formaldehyde catalysis can be very roughly estimated as six orders of magnitude in terms of k_{cat}/k_{non}. Taking into account the molarity of water (55 M) in the uncatalyzed pathway, a value of approximately 10^6-10^8 M is then deduced for the EM of the hydroxy group of formaldehyde adduct **13**.

These examples show that EMs estimated for covalent adducts of carbonyl compounds are in agreement with those found for the cyclization of stable linear precursors giving five-membered rings.[33] Measured on a logarithmic scale, the benefits of intramolecularity in the case of formaldehyde-catalyzed hydrolysis of unactivated amide **1f** (six orders of magnitude) correspond to approximately one half of the rate enhancements provided by proteases[72] ($k_{cat}/k_{non} = 10^{10}$ to 10^{13}).

Induced Intramolecularity in Carbonyl Compound Catalysis

At low concentrations of substrate and catalyst (where $v = k_{intra}\cdot K_{CS}\cdot[C]\cdot[S]$), kinetics are dependent both on the equilibrium constant K_{CS} and on the rate constant k_{intra} (Scheme 2). Binding energy therefore contributes to the rate both by increasing the amount of the reactive intermediate C·S and by making the reaction intramolecular. It must be emphasized that neither substrate and catalyst concentrations nor the equilibrium constant K_{CS} have any effect on the latter contribution (induced intramolecularity) that can be measured by the ratio $EM = k_{intra}/k_{inter}$. The dependence of rate increases provided by induced intramolecularity on the nature of the linkage (in terms of the precise arrangement of reacting centers), but their apparent independence of the free energy corresponding to the binding step, has several important consequences that are analyzed in the following discussion of enzymatic catalysis.

The intramolecular reactions of covalent adducts reviewed here provide a quantitative way to assess the difference between intramolecular and intermolecular processes. The formation of a covalent bond in the adduct results in the loss of global rotation and translation entropy. As long as enthalpy/entropy compensations, related to differences in the solvation of the transition state and the substrate,[39] do not become predominant, a negative value is to be expected for the overall catalytic process. Such a value would be evidence of this kind of catalysis, and indeed, strongly negative values of $\Delta S^{\ddagger}$ (-140 to -230 $J\cdot K^{-1}\cdot mol^{-1}$) have been found for carbonyl compound catalysis of the hydration of α-amino nitriles.[20] In these examples the observed loss of entropy is clearly connected with the rate increase in the intramolecular reaction of the adduct. This is a clear additional argument in support of the explanation of rate increases in intramolecular reactions based on the entropic disadvantage of bimolecular reactions, which is still today the object of criticism[74–76] in spite of its solid foundations.[34–36,77]

A Tool for the Representation of Induced Intramolecularity

This concept, previously introduced by Jencks and coworkers in the context of enzymatic catalysis,[31][59] is associated with the formation of complexes of catalysts with substrates. It results in the addition of an association step (binding of substrate) and a dissociation step (release of product), which is very similar to the usual representation of enzymatic catalysis (Figure 1). As with many concepts, a convenient representation is needed to make it easily understandable. However, the two-dimensional free energy diagrams customarily used to describe enzymatic catalysis are not useful to bring to light the role of induced intramolecularity. This is because the reaction progress corresponding to first-order and second-order processes is merged into a single coordinate, which can be misleading if the fact that such a procedure needs the definition of a standard state is disregarded.[78] Many problems are associated with neglect of this point. A rigorous resolution of these problems is introduced here through the use of independent coordinates for the association step and the chemical step to build an energy diagram (Figure 2). Although the limitation to a three-dimensional space confines the use of this representation to a reaction involving one catalyst and one substrate only, it is used here to clarify the notions discussed below. The energy diagram corresponding to the reference unimolecular reaction is shown in the background, whereas the reaction involving bound species (intramolecular reaction) is shown in the foreground. Binding or release steps correspond to a transformation from one plane to the other one. Any comparison of background with foreground free energy levels requires the definition of a standard concentration. Therefore, the observer (who defines the standard state) cannot be neutral in this comparison and his/her point of view can be pictured by a sliding motion of one plane relative to the other one, or, more illustratively, by the angle from which the scheme is looked at (Figure 3).

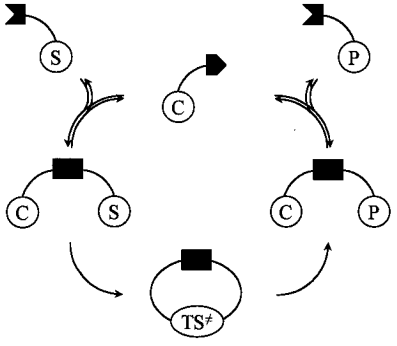


Figure 1. Catalysis through induced intramolecularity

An additional difficulty in this representation is that visualization of the advantages of intramolecularity requires comparison of the intramolecular reaction with a bimolecular one. This can be achieved by applying a three-dimensional diagram to catalysis by induced intramolecularity and mentally conceiving the consequences of the removal of binding interactions in the system (Figure 4). As these interactions decrease, the energy level of the C·S complex must increase up to the level of the improbable state C·S*, characterized by a complete absence of stability. This state corresponds to a fixation of reactants relative to each other in precisely the correct position for reaction but without any binding interaction between the catalyst and the substrate, which can be assigned to a truly bimolecular process.

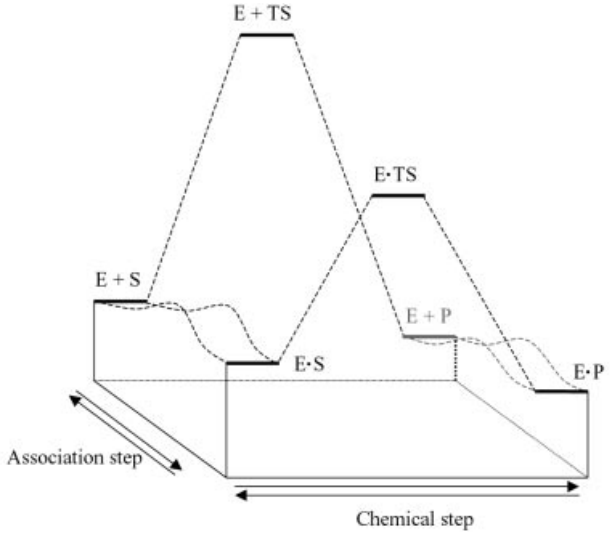


Figure 2. Three-dimensional free energy diagram illustrating the enzymatic catalysis of a unimolecular reaction

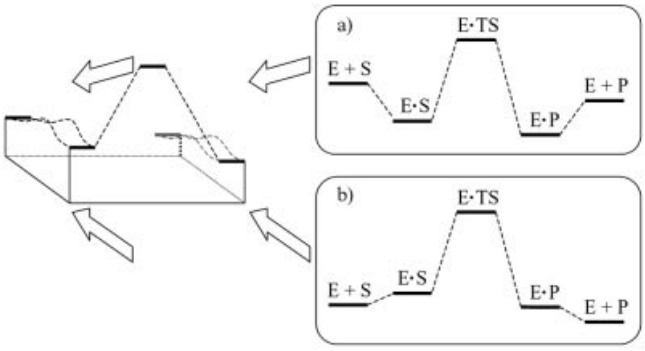


Figure 3. Comparison of Gibbs free energy levels of bound species with those of the free reactant or product; effect of the choice of a standard concentration for the conversion of a three-dimensional diagram into the usual two-dimensional diagram (the levels of diffusion barriers and that of the reference reaction have been omitted): (a) standard concentration = 1 M; (b) standard concentration = physiological concentration of substrate

As a result, these diagrams are suitable for the thermodynamic description of bimolecular reactions, which raises the question of the definition of the C·S* state in these processes and that of its physical reality. A conservative definition of the nature of C·S*, the product of the "association" step in a bimolecular reaction, can be that of an *encounter complex* (matching proximity requirements) *but one in which all motions hampering the conversion into transition state have been lost* (mainly overall rotation and translation motions for reactions with tight transition states[36]). In this situation, the rate constants for a reaction would be independent of the bimolecular, intramolecular, or enzymatic nature of the system, disregarding the specific stabilization of the transition state by enzymes that is independently responsible for an essential part of their power through the pre-organization of the active site.[79]

It seems obvious that two species reacting in a second-order reaction must come into contact with each other before reaction can occur. In many theoretical descriptions such reactions are formulated as occurring in two elementary steps. For instance, Jencks proposed the following thought experiment: "the nonenzymic reaction can be separated into two parts: first, the bringing together of the reactants into exactly the right position to react, [...] and second, the activation process to reach the transition state".[80] Moreover, the necessity for reactants to be brought together and for products to diffuse away from each others is essential in the theory of Marcus,[81] and associated with the work terms w_r and w_p, respectively. Finally, the definition of the C·S* state could be compared to that of the Near Attack Conformers (NACs) defined by Bruice[82] as "the required conformation for juxtaposed reactants to enter the transition state". However, NACs require the arbitrary choice of precise limits in terms of angle of approach and distance. This is not necessary with use of the present definition of encounter complexes, since the demand may be different from one reaction to another according to the "tight" or "loose" character of the corresponding transition state.

Experimental evidence for the definition for encounter complexes developed here can be found in the existence of double-well potential energy surfaces in gas-phase reactions such as nucleophilic substitution at a carbon atom.[83] In the gas phase, the association of two *neutral* molecules to produce an encounter complex with a proper orientation for reaction must be governed only by entropy, provided that weak (dipole−dipole and van der Waals) interactions are negligible. The product of this "association" step is an improbable state that may be defined as a "hidden intermediate"[84] that does not occupy a local minimum on the potential energy surface. However, as soon as favorable binding interactions are present, such as ion−dipole interactions in the gas-phase reaction between halide ions and halomethanes,[83] encounter complexes are stabilized and can correspond to true intermediates. The rates of these gas-phase reactions can be much higher than the corresponding rates in solution as a result of this induced intramolecularity in the complex, because of the utilization of binding energy to pay for the entropy loss. In solution, the advantage is cancelled because of the occurrence of independent interactions of reactants with the solvent.

Any binding interaction stabilizing the encounter complex must increase its lifetime by raising the energy barrier corresponding to the binding step. Induced intramolecularity is therefore connected with the formation of true intermediates corresponding to the use of intrinsic binding energy[39] to stabilize bound species C·S*, C·TS, and C·P*, rendering these states less improbable. As shown above for gas-phase reactions, the occurrence of stabilized complexes with reactants along the reaction path can therefore be considered as a manifestation of induced intramolecularity. It has also been applied to the detection of induced intramole-

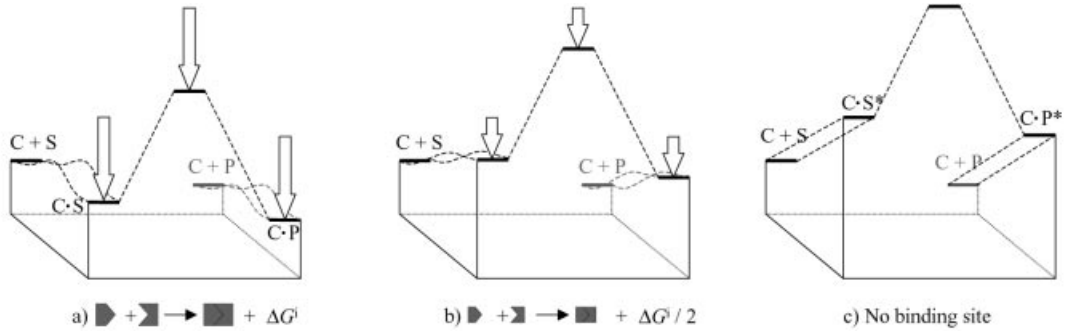


Figure 4. The reduction of the intrinsic binding free energy (ref.[39]) corresponding to interactions at the binding site as defined in Figure 1; as interactions decrease, the free energy level of the C·S complex increases, reaching the unstable encounter complex C·S* in c)

cularity in concerted processes of general acid−base catalysis at centers that are ordinarily hydrogen-bonded.[85]

By use of the three-dimensional diagrams, induced intramolecularity can easily be represented by a downward shift of energy levels in the foreground (Figure 5). Induced intramolecularity can thus be defined as the consequence of uniform binding according to the description of Albery and Knowles.[86] Although it is more easily understood when binding takes place at a site other than the reacting group, the specific feature of induced intramolecularity is associated with binding energy. This binding energy compensates for the cost of freezing the reactants in the proper position for reaction,[39] is maintained at the transition state (which accounts for catalysis), and disappears only when the product is released. In other words, binding interactions that are not specific of the transition state can efficiently bring about catalysis by converting the reaction into an intramolecular one. However, it must be stressed at this point that induced intramolecularity is not equivalent to binding, but is its consequence. It results in a lowering of the chemical step barrier because it is related to the loss of entropy of bound states and not to the free energy of the binding step. This nonequivalence is manifest when excess binding energy is released in the association step: it becomes useless, since no further advantages can be obtained, as soon as the free energy level of the bound substrate is below that of the free substrate. Moreover, favorable binding does not result in a complete loss of entropy if it corresponds to a large number of equivalent states.[80]

This description of induced intramolecularity as the uniform stabilization of bound species is also in agreement with recent examples of noncovalent catalysis in supramolecular complexes,[87] in hydrophobically stabilized encounter complexes,[88] or resulting from prebinding of substrates.[89]

Can Association and Chemical Steps be Concerted?

With three-dimensional energy diagrams, the reaction progress corresponding to these processes is represented by two different coordinates, which raises the question of the occurrence of concerted processes that are generally ana-

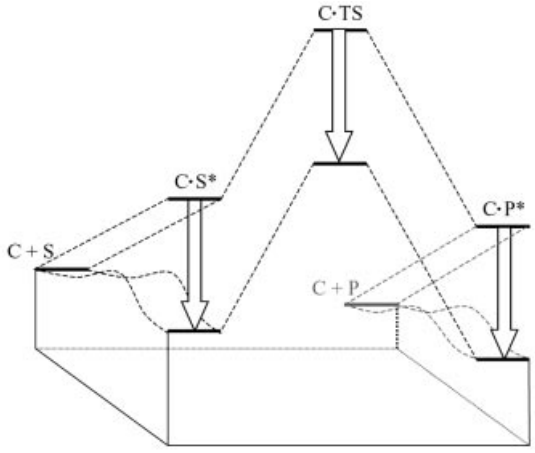


Figure 5. Catalysis by induced intramolecularity as the consequence of uniform binding (uniform stabilization of bound states)

lyzed by use of the closely related More O'Ferrall−Jencks diagrams.[90,91] The question of the physical existence of the encounter complex C·S* is changed into that of the concerted or stepwise character of "association" and chemical steps (does the bimolecular reaction proceed through the left-hand foreground corner of Figure 5?). According to the principle of microscopic reversibility this question is related to that of a finite lifetime for the complex C·S* in the reverse direction from the transition state back to the substrate. Comparison of the decay of transition states, usually associated with the universal frequency factor ($k_BT/h \approx 6 \times 10^{12}$ s^{-1}) according to transition state theory,[92] with the upper limit for the dissociation of bimolecular complexes (10^9 to 10^{12} s^{-1})[1] rules out a fully concerted mechanism. This analysis is consistent with a relaxation time for the environment that can be longer than the decay of transition states.[93][94]

Another interesting aspect of this dynamic description is that the need for "tight" binding, which has been associated with the efficiency of intramolecular[34−36] and enzymatic catalyses,[80] can be defined more clearly by introducing the question: what are the motions that are not compatible with the reaction and that cannot be lost in a concerted manner with the chemical step to enter the transition state? This

approach may be helpful in recognition of the detrimental effect of motions that have important contributions to the entropy of the system, namely translations or global rotations in the gas phase and related low frequency motions ($\leq$ 100−400 cm^{-1}) in condensed phases or in loose complexes.[34] "Tight" binding may thus be related to the formation of an intermediate along the reaction path resembling the C·S* state but *with a lifetime that is sufficiently long for the corresponding energy to be redistributed* to the internal degrees of freedom or to the environment in a reversible step that is independent of the activation process. This proposition means that to take advantage of induced intramolecularity would need the conversion of an ordinary (loose) encounter complex in which low-frequency motions are still present into an intermediate defined by a well in the potential energy surface. According to this hypothesis, motions that contribute to entropy cannot be lost within the lifetime of the transition state, so complementarity of enzymes to transition states would not be efficient in overcoming the entropy barrier. The achievement of the full binding energy in transition states would then require prebinding of substrates, which is in agreement with the widespread observation of complexes of enzymes with their substrates.

A New Energetic Representation of Intramolecular Reactions

An unexpected outcome of the use of these three-dimensional diagrams is their suitability for the description of intramolecular reactions as well. In this description, *any* intramolecular reaction can be described as corresponding to a partial progress of the association step as early as in the ground state (Figure 6). For highly efficient intramolecular systems (such as the formation of five- or six-membered rings) the association step can be viewed as almost complete in the ground state because of its sufficient similarity with the C·S* state with respect to the above description of "tight" binding and the obvious absence of lifetime restriction. This description emphasizes the importance of intramolecular reactions as unique tools to provide information on the ultimate events of chemical reactions and the ability of covalent bonds in achieving tight binding.

The Emergence and Evolution of Enzymes

The high rates of the intramolecular reactions of covalent adducts and the fact that some enzyme-catalyzed reactions involve covalent recognition of substrates[95] raise the question of the importance of this process in early enzyme evolution. To ensure tight binding, modern enzymes take advantage of multiple non-covalent interactions with their three-dimensional folded structure. The spontaneous emergence of such evolved catalysts seems improbable, since their structures are associated with huge amounts of information not only in the definition of the position of functional groups in the sequence but also in the encoding of the final three-dimensional structure and the folding pathway. Induced intramolecularity through covalent binding may be an alternative and less improbable mechanism, since the chemical reactivity of only a single functional group is needed to take advantage of the high rates of intramolecular reactions. However, except when they facilitate chemical reactions, these covalent interactions could not have been conserved by natural selection, as enzymes became more efficient, requiring fast turnover. In fact, the formation of covalent bonds is inevitably associated with bond-breaking, which gives rise to significant energy barriers, and the corresponding rates are intrinsically slower than diffusion-limited non-covalent association.

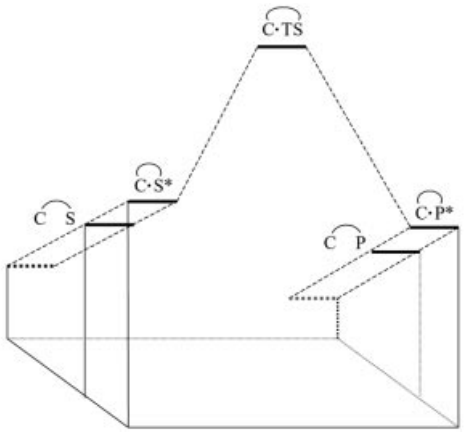


Figure 6. A representation of intramolecular reactions by use of three-dimensional free energy diagrams

Rudimentary molecules such as carbon dioxide and aldehydes (formaldehyde, glycoaldehyde, glyceraldehyde, and other simple carbohydrates) as well as metal ions were probably present on the primitive earth. The examples mentioned here demonstrate their ability to catalyze some reactions of bifunctional substrates very efficiently (a characteristic of most biochemical compounds), α-amino acid derivatives in particular. A striking indication of the involvement of these molecules in the processes from which life arose is the fact that aldehyde catalysis was demonstrated as the predominant pathway[20,96] for the hydration of α-amino nitriles in Miller's experiment showing the formation of amino acids under prebiotic conditions.[97] The hypothesis that catalysis through induced intramolecularity has played an important role in the processes resulting in the emergence of life therefore seems quite reasonable. A connection between peptide chemistry and nucleic acid chemistry in prebiotic systems has already been proposed.[98,99] According to that hypothesis, based on a new retrosynthetic disconnection, simple aldehyde precursors of nucleic acids were key intermediates. These species, together with metal ions, could also have played a catalytic role through covalent binding. A further possible means of enhancement of their efficiency may have resulted from their covalent or non-covalent association with a folded polymer (protein or nucleic acid). On the basis of that hypothesis, primitive protein catalysts

would have evolved as tools to improve the activity of primitive cofactors by processes for which the requirements are not as severe as in nucleophilic catalysis, such as electrostatic or acid−base catalyses and microenvironment effects.[100] This is in agreement with the proposition that induced intramolecularity is already involved in intermolecular general acid−base catalysis at electronegative centers.[85] Relatively short polypeptides might have been capable of achieving these functions. In comparison with self-sufficient protein catalysts, these short sequences may have required a less complicated translation machinery and may have been encoded by a simplified system. The relationship between the term "uniform binding" introduced to account for the process of enzyme evolution[86] and induced intramolecularity has been emphasized above. Even more relevant to this issue is the fact that this less sophisticated kind of binding has been proposed as giving rise to the "primordial catalysts" of enzyme evolution.[101] This is consistent with the hypothesis of covalent binding of a substrate to a cofactor, resulting in catalysis by induced intramolecularity, the full advantage of which is achieved as soon as the adduct is formed. The more evolved action of a peptide or RNA component capable of improving catalysis may involve specific recognition of the transition state.

Summary and Outlook

The catalytic power of enzymes is still a subject of controversy,[76,78,102−104] perhaps because of the relative neglect of the reference solution reactions to which they are compared.[85,93,94] The catalytic reactions of covalent adducts reviewed here provide a simple and schematic model to assess the pertinence of concepts elaborated to account for enzyme efficiency. The use of covalent binding energy by these unusual but ordinary chemical catalytic mechanisms is very similar to the use of noncovalent binding interactions with substrates by enzymes. Very efficient rate accelerations are brought about by these simple systems because of their capacity to take advantage of intramolecularity. By doing so, they reproduce the first of two important features of enzyme catalysis: namely binding of substrate and specific stabilization of the transition state. Binding of substrates at sites different from the reaction center thus brings about impressive rate increases, emphasizing the need for enzymes to hold their substrate tightly in the active site.[39] Rather than being the result only of complementarity to the transition state, enzymatic catalysis may therefore be interpreted in terms of compromise between contradictory requirements (namely, specific transition state stabilization needed for catalysis, although that is not considered here to be effective in lowering the entropy barrier, complementarity to nonreacting portions of the substrate, which is counterproductive in lowering the free energy barrier when associated with excess binding energy, and high turnover rates, which are incompatible with high ratios of enzyme−substrate and enzyme−product complexes). This description is consistent with some of the recent proposals of Benkovic and co-workers about the dynamics of enzymatic catalysis,[93,94,105] but also takes into account the entropy barrier introduced by making the reaction catalytic, previously analyzed by Jencks.[39] The fact that the contribution of enzyme−substrate complementarity (or, more accurately, binding that is not specific of the transition state) may have been underestimated in their design raises the question of the extent to which enzyme mimics based on non-covalent recognition, including catalytic antibodies directed against transition states, are capable of tight fixation of substrates.[106]

The further introduction of specific recognition of the transition state should, in principle, give rise to an increase in the rates of catalytic reactions of covalent adducts and thus to the design of artificial catalysts achieving rate enhancements similar to those of enzyme reactions. Apart from the covalent nature of the chemical interaction, such investigations are, however, associated with intrinsic limitations. For instance, the synthesis of catalysts designed to involve multiple catalytic groups may be difficult.[106] Furthermore, the simulation of the preorganized environment for transition-state stabilization, responsible for an important part of the power of enzymes, appears out of the range of present-day chemical tools both in design and synthesis. An alternative method to improve the activity of chemical catalysts acting through covalent binding as reviewed here might be that of associating them with a protein, just as proposed above for the emergence of protoenzymes. Eliciting catalytic antibodies against transition-state isosters of reactions catalyzed by induced intramolecularity seems a very accessible perspective. This possibility may be an example of combining the intrinsic reactivity of a cofactor with a catalytic antibody, "a strategy that has been surprisingly underutilized",[107] to provide access to artificial enzymes with many applications in fields such as, for instance, stereoselective or enantioselective reactions of amino acid derivatives.

Acknowledgments

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