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Acceleration of Reactions by Enzymes

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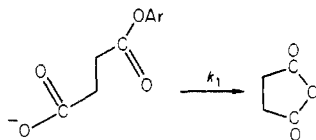
In those enzymes that accelerate reactions by the largest factors, 10^{14} or more,[1] there is a fairly extensive region of interaction with substrate. For example, in carboxypeptidase A the enzyme recognizes the five C-terminal peptides as it cleaves the final peptide bond of its substrates.[2,3] In this Account I shall refer to the various known, partly separable, contributions to these large rate enhancement factors in enzymatic catalysis and then comment on carboxypeptidase A, as a specific case.

Factors in Enzyme Catalysis

Entropy Changes. Observed entropy changes are dominated by compensation effects involving solvent.[4] For example, ΔG_u, $\Delta H°$, and $-T\Delta S_u$ values[5] for the binding of 1,6-bisphosphate to aldolase of rabbit muscle are, in kcal/mol, –6, 23, and –29 at 5 °C, –7, 1, and –8 at 25 °C, and –7, –21, and 14 at 45 °C, respectively. [Unitary values, u, have been corrected for the entropy of unmixing,[6] $-R \ln 55.5^{-1}$]. This phenomenon, associated with large negative $\partial(\Delta C_p)/\partial T$, makes it difficult to deal with structural aspects of enzyme–substrate interactions except insofar as they involve solvent interactions and changes in low frequency modes as the complex is formed.[7] Nevertheless, some other aspects of entropy changes must also be present, such as loss of translational and rotational degrees of freedom and displacement of solvent from the active site, including dehydration of charged groups. Because of the compensation of $\Delta H°$ and $-T\Delta S_u$ to yield ΔG_u almost independent of temperature, these other aspects are not easily discernible. However, they must be present. The approximate cancellation, in an association reaction, of entropy losses with gains when a solvent cage is lost can result in a ΔH effect because of the compensation relationship.[8]

These entropy losses upon association have been estimated from model reactions. For example, the unimolecular (k_1) and bimolecular (k_2) rate constants for the formation of anhydride from the succinate half-ester



or from the acetate ester and anion

$$CH_3CO_2Ar + CH_3CO_2^- \xrightarrow{k_2} (CH_3CO)_2O$$

show[9] that k_1/k_2 is 10^5 M. For molecules that lack the internal rotational freedom of the succinate half-ester, and which are unstrained, this ratio approaches[10] 10^8 M. Similar estimates have been made for other typical organic reactions in solution.[11-13]

The binding of a substrate to an enzyme is analogous to the above transformation of a second-order reaction to a first-order, intramolecular reaction.[14] If the losses of the translational and rotational degrees of freedom are accounted for in the initial stages of binding of substrates, the enzyme-catalyzed reaction will have an entropic advantage over the uncatalyzed reaction by a factor that could be as high as 10^8 M for 1 M standard states or 10^{11} M for 10^{-3} M standard states. (We exempt enzyme–substrate reactions that are diffusion controlled, e.g., those involving carbonic anhydrase, triosephosphate isomerase, and a few others).

There are, of course, other aspects of entropy changes that are of importance in enzyme catalysis, including desolvation of water molecules adjacent to charged groups, displacement of water from the active site when substrate binds, entropy cost in organizing the close fit of the enzyme–substrate interaction, losses of low-frequency modes as the complex forms, and perhaps changes in hydration or structure in regions other than the substrate binding region.

Some flexibility is required in both enzyme and substrate so that the chemical reactions can occur. While a well-formed active site is needed for steric specificity, enzymes cannot be regarded as rigid. On the other hand, if they are so flexible around the active site that the cost in entropy for organizing the active site exceeds the binding free energy, substrates will not be bound to a significant extent. An example is the inactive proenzyme, trypsinogen, in which the active site is fully available but is disordered.[15] The cost of organizing this active site of trypsinogen may be estimated from the free energies of binding of pancreatic trypsin inhibitor to trypsinogen (9.5 kcal/mol) and to

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trypsin (18.5 kcal/mol) in which the active site is fully formed. This difference (9 kcal/mol) is larger than the free energy of binding of most substrates of trypsin (about 6 kcal/mol). More usually, the conformation changes are small, of the order of a few angstroms, and when they are as large as in carboxypeptidase A the changes occur between two (or a few) states rather than among a large number of conformational states.

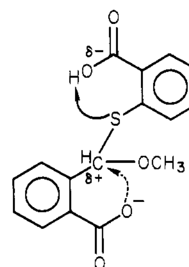
"Transition-State" Binding. The pre-transition-state binding mode of large substrates to enzymes normally involves a number of interactions aside from those of groups that carry out the chemical steps. It is these other interactions that allow overall binding of substrate in spite of some destabilization of the catalytic groups. This destabilization takes the form of (a) desolvation of a charged group that can then polarize or react more strongly as the reaction proceeds, (b) changes of pK_a, which increase nucleophilicity of a catalytic group or which increase the percentage of enzyme in a reactive form, and (c) exclusion of water when charged atoms in the precatalytic complex becomes neutral as the transition state is formed. Similar effects, including strain, have been discussed elsewhere.[12]

The use of these other interactions to promote a well-formed "transition state" is a characteristic of specific substrates, which are generally cleaved more rapidly than nonspecific substrates. One aspect in carboxypeptidase A is the binding mode of the penultimate peptide bond of the peptide substrate, as we shall see. The selection by Nature of enzymes that bind the "transition state", or at least, some intermediate configuration along the reaction pathway, is an early idea of enzyme action. Haldane[16] said in 1930, "Using Fischer's lock and key simile, the key does not fit the lock perfectly, but exercizes a certain strain on it". Pauling,[17] in 1948, commented, "I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze". This emphasis on strain of the substrate has given way to the view that these other interactions promote the juxtaposition and activation of reactive groups on the substrate and enzyme. Thus, a balanced view of moderate flexibility suggests at most small strain, for example, in lysozyme.[18,19]

If, then, binding of the "transition state" is preferred to that of substrate, it should be possible to design enzyme inhibitors that have some of the features resembling the "transition state". Recent summaries[20-22] including some 60 examples show a few which are bound to the enzyme by factors of 10^4 to 10^5 more strongly than substrate. Examples are the binding of conformycin to adenosine deaminase, 2-phosphoglycolohydroxamate to aldolase of yeast, L-valinol AMP ester to valyl-tRNA ligase, and oxalate to lactate dehydrogenase. Many nonconvalently bound analogues yield enhanced binding by factors of about 10^3. Of course, the "transition state" is never reproduced in these analogues, which therefore may yield underestimates of this factor. Probably, a suitable guess is a rate enhancement factor for real substrates of about 10^4 (or 10^5) as the true transition state is approached.

The combined entropy and "transition-state" effect, including solvent displacement but not covalent catalysis, is illustrated in a comparison of the enzymatic and nonenzymatic S_N1 hydrolysis of glycosylpyridinium salts by β-galactosidase.[23] Electrophilic catalysis of the leaving group is not required for these substrates, and the α deuterium isotope effects indicate that both the catalyzed and uncatalyzed reactions involve a galactosyl cation. Thus, covalent bond formation with a nucleophile is not indicated. Because both the catalyzed and uncatalyzed reactions are S_N1, the comparison is independent of standard states. The enzyme β-galactosidase increases the rates of these substrates by factors ranging from 10^{10} to $10^{12.5}$, in agreement with the combined tentative estimates made above for these two effects. It is likely that the enzyme stabilizes the galactosyl carbonium ion intermediate, as it does in lysozyme, where it is known that the other noncovalent interactions bring a region of neutral substrate near Asp-52, displacing solvent. The development of a carbonium ion adjacent to Asp-52 yields local electroneutrality as the transition state and intermediate develop.

Chemical Catalysis. Most enzymes participate in the chemistry of substrates. Hydrolytic enzymes, for example, frequently use both general acid and general base catalysis in their mechanisms, and some form covalent intermediates. The general acid may involve proton donation from histidinium ion, tyrosine, or a carboxyl group, while the general base or nucleophile may be serine (incipient SerO$^-$), histidine, or carboxylate anion. The very large rate enhancement factors in strained models are probably not relevant here, and so we examine concerted bifunctional catalysis in an unstrained system. Fife and Przystas[24] observe that in the thioacetal



the occurrence of facile bond breaking (at C–S) catalyzed by the acid group conflicts with the development of extensive carbonium ion character (stabilized by the anionic carboxylate) in the transition state as this bond stretches. Consequently, if both types of catalysis occur, neither can show a large rate enhancement factor. In this example, the carboxyl group and carboxylate anion contribute rate enhancement factors of about 10^2 and 10^3, respectively, in 50% dioxane in water as solvent.

If this result is general for unstrained systems, enzyme-catalyzed hydrolytic reactions probably are en-

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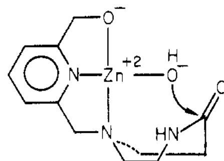
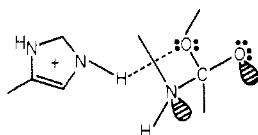
hanced in rate by about a factor of 10^4–10^6 by general acid and general base catalysis. The standard free energy of activation, $\Delta G^{\ddagger} = -RT \ln K^{\ddagger}$, indicates that a factor of 10^5 corresponds to a lowering of the free energy of activation by 7 kcal/mol.

An intramolecular, noncatalytic model[25] does involve Zn^{2+}, where the attack of OH^- on the carbonyl carbon of a peptide bond occurs with a rate enhancement factor of over 10^5.



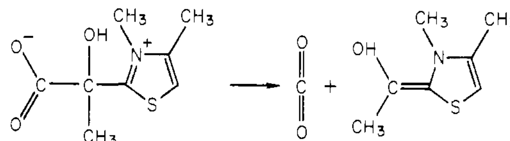
In this lactam complex, the expulsion of nitrogen in the cleavage is favored stereoelectronically by the antiperiplanar orientation of lone pairs of both oxygens of the tetrahedral intermediate. The high rate is associated with the attack of OH^- perpendicular to the plane of the amide bond. A mechanism of this type has not been eliminated for carboxypeptidase A, and is likely for the hydrolysis of the acyl enzyme, as described below.

Other Effects. Another example of stereoelectronic effects[26,27] occurs in chymotrypsin. The hydroxyl group of Ser-195 attacks the carbonyl carbon of the substrate's scissile peptide bond and donates its proton to His-57. The stereochemistry of this intermediate has a preferred geometry in which the lone pairs indicated by shaded orbitals are antiperiplanar to the newly formed C–O bond.[28]



Specific changes of pK_a's of catalytic groups may increase the fraction of reactive enzyme. In acetoacetate decarboxylase the lysine at the active site forms a Schiff base with the carbonyl group of acetoacetate.[29] The enzyme reduces the pK_a of this lysine from a "normal" value of 10–11 to 6, thus increasing[30,31] the reactive ϵ-NH_2 form over the unreactive ϵ-NH_3^+ form by a factor of 10^4 to 10^5. If this advantage is not to be lost, the protonation of the resulting ketimine must be coupled either to the decarboxylation or to a conformational change in the complex. Because decarboxylation of β-imino acids is some 10^6 times faster than that of β-keto acids, chemical catalysis contributes substantially to the rate enhancement.[32]

Promotion of reactivity of charged groups by creation of a partially hydrophobic environment is illustrated above by the choice of 50% dioxane–water for the hydrolysis of a thioacetal.[24] Another example is the decarboxylation



which proceeds faster by a factor of 10^4 to 10^5 when the solvent is ethanol rather than water.[33]

Finally, changes in hydration[34] may occur as a loose Michaelis complex transforms to a tighter transition state. These changes could be reflected as an entropy effect or an energy effect depending on the temperature and may extend over a substantial part of the enzyme or over a region near the active site.[35] This transformation may also enhance dynamical aspects of the reaction.

Mechanisms of Carboxypeptidase A

This enzyme cleaves the C-terminal amino acid (or ester) from peptide (or depsipeptide, or ester) substrates. The most rapidly cleaved substrates have values of k_{cat} near 10^2 s^{-1} for peptides and 10^3 s^{-1} for esters. While the increase in rate over that of the uncatalyzed hydrolysis, which proceeds by a different mechanism, is probably at least 10^{12}, no reliable estimates are known to this author. The binding region[2] extends over five amino acids of the substrate, and hydrolysis of longer substrates is not subject to large deviations from Michaelis–Menton kinetics.[2]

A region near the active site (Figure 1) shows the large cavity[36] available for substrates, exemplified by Gly-L-Tyr[37] (Figure 2), (–)-2-benzyl-3-(*p*-methoxybenzoyl)propionic acid[38] (Figure 3), and the four C-terminal amino acids of the 39 amino acid inhibitor of the potato[37,39] (Figure 4). Carboxypeptidase A shows a large conformational change of Tyr-248 and associated polypeptide chain when substrates or inhibitors that have a C-terminal carboxylate interact with the enzyme (Figure 5). This was the first example relating to the "induced fit" hypothesis[40] and involving substantial changes in the enzyme. This result emphasizes the closeness of the stereochemical relationship between enzyme and substrate.

Both Gly-Tyr (Figure 2) and the ketonic analogue for ester substrates (Figure 3) have the C-terminal carboxylate bound by two hydrogen bonds to Arg 145, the aromatic side chains in the pocket of the enzyme, and the carbonyl group bound to Zn^{2+}. Stabilization of product is clearly suggested by the interactions of Pro-Tyr-Leu of the potato inhibitor now that the C-

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Figure 1. Stereoview of the active site of carboxypeptidase A, showing about one-fifth of the enzyme. The larger isolated circle is the Zn^{2+} ion. Gly-270 is to the left of Zn^{2+} and toward the reader, while tyr-248 is shown almost edge-on in the upper-middle right, closest to the reader. The Zn^{2+} ligands are His-69, Glu-72 (both oxygens), and His-196. A water molecule, the only nonprotein ligand to Zn^{2+}, is not shown.

Figure 2. Stereoview of the binding of Gly-L-Tyr to the same region as shown in Figure 1. The side chain of Tyr-248 has rotated by about 120° about the C_α–C_β bond so that the phenolic side chain extends away from the reader toward the substrate. The carbonyl oxygen of Gly-Tyr is bound to Zn^{2+}, while the N terminus is statistically bound to Zn^{2+} (as shown here) on hydrogen bonded to Glu-270 in an alternative orientation achieved by rotation about the CH_2–CO bond. The carboxylate group of this poor substrate makes two hydrogen bonds to Arg-145 (upper middle-right).

terminal Gly has been cleaved between S_1' (pocket) and S_1 (Leu-38): (a) the newly formed carboxylate anion has one oxygen bound to Zn^{2+} while the other receives a hydrogen bond from Tyr-248; (b) the Tyr-37–Leu-38 peptide bond donates a hydrogen bond (NH) to Tyr-248 and receives a hydrogen bond from Arg-71. These interactions make it clear that the peptide bond in the penultimate position of peptide and depsipeptide substrates supplies important interactions that aid progress toward the transition states and products.

Entropy factors related to the binding-catalytic steps are difficult to exhibit because of the large compensation effects of solvent described above. However, the structure shows clearly the rather precise fit which limits rotational and translational degrees of freedom of substrate. These large entropy decreases are balanced in part by the binding interactions and by the release of about a dozen well-ordered molecules in the region of the active site. To some degree, the regions around the charged groups (Zn^{2+}, Glu-270, Arg-145, Arg-127, Arg-71, and the carboxylate of substrate) have been desolvated.

It is also difficult to assess the extent to which the enzyme favors binding of the transition state as compared to substrate. The phosphoramidate,[41] *N*-[[[(benzyloxycarbonyl)amino]methyl]hydroxyphosphinyl]-L-phenylalanine,

$$\text{Cbz—NHCH}_2\overset{\overset{\text{O}}{\|}}{\underset{\underset{\text{O}^-}{|}}{\text{P}}}\text{—NH}\overset{\text{H}}{\underset{\underset{\text{CO}_2^-}{|}}{\text{C}}}\text{—CH}_2\text{C}_6\text{H}_5$$

(41) N. E. Jacobsen and P. A. Bartlett, *J. Am. Chem. Soc.*, **103**, 654–657 (1981).

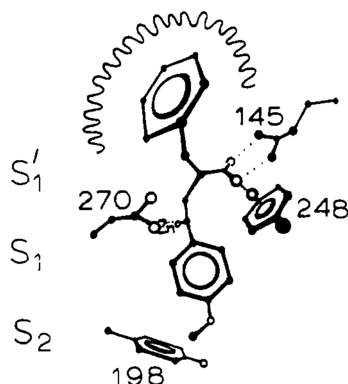
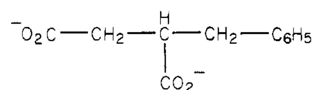


Figure 3. Binding of (–)-2-benzyl-3-(*p*-methoxybenzoyl)propionic acid to the active site of carboxypeptidase A. Here, the amide NH or ester O of a cleavable substrate is replaced by a CH_2 group which exchanges its R proton (that nearer Glu-270) stereospecifically.

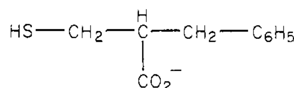
shows a K_i of 9×10^{-8} M at pH 7.5 and 6×10^{-9} M at pH 6. No structural study is available, although it is assumed that the CO_2^- binds to Arg-145 and the O^- of P to Zn^{2+}. The tetrahedral bonding about P is analogous to a first intermediate. As the pH is lowered a hydrogen bond may develop between the other oxygen on P and Glu-270 as the latter becomes protonated.

The inhibitor, L-benzylsuccinate,[42]



has a K_i of 4.5×10^7 M at pH 7.5 and also binds about an order of magnitude more strongly at pH 6.5. Here, also, no structure is available, although it is probable that the carboxylate at the asymmetric carbon is bound to Arg-145, while the other carboxylate displaces water as it binds to Zn^{2+}.

The compound[43] 2-benzyl-3-mercaptopropanoic acid (SQ 14603)



has a K_i of 1.1×10^{-8} M for binding to carboxypeptidase A and presumably has its CO_2^- bound to Arg-145 and the S^- bound to Zn^{2+}.

Typical K_i or K_s values are about 10^{-5} M. While the K_i values of these three compounds are suitably smaller than those for substrates, a major factor here is the presence or development of negative charge on the group which presumably binds to Zn^{2+}.

In view of the structures, the primary chemical transformations of catalysis are limited to Glu-270, Tyr-248, Zn^{2+}, H_2O, and the substrate. The optimal pH of about 7.5 suggests that the nucleophile is either Glu-270 (Figure 6) or H_2O promoted by Glu-270 (Figure 7) and that the proton donor is Tyr-248 (H_2O is less likely because of the apparent tightness of the complex). However, mechanisms involving $Zn(H_2O)$ or ZnOH attack at the carbonyl carbon, either with or without displacement of the carbonyl oxygen from Zn^{2+}, have not been eliminated. Several other proposed mechanisms will be mentioned below.

The anhydride mechanism[36] has been shown to occur for the ester substrate *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate.[44,45] In the deacylation step at –40 °C the pK_a of 7.65 for the $Zn(OH_2)$ ionization of the Zn enzyme becomes 6.33 for the Co enzyme; a five-coordinated metal ion has been suggested for this acyl intermediate.[46] The further observation[47,48] that ^{18}O is exchanged from substrate at the rate that resynthesis of the peptide bond by the enzyme and products occurs suggests either that the general base pathway prevails or that the water molecule involved in hydrolysis is sequestered from solution throughout the course of the anhydride mechanism.

Thus far the chemistry of peptide, and probably some ester, hydrolysis is ambiguous about the position of the water molecule if the general base pathway occurs: whether it is near Glu-270, Zn^{2+}, or Tyr-248 or between any two of these three. On the question of different pathways for different classes of rapidly cleaved substrates, enzymologists tend to believe that evolution of such an efficient process yields only one mechanism, which may have different rate-controlling steps,[49] while bioorganic chemists lean toward different mechanisms.[48]

The covering of the active site by the large conformational change of Tyr-248 as described in the X-ray diffraction work produces a largely hydrophobic environment, which probably enhances the effects of charges. For this reason, the tetrahedral intermediates of Figures 6 and 7 have been drawn so that all charges have been neutralized, either as a salt link or by formation of covalent bonds.

Inactivation of Glu-270 by *N*-ethyl-5-phenylisoxazolium-3′-sulfonate[50,51] and by *N*-(bromoacetyl)-*N*-methyl-L-phenylalanine[52,53] shows a pK_a of about 7, about 2.5 units above the usual range for Glu. If a value of about 0.8 for the β in Brønsted's equation for acylation is assumed, the expected contribution of this change of pK_a to the rate enhancement is a factor of about 10^2.

Also, the hydrophobic environment can be expected to increase the susceptibility of the carbonyl carbon to attack by a nucleophile, because of the polarization of this bond by Zn^{2+}. In a molecular orbital model calculation,[54] a nucleophile was allowed to approach the carbonyl carbon of formamide. As the tetrahedral intermediate was formed, the bond order of the carbonyl bond decreased from 1.86 to 1.13, while the CO···$(Zn L_3)^+$ bond order increased slightly from 0.38 to 0.63. This polarization role for Zn^{2+} does not exclude other functions in later stages of the reaction, including

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Figure 4. Stereoview of the four C-terminal amino acids (Pro-Tyr-Leu-Gly) of potato carboxypeptidase A inhibitor as they bind after cleavage of the C-terminal Gly. This view is similar to that of Figures 1 and 2, although less of the enzyme is shown for clarity.

Figure 5. Stereoview of the active site showing the native enzyme as solid lines, and the same region after the potato carboxypeptidase inhibitor binds as dotted lines. The inhibitor has been omitted, and the Zn^{2+} is designated as 308.

Figure 6. Anhydride formed by the acyl enzyme pathway, as found for the ester substrate, *O*-(*trans*-*p*-cinnamoyl)-L-β-phenyllactate at -40 °C. Deacylation may then occur when H_2O or OH^- adds to Zn^{2+}.

Figure 7. General base pathway, in which Glu-270 promotes the attack of H_2O on the carbonyl carbon of the substrate. It is not certain whether or not this water is also associated with Zn^{2+} or with Tyr-248.

participation in deacylation if indeed an anhydride intermediate is shown to be a general mechanism. This deacylation step would probably be greatly facilitated if the Zn^{2+}-bound carbonyl group of the anhydride were to shift about 1 Å, away from Zn^{2+} to Arg-127. The lower coordination at Zn^{2+} would lower the pK_a of the $Zn^{2+}(OH_2)$ group and facilitate attack of $Zn^{2+}OH^-$ perpendicular to this displaced carbonyl group.

Finally, multiple roles for catalytic and participating groups are indicated. Besides acting as a nucleophile or promoter of attack of water by abstracting a proton, in this latter pathway Glu-270 may also donate[48] a proton to Tyr-248 anion after Tyr-248 delivers a proton to the leaving NH group of the scissile bond. Also, before substrate is bound Glu-270 receives a hydrogen bond from a Zn-bound H_2O, which is largely un-ionized below pH 9 because of proximity to Glu-270. Multiple functions are indicated for Tyr-248: as a proton donor; as a recipient of a hydrogen bond from the penultimate peptide bond; and as a donor of a hydrogen bond to the newly formed carboxylate anion of product, and to the C-terminal carboxylate anion at the penultimate stage of transfer of substrate along the subsites of the enzyme before cleavage.

These studies, and similar ones on other enzymes, have thus provided three-dimensional models from which plausible mechanisms may be presented for further chemical studies.

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Appendix

Aspects of controversy concerning the mechanism include (a) the essential requirement of two tyrosine residues in catalysis,[55] (b) different sites for peptide and ester hydrolysis,[55–58] (c) the conclusion that during the catalytic process Tyr-248 moves away from Zn to which it is initially bound,[59] and (d) comments[59–62] on the mechanisms of the X-ray study on relatively active crystals ($^1/_3$ of solution activity,[63] reduced by loss of substrate activation[37]) on the basis of experiments[59–62] on relatively inactive crystals ($^1/_{300}$ of that in solution[64,65]) that have different unit cell dimensions.[63]

Only side chains of Tyr-248 and Glu-270 can approach atoms of the substrate's scissile peptide (or ester) bond. The conformational changes proposed[66] for Tyr-198 have not been observed in any X-ray diffraction study. While Tyr-198 lies in the extended binding region, it is not near enough to the scissile bond to participate in the catalytic steps.

The binding of an ester analogue (Figure 3) is like that of the peptide (Figure 2) in the region of the active site, and thus offers no support to the proposal of different cleavage sites for esters and peptides.

Crystals that have been grown at pH values of 7.5, 8.0, 8.5, and 9.0 show no binding of Tyr-248 to Zn.[67] Although a few percent would not be detected, the probable value is zero in the unmodified enzyme. The binding of Tyr-248 to Zn in the arsanilazo-Tyr-248 derivative is most probably due to the additional interactions supplied by the arsanilazo group.

Aside from the movements which occur when substrates bind (Figure 5), the structure of the enzyme is the same in several different crystallographic environments in crystals containing about 45% of water. It is therefore likely that the molecular conformations in these crystals are similar to those available in solution on the time scale of rapidly cleaved substrates.

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Determination and Significance of Transition-Metal–Alkyl Bond Dissociation Energies

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Transition-metal–alkyl bond dissociation energies are of importance in the context of a variety of organometallic, biochemical, and catalytic systems. One such context relates to the stabilities of transition-metal alkyl compounds. Until fairly recently such compounds were relatively rare, giving rise to speculation about the thermodynamic and/or kinetic instability of transition-metal–carbon σ bonds and about the possible origin of such instability.[1–4] It is now apparent that many transition metal alkyl compounds, both binary (homoleptic), i.e., of the type MR_n (where R is an alkyl group), and complex, i.e., of the type L_xMR_n (where L is a ligand such as CO, CN^-, PR_3, etc.), are sufficiently stable to resist decomposition below 100 °C.[4–7] Furthermore, when decomposition does occur, routes other than metal–carbon bond homolysis often intervene, for example, olefin elimination (with β-hydrogen transfer to the metal), reductive elimination, etc.[4–8] The metal–alkyl bond dissociation energies in such cases clearly must exceed ca. 20 kcal/mol but could still lie in a relatively low range.

One context in which transition-metal–alkyl homolytic bond dissociation has been invoked is that of coenzyme B_{12} promoted enzymatic rearrangements.[9,10]

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