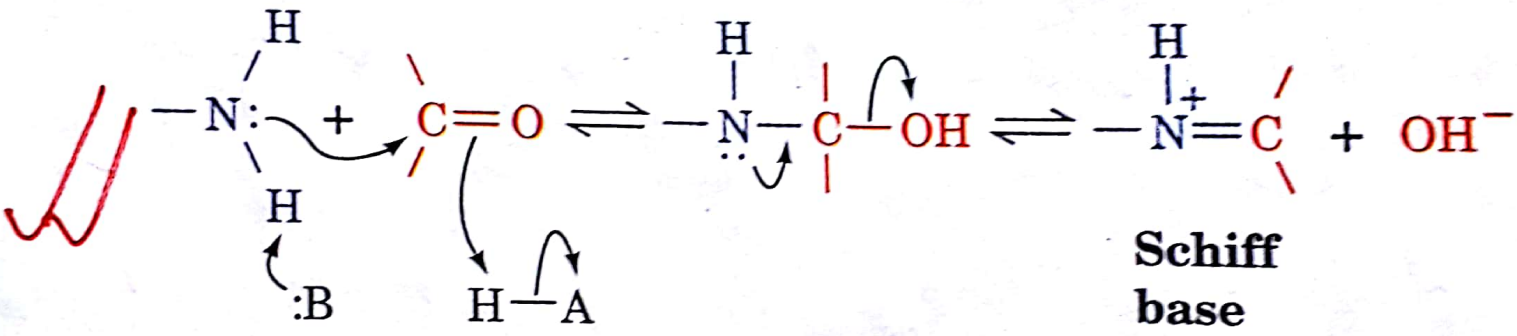
B. *Covalent Catalysis*

Covalent catalysis *involves rate acceleration through the transient formation of a catalyst–substrate covalent bond.* The decarboxylation of **acetoacetate,** as chemically catalyzed by primary amines, is an example of such a process (Fig. 15-4). In the first stage of this reaction, the amine nucleophilically attacks the carbonyl group of acetoacetate to form a **Schiff base** (imine bond).

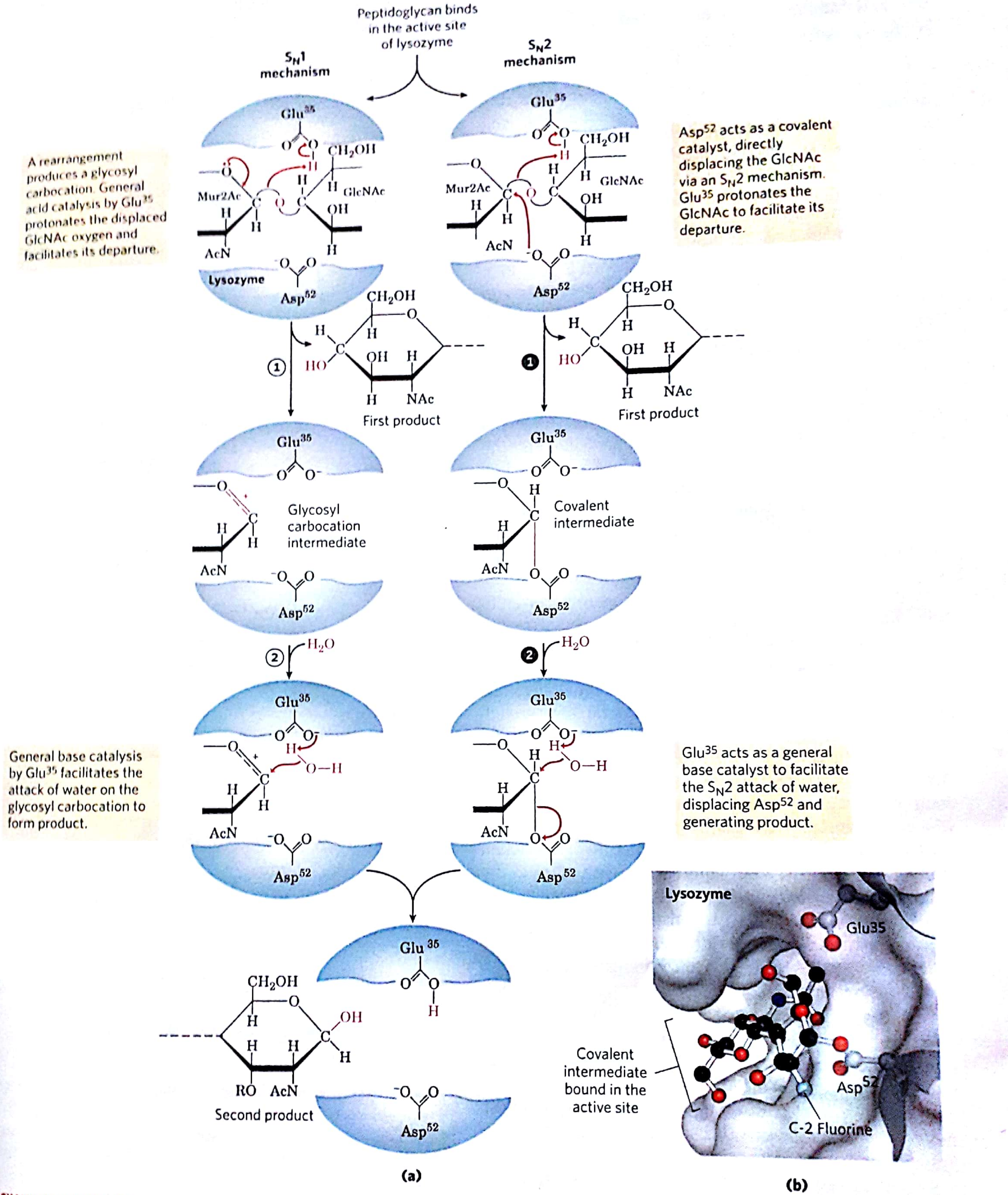


The protonated nitrogen atom of the covalent intermediate then acts as an electron sink (Fig. 15-4, bottom) so as to reduce the otherwise high-energy enolate character of the transition state. The formation and decomposition of the Schiff base occur quite rapidly, so that these steps are not rate determining in this reaction sequence.

Reaction mechanisms are somewhat arbitrarily classified as occurring with either **nucleophilic catalysis** or **electrophilic catalysis** depending on which of these effects provides the greater driving force for the reaction, that is, which catalyzes its rate-determining step. The primary amine–catalyzed decarboxylation of acetoacetate is clearly an electrophilically catalyzed reaction since its nucleophilic phase, Schiff base formation, is not its rate-determining step. In other covalently catalyzed reactions, however, the nucleophilic phase may be rate determining.

The nucleophilicity of a substance is closely related to its basicity. Indeed, the mechanism of nucleophilic catalysis resembles that of general base catalysis except that, instead of abstracting a proton from the substrate, the catalyst nucleophilically attacks it so as to form a covalent bond. Consequently, if covalent bond formation is the rate-determining step of a covalently catalyzed reaction, the reaction rate tends to increase with the covalent catalyst's basicity (pK).

An important aspect of covalent catalysis is that the more stable the covalent bond formed, the less facilely it will decompose in the final steps of a reaction. A good covalent catalyst must therefore combine the seemingly contradictory properties of high nucleophilicity and the ability to form a good leaving group, that is, to easily reverse the bond formation step. Groups with high polarizabilities (highly mobile electrons), such as imidazole and thiol functions, have these properties and hence make good covalent catalysts.



Peptidoglycan binds in the active site of lysozyme
S_N1 mechanism
S_N2 mechanism
A rearrangement produces a glycosyl carbocation. General acid catalysis by Glu[35] protonates the displaced GlcNAc oxygen and facilitates its departure.
Asp[52] acts as a covalent catalyst, directly displacing the GlcNAc via an S_N2 mechanism. Glu[35] protonates the GlcNAc to facilitate its departure.
Glu[35]
Mur2Ac
GlcNAc
AcN
Lysozyme
Asp[52]
First product
Glycosyl carbocation intermediate
Covalent intermediate
General base catalysis by Glu[35] facilitates the attack of water on the glycosyl carbocation to form product.
Glu[35] acts as a general base catalyst to facilitate the S_N2 attack of water, displacing Asp[52] and generating product.
Second product
(a)
Lysozyme
Glu35
Asp52
C-2 Fluorine
Covalent intermediate bound in the active site
(b)

MECHANISM FIGURE 6-28 Lysozyme reaction. In this reaction (described in the text), the water introduced into the product at C-1 of Mur2Ac is in the same ... pathway (right) is the mechanism most consistent with current data. **(b)** A surface rendering of the lysozyme active site ...

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C. Metal Ion Catalysis

Nearly one-third of all known enzymes require the presence of metal ions for catalytic activity. There are two classes of metal ion–requiring enzymes that are distinguished by the strengths of their ion–protein interactions:

1. ***Metalloenzymes*** *contain tightly bound metal ions,* most commonly transition metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, or Co^{3+}.

2. ***Metal-activated enzymes*** *loosely bind metal ions from solution,* usually the alkali and alkaline earth metal ions Na^{+}, K^{+}, Mg^{2+}, or Ca^{2+}.

Metal ions participate in the catalytic process in three major ways:

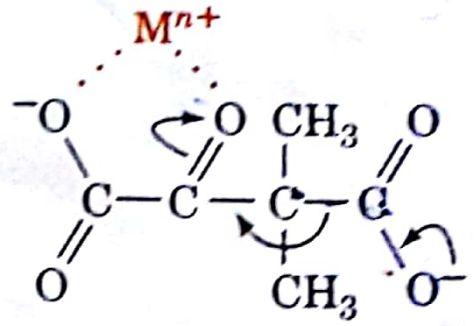
1. By binding to substrates so as to orient them properly for reaction.
2. By mediating oxidation–reduction reactions through reversible changes in the metal ion's oxidation state.
3. By electrostatically stabilizing or shielding negative charges.

In this section we shall be mainly concerned with the third aspect of metal ion catalysis. The other forms of enzyme-mediated metal ion catalysis are considered in later chapters in conjunction with discussions of specific enzyme mechanisms.

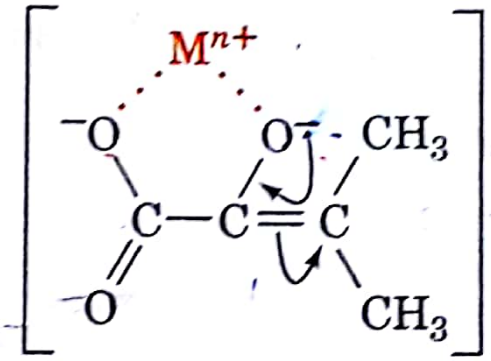
a. Metal Ions Promote Catalysis through Charge Stabilization

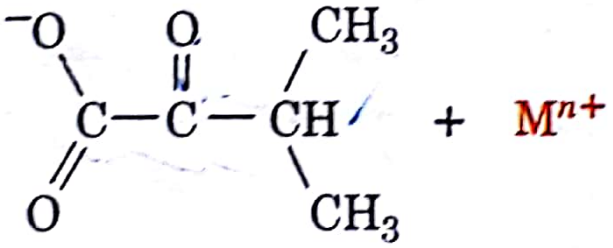
In many metal ion–catalyzed reactions, the metal ion acts in much the same way as a proton to neutralize negative charge, that is, it acts as a Lewis acid. Yet *metal ions are often much more effective catalysts than protons because metal ions can be present in high concentrations at neutral pH's and can have charges greater than +1.* Metal ions have therefore been dubbed "superacids."

The decarboxylation of **dimethyloxaloacetate,** as catalyzed by metal ions such as Cu^{2+} and Ni^{2+}, is a nonenzymatic example of catalysis by a metal ion:



Dimethyloxaloacetate





Here the metal ion (M^{n+}), which is chelated by the dimethyloxaloacetate, electrostatically stabilizes the developing enolate ion of the transition state. This mechanism

late oxaloacetate require a metal ion

b. Metal Ions Promote Nucleophilic Catalysis via Water-Ionization

A metal ion's charge makes its bound water molecules more acidic than free H_2O and therefore a source of OH^- ions even below neutral pH's. For example, the water molecule of $(NH_3)_5Co^{3+}(H_2O)$ ionizes according to the reaction:

$$(NH_3)_5Co^{3+}(H_2O) \rightleftharpoons (NH_3)_5Co^{3+}(OH^-) + H^+$$

with a pK of 6.6, which is ~9 pH units below the pK of free H_2O. *The resulting metal ion–bound hydroxyl group is a potent nucleophile.*

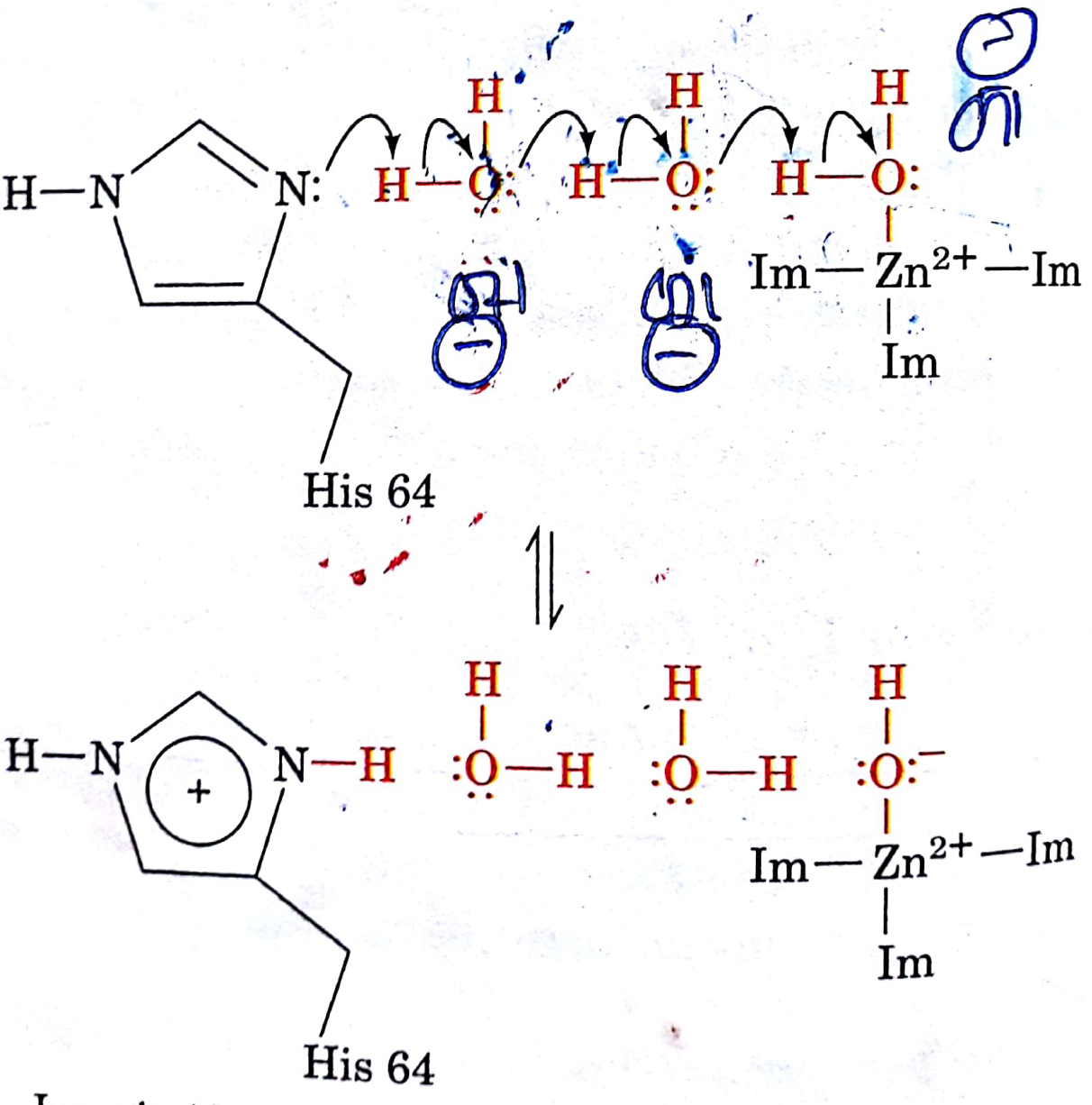
An instructive example of this phenomenon occurs in the catalytic mechanism of **carbonic anhydrase** (Section 10-1C), a widely occurring enzyme that catalyzes the reaction:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

Carbonic anhydrase contains an essential Zn^{2+} ion that lies at the bottom of an ~15-Å-deep active site cleft (Fig. 8-41), where it is tetrahedrally coordinated by three evolutionarily invariant His side chains and an O atom of either an

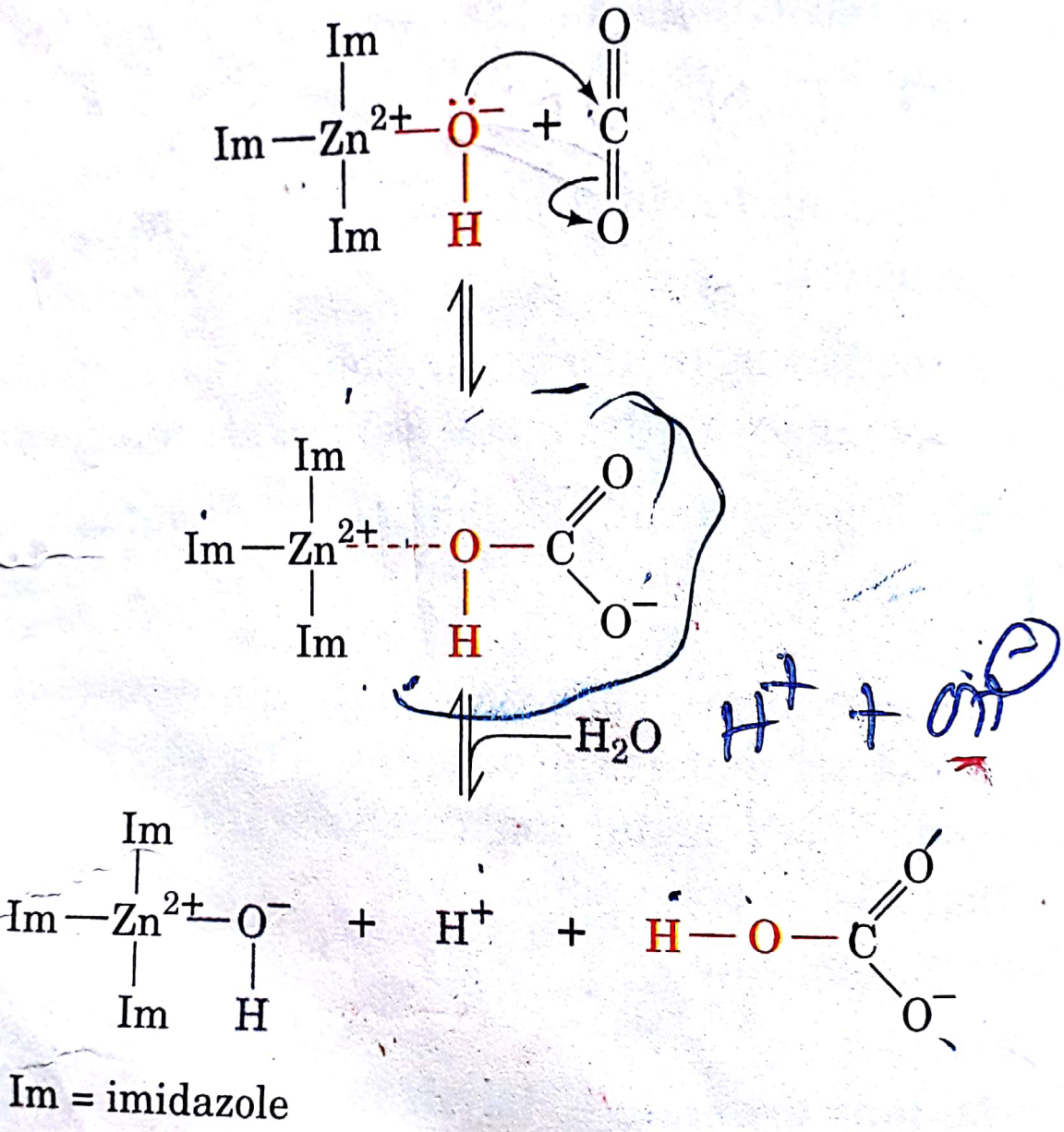
HCO_3^- ion (Fig. 15-5*a*) or a water molecule (Fig. 15-5*b*). The enzyme has the following catalytic mechanism:

1. We begin with a water molecule bound to the protein in the Zn^{2+} ion's fourth liganding position (Fig. 15-5*b*). This Zn^{2+}-polarized H_2O ionizes in a process facilitated through general base catalysis by His 64 in its "in" conformation. Although His 64 is too far away from the Zn^{2+}-bound water to directly abstract its proton, these entities are linked by two intervening water molecules to form a hydrogen bonded network that is thought to act as a proton shuttle.



Im = imidazole

2. The resulting Zn^{2+}-bound OH^- ion nucleophilically attacks the nearby enzymatically bound CO_2, thereby converting it to HCO_3^-.



Im = imidazole

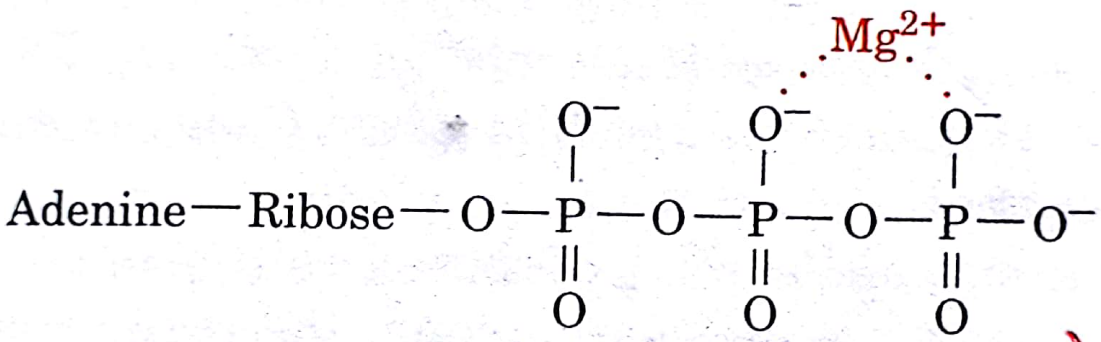
In doing so, the Zn^{2+}-bound OH^- group donates a hydrogen bond to Thr 199, which in turn donates a hydrogen bond to Glu 106 (Fig. 15-5*a*). These interactions orient the OH^- group with the optimal geometry (see below) for nucleophilic attack on the substrate CO_2.

3. The catalytic site is regenerated by the exchange of the Zn^{2+}-bound HCO_3^- reaction product for H_2O together

with the deprotonation of His 64. In the latter process, His 64 swings to its "out" conformation (Fig. 15-5*b*), which may facilitate proton transfer to the bulk solvent.

c. Metal Ions Promote Reactions through Charge Shielding

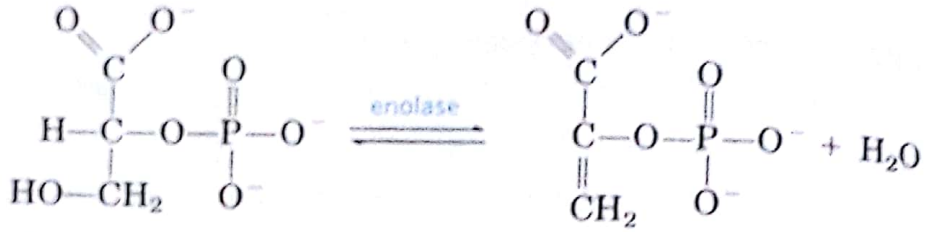
Another important enzymatic function of metal ions is **charge shielding.** For example, the actual substrates of **kinases** (phosphoryl-transfer enzymes utilizing ATP) are Mg^{2+}–ATP complexes such as



rather than just ATP. Here, the Mg^{2+} ion's role, in addition to its orienting effect, is to shield electrostatically the negative charges of the phosphate groups. Otherwise, these charges would tend to repel the electron pairs of attacking nucleophiles, especially those with anionic character.

The Enolase Reaction Mechanism Requires Metal Ions

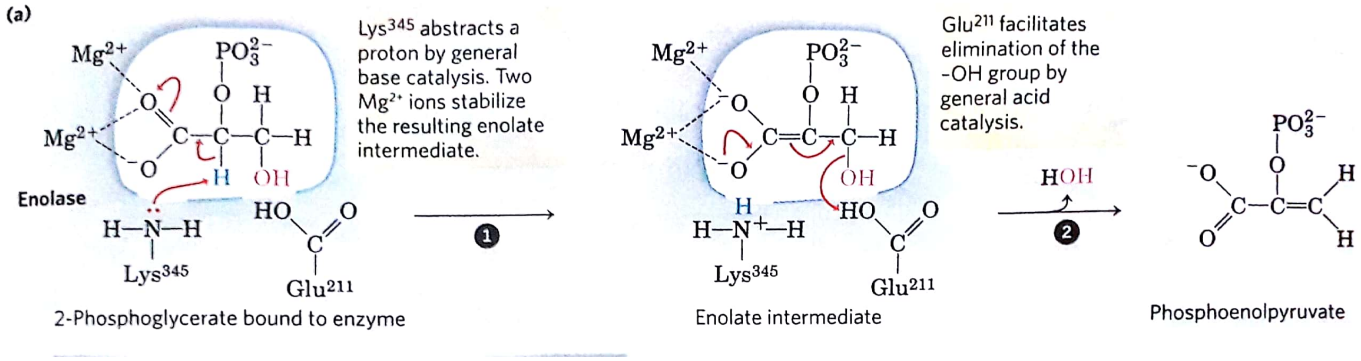
Another glycolytic enzyme, enolase, catalyzes the reversible dehydration of 2-phosphoglycerate to phosphoenolpyruvate:



2-Phosphoglycerate Phosphoenolpyruvate

The reaction provides an example of the use of an enzymatic cofactor, in this case a metal ion (an example of coenzyme function is provided in Box 6–3). Yeast enolase (M_r 93,316) is a dimer with 436 amino acid residues per subunit. The enolase reaction illustrates one type of metal ion catalysis and provides an additional example of general acid-base catalysis and transition-state stabilization. The reaction occurs in two steps **(Fig. 6–26a)**. First, Lys^{345} acts as a general base catalyst, abstracting a proton from C-2 of 2-phosphoglycerate; then Glu^{211} acts as a general acid catalyst, donating a proton to the —OH leaving group. The proton at C-2 of 2-phosphoglycerate is not acidic and thus is quite resistant to its removal by Lys^{345}. However, the electronegative oxygen atoms of the adjacent carboxyl group pull electrons away from C-2, making the attached protons somewhat more labile. In the active site, the carboxyl group of 2-phosphoglycerate undergoes strong ionic interactions with two bound Mg^{2+} ions (Fig. 6–26b), strongly enhancing the electron withdrawal by the carboxyl. Together, these effects render the C-2 protons sufficiently acidic (lowering the pK_a) so that one can be abstracted to initiate the reaction. As the unstable enolate intermediate is formed, the metal ions further act to shield the two negative charges (on the carboxyl oxygen atoms) that transiently exist in close proximity to each other. Hydrogen bonding to other active-site amino acid residues also contributes to the overall mechanism. The various interactions effectively stabilize both the enolate intermediate and the transition state preceding its formation.

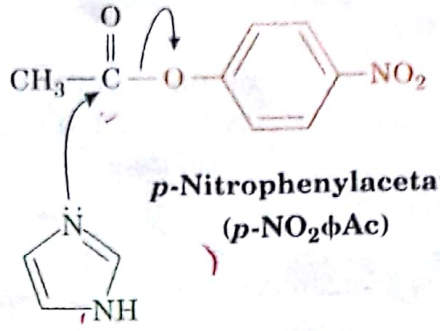
(a)



E. *Catalysis through Proximity and Orientation Effects*

Although enzymes employ catalytic mechanisms that resemble those of organic model reactions, they are far more catalytically efficient than these models. Such efficiency must arise from the specific physical conditions at enzyme catalytic sites that promote the corresponding chemical reactions. The most obvious effects are **proximity** and **orientation:** *Reactants must come together with the proper spatial relationship for a reaction to occur.* For example, in

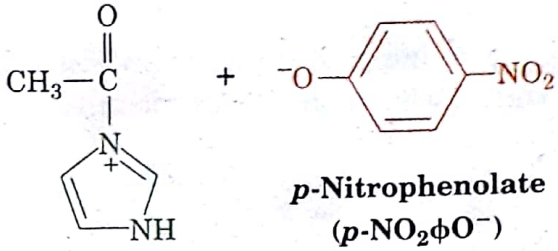
the bimolecular reaction of imidazole with *p*-nitrophenylacetate.



***p*-Nitrophenylacetate**
(*p*-$NO_2\phi$Ac)

Imidazole

k_1



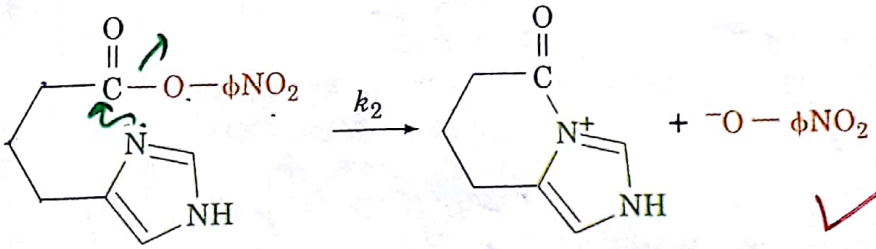
***p*-Nitrophenolate**
(*p*-$NO_2\phi O^-$)

***N*-Acetylimidazolium**

the progress of the reaction is conveniently monitored by the appearance of the intensely yellow ***p*-nitrophenolate ion:**

$$\frac{d[p\text{-}NO_2\phi O^-]}{dt} = k_1[\text{imidazole}][p\text{-}NO_2\phi Ac] = k_1'[p\text{-}NO_2\phi Ac] \quad [15.4]$$

where ϕ = phenyl. Here k_1', the pseudo-first-order rate constant, is 0.0018 s^{-1} when [imidazole] = 1*M*. However, for the intramolecular reaction



the first-order rate constant k_2 = 0.043 s^{-1}; that is, $k_2 = 24k_1'$. Thus, when the 1*M* imidazole catalyst is covalently attached to the reactant, it is 24-fold more effective than when it is free in solution; that is, *the imidazole group in the intramolecular reaction behaves as if its concentration is 24M.* This rate enhancement has contributions from both proximity and orientation.