

### Mechanism of Enzyme Action

We have been approaching, by kinetics and chemical modification, the central question about enzymes: how do they work? How do they catalyze reactions? Some of the considerations we shall cover apply also to other functions of proteins, such as electron transfer, conformational change which transmits a hormonal message through a membrane, etc.

Jencks has noted that enzyme mechanisms may be approached in three ways: general theories (and perhaps experiments based on them); small molecule model reactions; and details of specific mechanisms. The last is the proof of the pudding, but may not be generally applicable; indeed, we shall see that different catalytic factors play roles of varying importance in different enzyme mechanisms. In the next several lectures I shall discuss general theories and lessons from small molecules, then go on to specific mechanisms.

First, what do we mean by catalysis? **Increase of rate** of a reaction, brought about by something (the catalyst) which is **not consumed** in the reaction, may be reused. A reaction may be looked at as passing from one valley, representing stable reactants, over a mountain pass to another valley, the products. The pass between them is called the **transition state**, the state from which the molecules may with equal probability go ahead to products or back to reactants. Energy must be put in to a reaction to raise the reactants to the top of the pass, the transition state; this energy is called the **free energy of activation**,  $\Delta G^*$ , always **positive** because it represents energy which must be **put in** to reach the unlikely transition state. The role of the catalyst is to **find a lower pass** over the mountain range, a pathway with a lower activation energy.

The transition state may be described as a condition of the reactants - let's just use one, call it  $A^*$  - in **equilibrium** with the ground state  $A$ , so that  $K^*$ , the equilibrium constant of activation,  $= [A^*]/[A]$ . An alternative way of looking at the role of a catalyst is that it **stabilizes the transition state**, increases the ratio  $[A^*]/[A]$ . The rate *constant* of the overall reaction,  $k$ , by which  $[A]$  is multiplied to get the rate, is proportional to the amount of the reactant in the transition state,  $k = (\kappa T/h)K^*$ , where  $\kappa$  is Boltzmann's constant and  $h$  is Planck's constant. The factor  $\kappa T/h$  is the frequency of decomposition of the transition state, which is the same as the vibrational frequency  $\nu$  of the bond breaking. At 25° C  $\nu = 6.212 \times 10^{12} \text{ s}^{-1}$ .

The  $\Delta G^*$  of activation is of course related to the equilibrium constant  $K^*$  in the usual way,  $\Delta G^* = -RT \ln K^* = -RT \ln([A^*]/[A]) = -RT(\ln[A^*] - \ln[A])$ ,  $-(\Delta G^*/RT) = \ln[A^*] - \ln[A]$ ,  $-(\Delta G^*/RT) + \ln[A] = \ln[A^*]$ . Taking antilogs of both sides,  $[A]e^{-\Delta G^*/RT} = [A^*]$ . This relates the conc. of  $[A^*]$  to the conc. of ground-state  $A$  and the difference in free energy between the ground state and the transition state. Since the exponent is  $-\Delta G^*/RT$ , a negative number,  $e^{-\Delta G^*/RT}$  is a number  $< 1$ , a small fraction, and the larger  $\Delta G^*$  is, the smaller  $e^{-\Delta G^*/RT}$ , and the smaller the fraction of  $A$  in the transition state.

The  $\Delta G^*$  may further be separated into enthalpy and entropy,  $\Delta G^* = \Delta H^* - T\Delta S^*$ ,  $k = (\kappa T/h)e^{-\Delta H^*/RT}e^{\Delta S^*/R}$ . The entropy term is frequently the most important; we shall return to it.

If one can measure the rate constant  $k$  of reaction as a function of temperature, one can obtain (although not terribly accurately) values for  $\Delta G^*$ ,  $\Delta H^*$  and  $\Delta S^*$  from the following rearrangements of the above reaction:  $\Delta G^* = -RT \ln(k h/\kappa T)$ ,  $\Delta H^* = -R([d \ln k/d(1/T)] + T)$ ,  $\Delta S^* = R[(T d \ln k/dT) + \ln(k h/\kappa T) + T]$ . More commonly, rate  $k$  is plotted vs  $1/T$ , an Arrhenius plot, and a change in the slope of the plot is taken as indicating a change in the rate-limiting step of the reaction, i.e.  $\Delta G^*$  has one value in one temperature and another value in another temperature range.

The catalyst may decrease the activation energy to reach, or increase the stability of, the same sort of transition state reached in the uncatalyzed reaction, or it may provide an entirely different, if usually more complicated, pathway of reaction. It is generally assumed that *some* chemical pathway can be observed, for the reaction of small molecules, which is analogous to the enzyme-catalyzed reaction. This approach is covered in the chemistry course Bio-organic

Mechanisms; we are here concerned primarily with studying the rates of *enzyme*-catalyzed reactions, and obtaining from then evidence which may aid in selecting the best small-molecule reaction as model. Once one has chosen an appropriate pathway, one is defining how the transition state is stabilized compared to a simpler, less catalyzed version of the reaction.

Some examples of the effect of an enzyme on the activation energy, albeit on  $\Delta H^*$  rather than  $\Delta G^*$ , are:

$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$ :	uncatalyzed	$\Delta H^* = 18.0$ kcal/mole
	+ catalase	$\Delta H^* = 5.5$ kcal/mole
casein hydrolysis:	uncatalyzed	$\Delta H^* = 20.6$ kcal/mole
	+ trypsin	$\Delta H^* = 12.0$ kcal/mole
ethyl butyrate hydrolysis:	uncatalyzed	$\Delta H^* = 13.2$ kcal/mole
	+ lipase	$\Delta H^* = 4.2$ kcal/mole.

Let's return to the equation  $k = (\kappa T/h)e^{-\Delta H^*/RT}e^{\Delta S^*/R}$ , and more specifically to the entropy term  $e^{\Delta S^*/R}$ . Entropy may be thought of as a measure of **disorder**, and one law of thermodynamics states that entropy tends always toward a maximum, **energy must be put in** to a reaction to reverse disorder. My classic example of this is that if I put out my garbage can the night before it is due to be collected, and a dog or a raccoon knocks it over and scatters the garbage over the street, it takes me much more energy to collect the garbage into the can again than it did the dog or raccoon to knock it over. Any **transition state** is likely to be a very ordered state of the molecule or molecules undergoing reaction, which means that entropy is **lost** compared to the ground state. If  $\Delta S^*$  is negative, the term  $-T\Delta S^*$  is positive, and  $\Delta G^*$  is increased, making the reaction more difficult. Furthermore, a reaction making more molecules out of fewer - an increase of disorder - will have a **favorable** overall entropy term, while one making fewer molecules will have an **unfavorable** entropy term. This implies that the formation of a transition state in the reaction of **two** or more molecules, once separate but brought together in reaction, has a very substantial negative entropy term - although in many cases this is partially balanced by the displacement of water molecules from a relatively ordered state around the reactants to the disordered state of bulk  $\text{H}_2\text{O}$ .

This is particularly true when two or more reactants must be brought together into a precise arrangement, having started from quite random positions in solution. The molecules must lose **translational** entropy - randomness of position in the solution - **rotational** entropy - freedom of the whole molecule & its parts to rotate - and some **vibrational** entropy, freedom of the parts of a molecule to vibrate with respect to each other. As Figure 2-5 from Fersht (**handout**) shows, two molecules condensing to form one lose the translational and rotational entropy of three degrees of freedom each, although the latter at least is compensated by internal rotation of the parts of the larger molecule. The table in the handout shows that the translational entropy change involved in bringing a molecule from just anywhere to somewhere specific is 120 to 150 joules/degree-mole (29 to 36 cal/degree-mole, or at  $25^\circ\text{C} = 298^\circ\text{K}$  8.6 to 10.7 kcal/mole as an energy term) for a one molar solution. Entropy increases only slightly with molecular weight - the range given is for molecules of mol. wt. 20 to 200 - but **decreases** with increasing concentration, since "anywhere" is a larger fraction of possible locations as concentration increases, so the *change* in order is less.

So when two molecules get together to form one there is an entropy loss in forming the adduct, which is seen both in the thermodynamic free energy change of forming the final product and in the free energy of **activation** of the reaction. For two molecules becoming one at  $25^\circ\text{C}$  the total entropy loss, translational and rotational, translates into an unfavorable free energy change of 13 to 14 kcal/ mole, though slightly offset by new internal rotational and vibrational entropy.

One way this is seen is that a catalytic group in the **same** molecule is immensely more effective than one in a **separate** molecule, because no entropy penalty, or only a small one involving the reduction of internal rotational freedom, must be paid in forming the transition state. For instance, as shown at the left of the handout, the hydrolysis of *p*-nitrophenyl acetate is

catalyzed by free acetate, with a second-order rate constant of  $4 \times 10^{-6} \text{ s}^{-1} \text{ M}^{-1}$ . Mono-*p*-nitrophenyl succinate hydrolyzes with a first-order rate constant of  $0.8 \text{ s}^{-1}$ . The ratio between these two rates is  $2 \times 10^5 \text{ M}$ , i.e. the other  $\text{COO}^-$  in the succinate acts like acetate at a concentration of  $2 \times 10^5 \text{ M}$ , which is clearly impossible. In effect, in the succinate case we are seeing close to the 'real' catalytic effectiveness of the succinate, without paying the tremendous entropy penalty for bringing in a separate acetate ion into the transition state. Succinate still has **rotational** entropy around the bond between the  $\text{CH}_2$  groups, so some ordering, and loss of entropy, still occurs in the transition state. For instance, if at the left of the other side of the handout we look at the rates of hydrolysis of monoesters of glutarate (with free rotation around two C-C bonds), succinate (with rotation around one bond) and *endo*-norbornenyldicarboxylic acid (with no free rotation), the rates increase 230-fold for each loss of a free rotation, which corresponds to 3.22 kcal/mole at  $25^\circ \text{ C}$  or 10.8 cal/degree-mole of entropy.

Bruice and Benkovic have compiled average values for the entropy of activation of comparable uni- bi- and ter-molecular reactions, as shown in the right two-thirds of the back side of the handout. They conclude that the entropy of activation divided by the kinetic order, the second column of numbers, is approximately constant, about 4.4 cal/degree-mole. This means that a **reduction in the kinetic order of the reaction** - making it intramolecular rather than intermolecular - will reduce the entropy of activation by 4 to 5 cal/degree-mole, and increase the rate by about 1000-fold. Of course it took more energy to synthesize the more complex molecules which have intramolecular catalysis, because they are more ordered and contain less entropy. The point is that because this order is **built into** these compounds, there is less difference in entropy between the reactants and the transition state, and thus less entropy of activation, which is this difference.

We can now see that a major part of the role of the enzyme in catalysis is to separate the entropy penalty from the activation energy of the reaction *per se*. One cannot escape the entropy penalty entirely, but with enzymes the entropy penalty is paid in the **formation of the enzyme-substrate complex**, in which energetically favorable binding interactions with negative  $\Delta H$  - charge attraction, van der Waals contacts, hydrogen bonding - are formed which compensate for the unfavorable entropy change. Not only can two or more molecules react with each other without paying the entropy penalty in forming the transition state, but **groups on the enzyme** can be involved in **chemical** catalysis of the reaction, in ways we'll discuss later. These catalytic involvements in small-molecule catalysis - such as the examples with  $n = 3$  in Bruice & Benkovic's table - must accelerate the reaction **more than 1000-fold** to overcome the entropy penalty and display net acceleration of the reaction. Binding to an enzyme allows a **number** of catalytic groups to act, each raising the rate another 100-1000 fold, **without** any entropy penalty for their involvement, until an otherwise unlikely reaction such as the rearrangement of succinate to methylmalonate can take place at a reasonable rate. Thus the decrease of activation entropy, thanks to binding, **allows the expression** of other mechanisms of catalysis. Of course a tremendous entropy penalty had to be paid in the synthesis of the large, highly ordered enzyme molecule, but **only once**, the enzyme molecule can be used for many, many reactions, while in small-molecule catalysis the transition state complex must be formed anew for each reaction. You could say that this is "the meaning of life" - the living system synthesizes catalysts which diminish the payment of entropy of activation by the **reusability** of the catalysts

Another point, of which much has been made by Koshland, is that molecules do not react just anywhere on their surfaces; only when precise portions of their electron orbitals are brought into contact will fruitful reaction take place. We may symbolize by drawing the reacting molecules as spheres with only some small area on the surface where reaction will be fruitful. This area over the total surface area of the sphere is a factor  $1/\theta$ ; the smaller the area over which reaction is fruitful, the larger is  $\theta$ . Of course the other reacting molecule also has a limited fruitful area, and the probability of reaction upon contact is  $1/\theta_A \times 1/\theta_B$ , or the **rate enhancement obtained** by binding reactants A and B to an enzyme in such arrangement - Koshland called it "orbital steering" - is  $\theta_A \theta_B$ . Koshland suggested that rate enhancements of up to  $10^6$  fold were

thus obtainable, but one can calculate that this would require fruitful areas to be only one square degree on the surface, and it is rather easy to prove from infrared absorption studies that bonds actually bend five degrees or so from their rest position quite easily without breaking, which implies that if you brought reacting molecules together even five degrees off from the best alignment they would still have at least a 50% chance of forming the new bond stably, which in turn implies that the fruitful area is ten degrees across or 78 square degrees. Consequently, this "orbital steering", or orientation as it is also called, is suggested to supply catalytic factors of the order of 100 to 1000, not  $10^6$ -fold, and Koshland stopped being able to dine out on the idea.

Another physical effect is **strain**, which was originally suggested to be the bending or stretching of a substrate molecule toward the transition state conformation when it bound to the enzyme, thus decreasing the enthalpy of formation of the transition state as well as the entropy. The classic example of this was the deformation of the ring of *N*-acetylglucosamine when it binds to lysozyme, toward the flattened, half-chair form which the protonated intermediate transition-state-like structure assumes in the acid catalyzed reaction (**draw out**). Fersht disagrees with this description, saying that the flattening out occurs only on going into the transition state, the enzyme **selects** the rare but naturally occurring flattened conformation and stabilizes it by forming new binding interactions and relieving unfavorable interactions which occur with the 'ground' state. The distinction is a little like that between **specific** acid or base catalysis, in which a proton is transferred to or from the substrate **before** going to the transition state, and **general** acid or base catalysis, in which at least partial transfer occurs **while** going to the transition state.

The description of this effect with which Fersht **would** agree is that **binding complementarity is best in the transition state**, the enzyme stabilizes the transition state by the improvement of the interactions with the substrates and holds them in the transition state long enough to greatly improve the chance that it will proceed to products. The improvement of the binding interactions provides negative  $\Delta G$  which balances the positive (unfavorable)  $\Delta G$  of distorting the substrate to the transition state structure. Note that improvement of binding interactions quite distant from the catalytic site can be used in stabilizing the transition state; the classic example is elastase, a protease which acts very poorly, low  $V_{max}$ , on simple substrates such as tosyl-alanine ethyl ester;  $V_{max}$  as well as binding improves as the substrate gets larger, being best for an N-blocked tetrapeptide.

This effect is studied by the use of **transition state analogs**: a structure for the transition state of the reaction is guessed at, and a compound with similar structure - if not always similar atoms, the transition state analog for chymotryptic hydrolysis of a substrate like phenylethancarboxylic acid esters is phenylethaneboronic acid, with  $-B(OH)_2$  replacing  $-COOH$  - is synthesized and shown to bind **much better** than the actual substrates, because less of the binding energy is used to approximate the transition state, so that the **net**  $\Delta G$  of binding is greater. Analogy: what one wants to hear and agree with is better received than bad news which must change your behavior.

(1999: 2nd lecture began here)

A final physical factor is **microenvironmental effects**, really a facilitation of chemical effects. Ionic groups and dipoles are **more effective**, interact more strongly with other ionic groups and dipoles, when they are isolated in hydrophobic areas - like oases in a desert. Perutz has pointed out that polar residues are not found in the interior of proteins, as determined by X-ray crystallography, unless there is a good reason in terms of function such as catalysis. (However, Dr. Kahn now argues that enzymes adjust their flexibility - generally needed for conformational changes during catalysis - by having some buried charged groups. Proteins are no more stable than they have to be for the temperature at which their organism lives. One can make a more stable protein by eliminating such buried charges, but it will be stiffer, less catalytic at moderate temperatures, even if highly active at higher temperatures.) Such an ionic group or dipole is likely to be a much more effective catalyst than one shielded by water molecules in aqueous solution; it is known that polar salts dissolved in nonpolar solvents can catalyze

reactions by several orders of magnitude, and reagents such as crown ethers (for which Charles Petersen received the Nobel Prize in 1987), which chelate a cation effectively in a nonpolar solvent, allow the free anion to react effectively in such solutions (for instance,  $\text{KMnO}_4$  in benzene with crown-18). The environmental effect of the enzyme would also include disrupting the solvation 'shells' of substrate molecules allowing them to interact more readily with each other as well as with groups on the enzyme.

But many of these **physical** effects also potentiate **chemical** catalysis by groups on the enzyme. We generally at least hope that the enzyme is not simply a template to which the substrates bind for ready reaction, but that groups on its surface - hydrophilic groups for sure - **participate in catalysis**. However, the amount of chemical participation varies widely among enzymes - the serine proteases have a lot of chemical participation, while tyrosyl tRNA synthetase has very little.

Very often the transition state of a reaction involves charge separation, which is energetically unfavorable. Any participation by a catalyst which spreads the charge more widely thus stabilizes the transition state and lowers the energy of activation. For instance, in the hydrolysis of an ester, the uncatalyzed reaction puts partial negative charge on the carbonyl, partial positive on the attacking  $\text{H}_2\text{O}$ . However, another electron-rich species, or base, can spread the charge more widely by pulling out a proton from the water, treating it as being part of the positive charge, which is neutralized by interaction with the electron-rich group.

Similarly the hydrolysis of an acetal, such as a glycoside, can be catalyzed by an acid which partially donates a proton to a leaving group which would otherwise be negatively charged:

This sequence also shows how general **base** catalysis could be involved in the same reaction, by helping to pull off the proton which is removed from the water which attacks the carbonium ion intermediate. Those who know the mechanism of action of lysozyme will recognize that the role of general acid catalysts is there played by glu-35. Asp-52, however, probably acts by forming an ion pair with the carboxonium ion intermediate and thereby stabilizing it, rather than by removing the proton from attacking water. This is called **electrostatic** catalysis. It is not very important with model compounds, principally because of the shielding effect of a high-dielectric medium such as water, which lessens the energy of interaction between charges. However, the nonpolar portions of the amino acid side chains of proteins provide a low-dielectric medium, and when water is excluded between and around interacting charges, the energies of interaction are much higher, so that the stabilization of charge in the transition state can be very substantial and lower the activation energy greatly; it has been calculated that the interaction of the carboxonium ion in the transition state of lysozyme hydrolysis with asp-52 lowers the energy of activation by 9 kcal/mole, equivalent to a rate enhancement of  $4 \times 10^6$ . (However, there are also claims that asp-52 actually forms a covalent acyl intermediate with the sugar;  $\text{H}_2\text{O}$  then attacks from above, displacing it.)

As I mentioned above, besides this sort of **general acid/base catalysis**, in which a proton is transferred in going through the transition state, there is **specific** acid/base catalysis, in which the appropriate ions from water,  $\text{H}_3\text{O}^+$  or  $\text{OH}^-$ , fully donate or remove a proton, generally to form a true intermediate rather than a transition state. In general acid/ base catalysis any proton-donating (acidic) or proton-accepting (basic) species can aid any reaction where a proton must be transferred; these are likely to be much weaker acids or bases than  $\text{H}_3\text{O}^+$  or  $\text{OH}^-$ , but present in much greater concentration at neutral pH, and in enzymes they simply are there. The effectiveness of the catalyst, measured as the rate constant of the second order reaction involving substrate and catalyst, is generally proportional to the strength of the acid or base, i.e. its  $\text{pK}_a$ :

$\log k_2 = A - \alpha pK_a$  for general acid catalysis

$\log k_2 = A + \beta pK_a$  for general base catalysis

The term A is the rate of the uncatalyzed reaction (or as catalyzed by neutral H<sub>2</sub>O), while  $\alpha$  and  $\beta$  indicate the sensitivity of the reaction to catalysis. If  $\alpha$  or  $\beta = 0$ , the reaction is not subject to acid or base catalysis; if  $\alpha$  or  $\beta = 1$ , only specific catalysis by H<sub>3</sub>O<sup>+</sup> or OH<sup>-</sup> is of importance.

For ester hydrolysis  $\beta$  is 0.3 to 0.5.

I should mention here the principle of kinetic equivalence, which prevents distinguishing among some types of catalysis. For instance, a reaction might be catalyzed by A<sup>-</sup>, or by HA and OH<sup>-</sup>; both will show the same dependence on total A concentration and on pH, since  $[A^-] = \frac{[HA][OH^-] K_a}{K_w}$ . The only way you can distinguish between the possibilities is when one would involve a second-order rate constant larger than the rate of diffusion of ions - if the reaction occurs fast enough at, say, pH 8.0, where  $[OH^-] = 10^{-6}$  M, that a  $k_2$  greater than  $10^8 \text{M}^{-1} \text{s}^{-1}$  would be necessary to achieve the observed rate. This is faster than the diffusion of the ion, and therefore impossible.

These have been mechanisms which involve lowering the activation energy of an already existing pathway. The alternative is to generate a basically different pathway, involving a new, generally covalent intermediate whose formation and breakdown both have a lower activation energy than the classical mechanism. The classical case is the hydrolysis of esters catalyzed by imidazole:

Imidazole is a better attacking nucleophile than water and a better leaving group than even *p*-nitrophenol, so that the overall reaction is faster than the uncatalyzed reaction in water. It should be noted that imidazole can also act as a general base catalyst, and indeed probably thus catalyzes the attack of another imidazole. One can distinguish nucleophilic alternate-pathway catalysis from general base catalysis in the following ways:

If an intermediate compound, such as acylimidazole, can actually be isolated, it is proof positive of nucleophilic catalysis. Less directly, the intermediate may react with alternate second attacking molecules, such as methanol and phosphate in stead of H<sub>2</sub>O, to an extent not seen for the uncatalyzed reaction. Such **partitioning** is also evidence for an intermediate compound.

On the other hand, general acid or base catalysis necessarily involves transfer of a proton in the transition state. This is harder if the hydrogen ion to be transferred is actually a deuteron <sup>2</sup>H<sup>+</sup>, or triton <sup>3</sup>H<sup>+</sup>, because the zero-point energy of the bond to hydrogen is lower and the  $\Delta G^*$  consequently higher; thus when proton transfer occurs in the transition state the reaction is **slower** for a deuteron, theoretically 7 fold but for other reasons anywhere between 2 and 15-fold. Such an **isotope effect** will be seen using deuterated or tritiated substrate if the proton is transferred **in the rate-limiting step** from a carbon atom, or using D<sub>2</sub>O as solvent if the proton is exchangeable, being on an oxygen, nitrogen or sulfur. On the other hand no primary hydrogen isotope effect will be seen in nucleophilic catalysis.

Small **secondary** isotope effects are seen when the deuterium substitution is at a position **adjacent** to where reaction occurs and the transition state has carbonium ion character, because the C-H substrate will more readily go into sp<sup>2</sup> hybridization than the C-D substrate. The maximum effect expected is a  $k_H/k_D$  ratio of 1.38, and ratios of 1.1 to 1.2 are accepted as indicating considerable carbonium ion character for an intermediate; the value is 1.11 for lysozyme acting on a substrate with a D on C-1, but only 1.01 for  $\beta$ -glucosidase.

Somewhat similar but much smaller effects can be observed using <sup>13</sup>C, <sup>15</sup>N and <sup>18</sup>O substrates; an isotope effect on the overall reaction indicates that breakage of a bond involving the heavy atom, rather than some other step, is largely rate-limiting. These isotope effects are not measured as direct effects on the rate - they are of the order of 1 or 2% - but by measuring the heavy-atom enrichment of the product using mass spectrometry; if there is an isotope effect,

the product will be depleted in the heavy isotope, because substrate with the heavy atom reacted less.

Metal ions can act as catalysts in a number of ways. The most obvious is electrostatic: as a stable positive charge, in the significant cases a divalent cation, they can stabilize negative charges in the transition state.  $Mg^{++}$  and  $Ca^{++}$ , which can interact with only four ligands, generally do nothing fancier than that, and indeed act largely as 'bridges' in binding negatively charged groups - for instance ATP nearly always reacts as its  $Mg^{++}$  complex. Zinc is the most often used as a strong positive charge which polarizes compounds coordinated to it and favors a negatively charged transition state, as in liver alcohol dehydrogenase.

A less obvious but related means for metal ions to catalyze reactions is to polarize a coordinated water molecule, which then attacks the substrate. For instance, Zn in carbonic anhydrase is coordinated to three histidines which attach it to the protein, but the fourth ligand is a water molecule which is believed to have a  $pK_a$  of 7 rather than 15.74 ( $K_w = 10^{-14}$  divided by 55 M). Metal-bound  $OH^-$ , though with a far lower  $pK_a$  than free water, reacts nearly as well as free  $OH^-$  - for instance,  $H_2O$  bound to  $Co(NH_3)_5^{+++}$  has a  $pK_a$  of 6.6, 9 pH units below that of free  $H_2O$ , but is only 40 fold less effective than an equimolar concentration of free  $OH^-$  in catalyzing the hydration of  $CO_2$ , though this rather disagrees with the Brønsted relationships for organic bases and acids, where effectiveness in catalysis follows  $pK_a$  rather well, i.e. if  $\log k_2 = A + \beta pK_a$  and  $\beta$  is 0.5, a decrease of  $pK_a$  by 9 units should mean a 4.5-fold decrease in  $\log k_2$  and thus a 30,000 fold decrease in rate.

The really catalytic metal ions are usually the transition metals,  $Mn^{++}$ ,  $Fe^{++}$ ,  $Co^{++}$ ,  $Cu^{++}$  and  $Zn^{++}$  - partly because they have a smaller ionic radius than  $Ca^{++}$  or  $Mg^{++}$ , more because they have more unfilled orbitals to coordinate ligands, hold them close while they work on them. Cu and Fe also can act as **redox** catalysts, shifting back and forth between different oxidation states, in the course of a reaction in which substrates are oxidized and reduced; in some cases oxidation states not seen free in solution, such as  $Cu^{+++}$  and  $Fe^{+4}$ , seem to be involved.