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Coenzymes and Cofactors

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Coenzymes and cofactors are molecules or ions that are used by enzymes to help catalyse reactions. Coenzymes are typically organic molecules that contain functionalities not found in proteins, while cofactors are catalytically essential molecules or ions that are covalently bound to the enzyme.

Functional Groups in Proteins

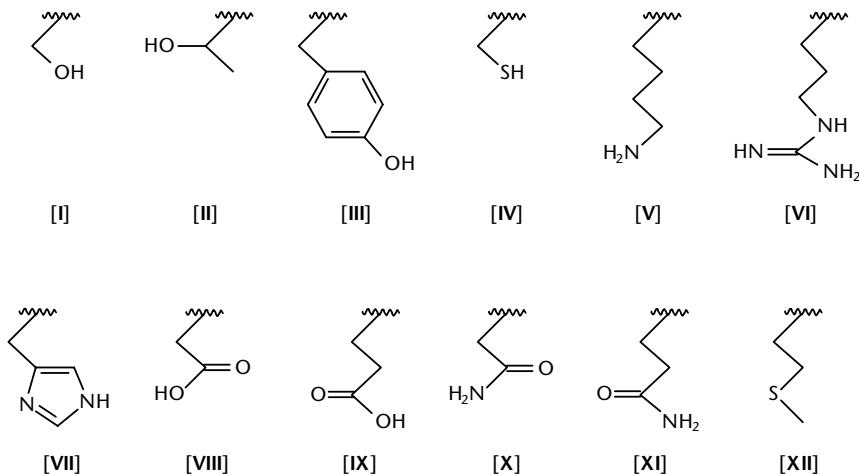
The 20 common amino acids that comprise the building blocks of proteins are characterized by their side-chains, which can range from simple aliphatic groups such as that found in alanine to more complex, functionalized side groups such as that of histidine. All of the amino acids play important roles in determining the three-dimensional structure of proteins through hydrophobic and hydrophilic interactions, ionic interactions and formation of disulfide bonds. Only a smaller subset of these amino acid side-chains, however, have been implicated in direct roles in enzymatic catalysis. Amino acids that are implicated directly in enzymatic catalysis are those with functional groups on the side-chains. Protein functional groups include hydroxyl (serine [I], threonine [II], tyrosine [III]), sulfhydryl (cysteine [IV]), ammonium (lysine [V] and arginine [VI]), imidazolium (histidine [VII]), carboxylate (aspartate [VIII] and glutamate [IX]), amide (asparagine [X] and glutamine [XI]), and thioether (methionine [XII]) groups. These groups can function in a variety of ways in enzymatic catalysis.

Work in recent years has pointed to the presence of a new type of protein functional group: the amino acid radical. A number of enzymes have now been identified that contain a catalytically essential protein radical (Stubbe and van der Donk, 1998). The most notable and best characterized

example at this point is the ribonucleotide reductase found in aerobic *Escherichia coli*, which contains a stable, catalytically essential tyrosyl radical (Jordan and Reichard, 1998). Other enzymes have been shown to require a stable tyrosyl radical for catalytic activity, including the photosynthetic oxygen-evolving complex of photosystem II (Barry and Babcock, 1987) as well as galactose oxidase (Babcock *et al.*, 1992). Other enzymes, including the ribonucleotide reductase from anaerobic *E. coli* (Mulliez *et al.*, 1993) and pyruvate formate-lyase (Unkrig *et al.*, 1989) contain a catalytically essential glycyl radical in their active forms. Because these protein-based radicals have distinct chemical properties and functionalities relative to the counterpart nonradical amino acid, they can be considered as protein functional groups distinct from the typical amino acid side-chains.

Catalytic Roles for Protein Functional Groups

Catalysis of reactions is perhaps the most central process in biology, since the great majority of reactions required by



living systems would occur far too slowly to be of any use to a living organism. From the simple hydrolysis of a peptide bond to the complex, multistep synthesis of purines and pyrimidines, it would be a slow world without enzymes! Uncatalysed reactions occur slowly because the substrates are relatively unreactive, and/or because one or more of the intermediates generated along the reaction pathway would be of high energy (i.e. unstable). The role of the catalyst, therefore, is to make the substrates more reactive, and/or to stabilize the reactive intermediates such that reaction will occur more rapidly.

Protein functional groups participate in catalysis by providing nucleophilic, electrophilic, acidic, or basic groups to aid in catalysis. A prevalent type of protein functional group is the nucleophile (the hydroxyl groups in [I], [II], and [III], and the sulfhydryl group in [IV], are all

good nucleophiles), and nucleophilic catalysis is commonly observed in enzymes. A classic example of nucleophilic catalysis occurs in the serine proteases (**Figure 1**). Note in this mechanism that the role of the serine hydroxyl is to form, via nucleophilic attack, an acyl enzyme intermediate that is more readily hydrolysed than the substrate amide bond. Note also the role of aspartate and histidine as general acid–base catalysts in this mechanism: (1) they serve to deprotonate the serine, making it more nucleophilic; (2) they protonate the amine, making it a better leaving group; (3) they deprotonate water, making it a better nucleophile; and (4) they reprotonate the serine, making it a better leaving group. This mechanism is a perfect example of how protein functional groups can aid in catalysis of enzymatic reactions through both nucleophilic and general acid–

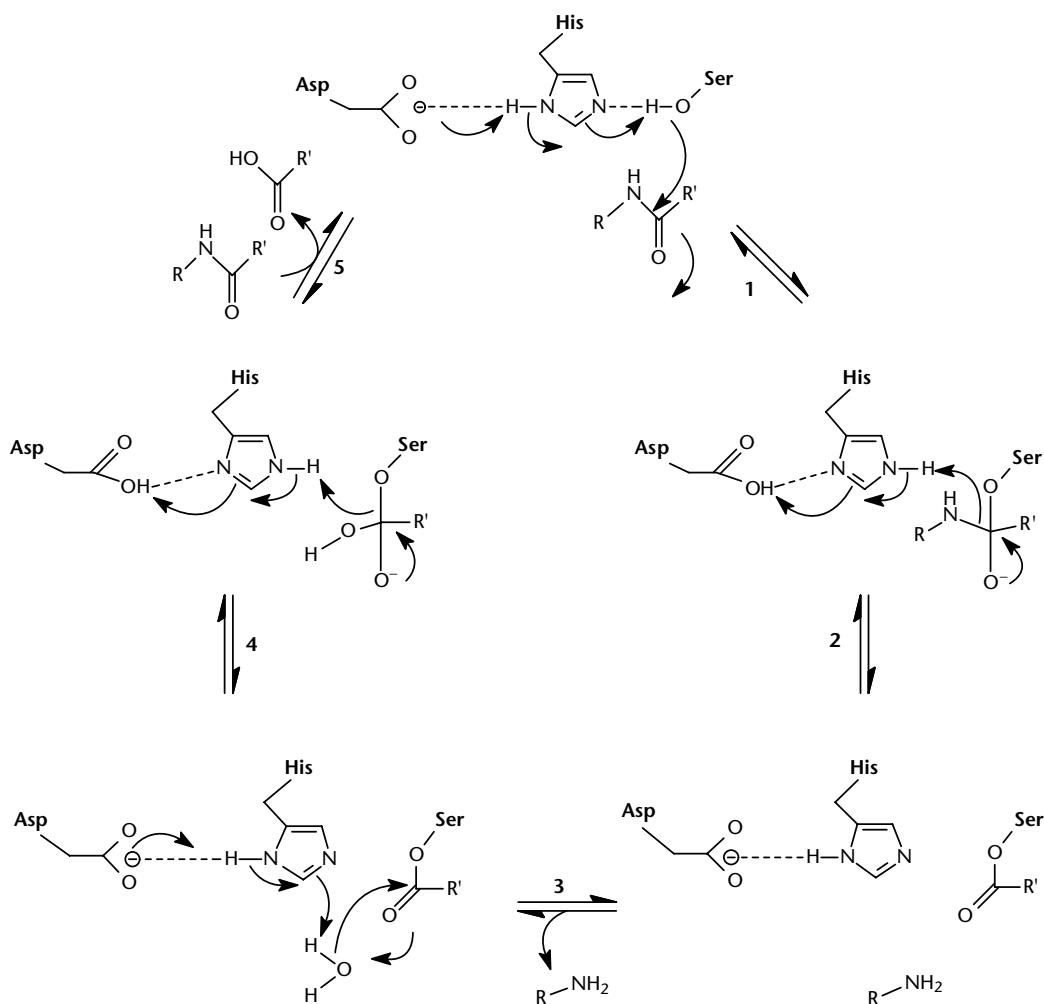


Figure 1 Mechanism of the serine proteases. Note the role of general acid–base catalysis in each step of the mechanism. Step 1, nucleophilic attack of the serine hydroxyl on the substrate. Step 2, collapse of tetrahedral intermediate to the acyl enzyme intermediate. Step 3, release of amine product. Step 4, deacylation resulting from nucleophilic attack of water to form a tetrahedral intermediate. Step 5, collapse of the tetrahedral intermediate and release of carboxyl product.

base catalysis. The carboxylate, histidine, and ammonium functional groups can all participate in general acid–base catalysis, and the amine side-chains can participate in electrophilic catalysis via Schiff base formation.

Amino acid radicals, the final type of protein functional group discussed in the previous section, appear to function in the initiation of radical chemistry by long-range electron transfer and/or hydrogen atom abstraction. Examples include the tyrosyl radical present in the active form of *E. coli* ribonucleotide reductase, which has been proposed to generate a radical at the active site via long-range electron transfer. This active site radical, in turn, initiates ribonucleotide reduction by abstraction of the 3' hydrogen of the substrate to generate an intermediate substrate radical (Stubbe and van der Donk, 1998). Other stable protein radicals, for example the glycy radical of pyruvate formate-lyase (Knappe *et al.*, 1993) and the tyrosyl radical of the photosynthetic oxygen-evolving complex (Hoganson and Babcock, 1997), have been proposed to initiate catalysis in similar ways, via initial H atom abstraction from the substrate.

Limitations of Protein Functional Groups in Biocatalysis

The functional groups in proteins are well-suited to general acid–base catalysis, nucleophilic and electrophilic catalysis, and in a few instances radical initiation, but certainly do not account for all of the types of catalytic reactions of enzymes. For example, an important class of enzymatic reactions are redox reactions, and in general protein functional groups are not suited to redox chemistry. One notable exception is cysteine, which can be oxidized by one electron per cysteine residue to form a disulfide bond, and the disulfide bond can be re-reduced by two electrons to form two cysteine sulfhydryls. The only other exceptions are the few instances in which amino acid radicals have been identified as protein functional groups, in which case the amino acid radical is perfectly suited to doing one-electron chemistry. Due to the limited number of different types of protein functional groups and the therefore limited chemistry in which they can be involved, it is not surprising that enzymes utilize the unique properties of a variety of nonprotein molecules and ions to assist in their catalytic chemistry.

Molecules or ions that are used by enzymes to help catalyse reactions are known as coenzymes or cofactors. Most coenzymes are organic molecules that contain functionalities not found in proteins, and therefore augment the functional groups and mechanisms available for enzyme catalysis. As will be discussed below, coenzymes are used in electrophilic catalysis, redox chemistry, rearrangements, group transfers, and other types of reactions. Some coenzymes, such as lipoic acid, contain

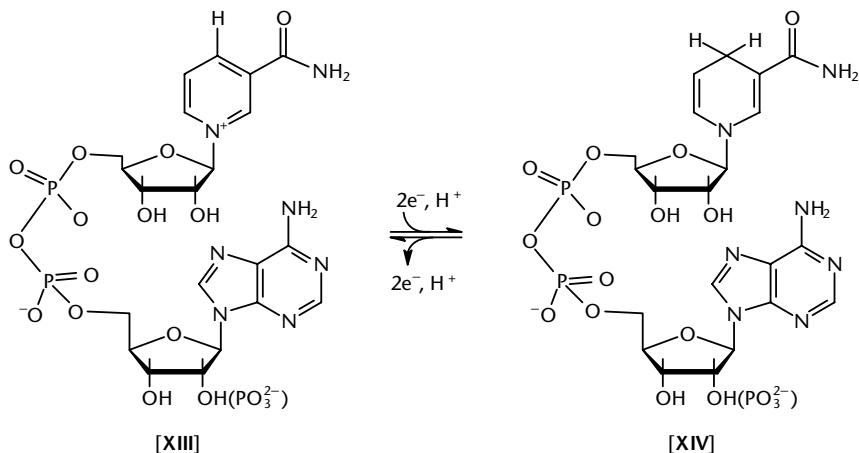
functional groups analogous to those found in proteins, but offer other properties, such as facile translocation, that are not possible for protein functional groups. Coenzymes are typically derivatives of vitamins or bacterial growth factors; for example, thiamin pyrophosphate (TPP) is a derivative of vitamin B₁ (thiamin), and coenzyme B₁₂ is a derivative of vitamin B₁₂. While coenzymes participate directly in enzymatic catalysis, they are not usually covalently bound to the enzyme with which they function. Coenzymes may, in fact, be only loosely associated with the enzyme during catalysis, or in other instances may be tightly, but noncovalently, bound to the enzyme.

The term cofactor is typically applied to catalytically essential molecules or ions that are covalently bound to the enzyme. Examples of enzyme cofactors include the unusual peptide-bound topa quinone in amine oxidase, in which a tyrosine residue has been oxygenated and oxidized to produce a catalytically essential and covalently bound cofactor, and the haem group found in many proteins and enzymes, including cytochrome *c* oxidase. In addition, the metal ions found in metalloenzymes, which are typically bound to the protein via coordination of amino acid side-chains, are cofactors in metalloenzyme catalysis. These metalocofactors can provide important characteristics to the enzyme, including sites for binding substrates via coordination, electrophilic centres to aid in catalysis, and multiple readily available oxidation states that facilitate involvement in redox and electron transfer chemistry.

Enzymatic Reactions that Require Coenzymes

Nicotinamide coenzymes

The nicotinamide coenzymes include nicotinamide adenine dinucleotide (NAD⁺, [XIII]) and nicotinamide adenine dinucleotide phosphate (NADP⁺), which differ only in the presence or absence of a phosphate group on the 2'-C of the adenosyl moiety. Both NAD⁺ and NADP⁺ serve as reversible carriers of reducing equivalents in the cell, and are utilized in a wide variety of enzymatic reactions including, for example, alcohol dehydrogenase and dihydrofolate reductase. The redox-active moiety is the nicotinamide base, which can be reduced by two electrons to generate NADH or NADPH [XIV]. In effect, the nicotinamide accepts a hydride ion equivalent from the substrate. Such a reaction requires a strongly electrophilic centre, something which is lacking among the functional groups of proteins, but present in the nicotinamide coenzymes due to the oxidized pyridinium ring. The reduced coenzymes, NADH and NADPH, in turn serve as reductants in other reactions, including the critical electron transfer processes that result in O₂ reduction and ATP synthesis.

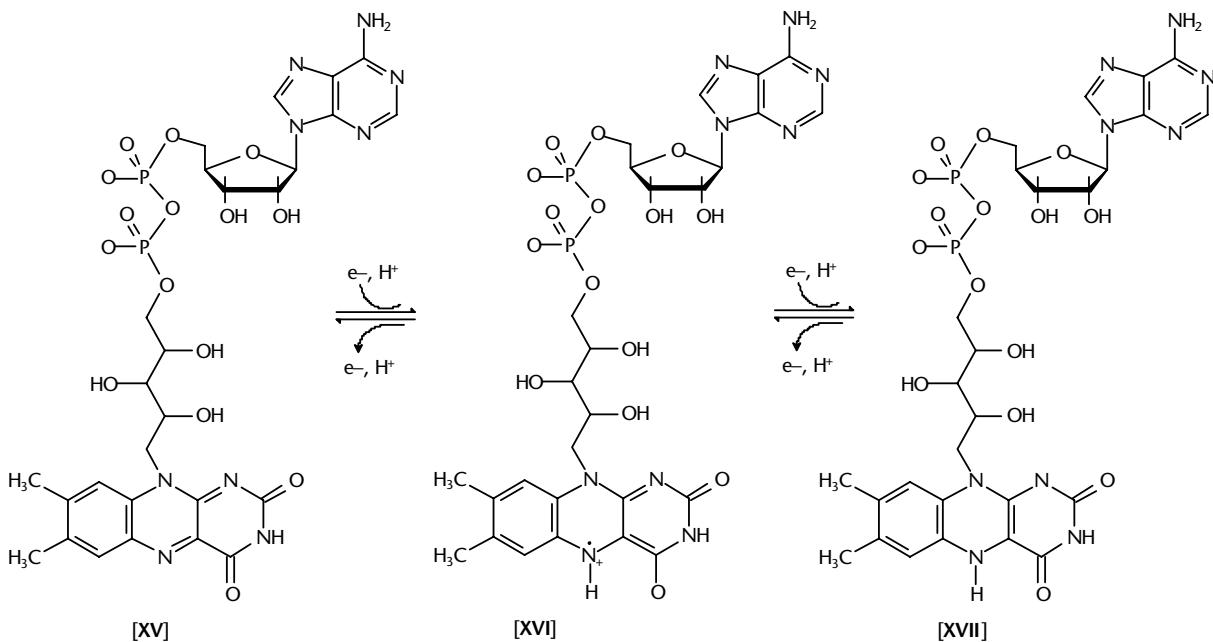


In many cases, such as those just discussed, the nicotinamide coenzymes are actually serving as cosubstrates, but they do serve as true coenzymes in some reactions, for example *S*-adenosylhomocysteinase. Here the coenzyme NAD^+ serves the same general function as in the reactions discussed above, i.e. it accepts a hydride ion equivalent from the substrate, but in this case the reduced coenzyme is an intermediate which is reoxidized to NAD^+ in generation of the product.

Flavin coenzymes

The flavin coenzymes, flavin–adenine dinucleotide (FAD [XV]) and flavin mononucleotide (FMN, which lacks the

second phosphate and the adenosyl moiety of [XV]), are derivatives of riboflavin (vitamin B_2). The business end of the flavin coenzymes is the flavin moiety, and FMN and FAD serve analogous functions in enzymatic reactions. Like the nicotinamide coenzymes, the flavin coenzymes participate in redox reactions; the flavin coenzymes, however, are more versatile in that they can participate in either one- or two-electron redox chemistry. The flavin coenzymes can exist in an oxidized form (FMN and FAD), a one-electron reduced semiquinone form (FMN \bullet and FAD \bullet [XVI]), and a two-electron reduced form (FMNH $_2$ and FADH $_2$ [XVII]). Because of this flexibility in oxidation states, the flavin coenzymes are able to participate in a wider variety of redox reactions, with a wider variety of



redox partners, than the nicotinamide coenzymes. Enzymes that utilize flavin coenzymes (often called flavoproteins) include dehydrogenases, oxidases and oxygenases. The direct redox partners of flavoproteins can include two-electron acceptors such as NAD^+ and NADP^+ or one-electron acceptors such as the ferric haems, iron–sulfur clusters and quinones.

B₁₂ coenzymes

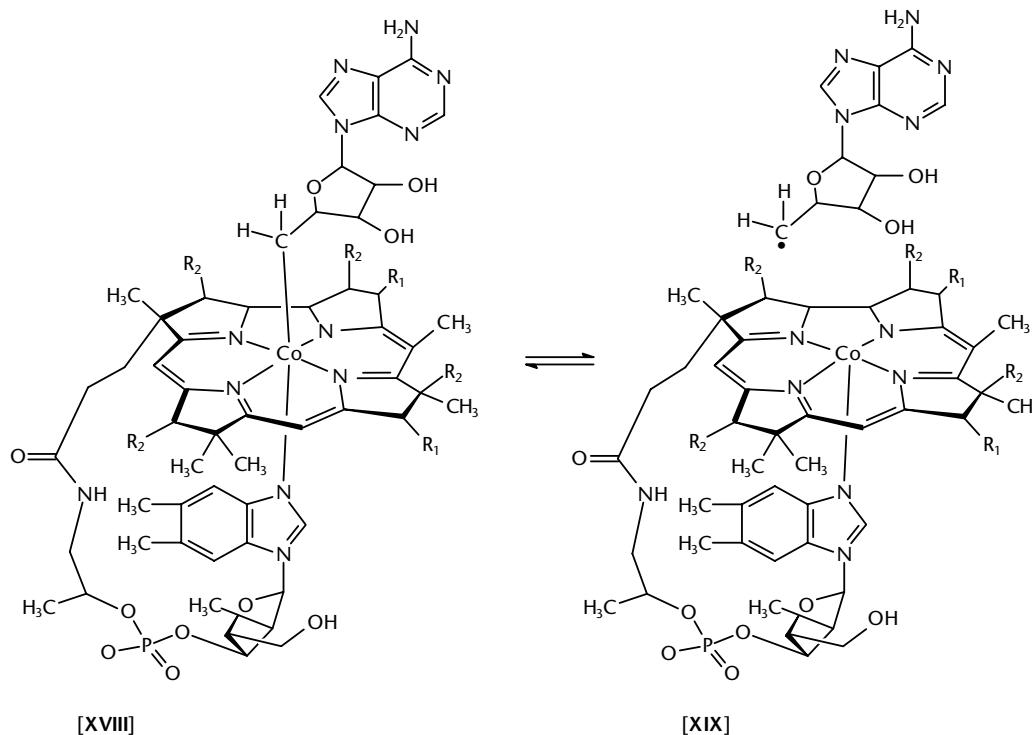
The B₁₂ coenzymes, adenosylcobalamin [XVIII] and methylcobalamin, consist of cobalt(III) bound to a substituted corrin ring and an alkyl group (either adenosyl or methyl) (Frey, 1990). These coenzymes are frequently involved in the catalysis of rearrangement reactions. By virtue of the direct bond between the cobalt and a carbon of the alkyl group, the B₁₂ coenzymes are organometallic coenzymes, and are the only well-characterized organometallic species in biology. The B₁₂ coenzymes function in rearrangements through reversible cleavage of the cobalt–carbon bond to generate a cobalt(II)–organic radical intermediate [XIX]. The organic radical intermediate then abstracts a hydrogen atom from the substrate, which rearranges and reabstracts the hydrogen atom from the 5'-deoxyadenosine intermediate to regenerate the 5'-deoxyadenosyl radical intermediate. This radical intermediate can then re-form the bond to the cobalt(II) to regenerate the coenzyme. One way to think of the role of the B₁₂

coenzymes is as reversible radical generators, coenzymes that can generate a radical on demand at the enzyme active site to aid in catalysis. In this sense, the B₁₂ coenzymes are more elegant versions of the amino acid radicals discussed above.

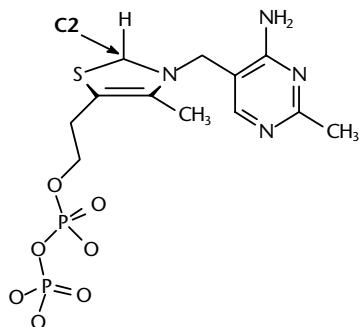
While the amino acid radicals are always present in the active enzymes and thus are subject to potentially damaging side reactions, the B₁₂ coenzyme radicals are 'stored' in a relatively innocuous form, the organometallic complex, and are generated only when and where needed through homolytic cobalt–carbon bond cleavage. Lysine 2,3-aminomutase utilizes another coenzyme, *S*-adenosylmethionine, to initiate radical chemistry via generation of an adenosyl radical intermediate (Baraniak *et al.*, 1989). In this case, no cobalt is required for the reaction, but the enzyme does contain an iron–sulfur cluster cofactor. The mechanism of generation of the adenosyl radical in this system has yet to be elucidated.

Thiamin pyrophosphate

Thiamin pyrophosphate (TPP [XX]), the pyrophosphorylated form of thiamin (vitamin B₁), plays a central role in a number of enzymatic reactions that involve the cleavage of carbon–carbon bonds, including the critical α -ketoacid decarboxylations such as that catalysed by pyruvate decarboxylase. Decarboxylation reactions require an electron acceptor to dissipate the negative charge that



builds up on the α -carbon, but α -ketoacids have no such intrinsic electron acceptor. In the case of most enzyme-catalysed α -ketoacid decarboxylations, TPP serves as an electron acceptor to promote decarboxylation.

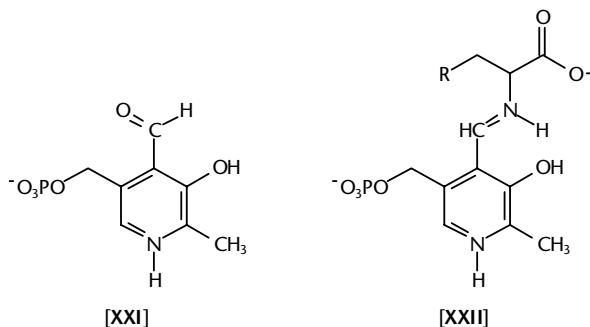


[XX]

It was shown by Breslow (1958) that the thiazolium C2 proton of TPP is very acidic, implicating the C2 carbanion of the thiazolium ring in the catalytic mechanism. TPP-dependent decarboxylation, for example in pyruvate decarboxylase, proceeds by attack of the thiazolium C2 carbanion on the α carbon of the substrate to produce a tetrahedral intermediate. Decarboxylation then proceeds, with delocalization of the resulting negative charge on to the thiazolium ring. The decarboxylated product is then produced and the thiazolium carbanion regenerated.

Pyridoxal 5'-phosphate

Like thiamin pyrophosphate, the role of pyridoxal 5'-phosphate (PLP [XXI]) in enzyme catalysis is to serve as an electron sink, to dissipate negative charge that builds up during turnover. PLP serves this role in conjunction with a large number of enzymes that catalyse diverse reactions involving the α , β or γ carbons of α -amino acids. Also like TPP, PLP dissipates negative charge via a covalent intermediate, in this case an aldimine formed between the amine of the amino acid substrate and the aldehyde of PLP [XXII]. In this way, PLP participates in the catalysis of decarboxylases, transaminases, aldolases, dehydratases and other enzymes that utilize α -amino acids as substrates.

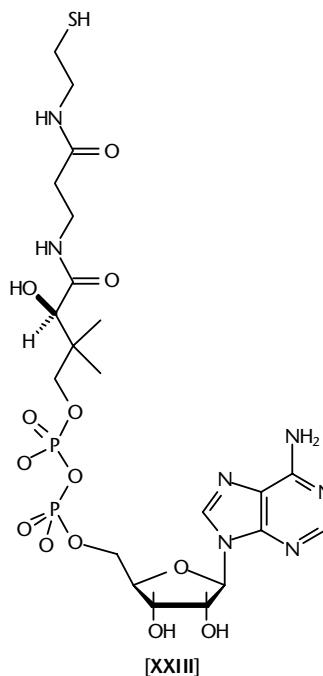


[XXI]

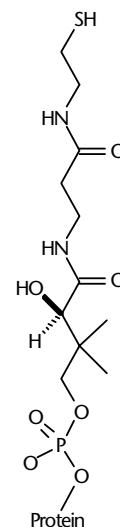
[XXII]

Phosphopantetheine coenzymes

The phosphopantetheine coenzymes include the ubiquitous coenzyme A (CoA [XXIII]), in which 4'-phosphopantetheine is attached to an adenosyl moiety, and acyl carrier protein (ACP [XXIV]), in which 4'-phosphopantetheine is attached to the small protein through a serine hydroxyl group. As can be seen from [XXIII] and [XXIV], the key feature of the phosphopantetheine coenzymes is a long, flexible chain terminating in a sulfhydryl group, and this sulfhydryl group is the key functional group of the coenzymes. The primary functions of the phosphopantetheine coenzymes are acyl group transfer and carboxylate activation, although other functions have been identified as well. These coenzymes serve as the intracellular currency of active acyl groups, and thus are central to many reactions, including fatty acid biosynthesis.



[XXIII]

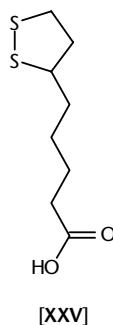


[XXIV]

Lipoic acid

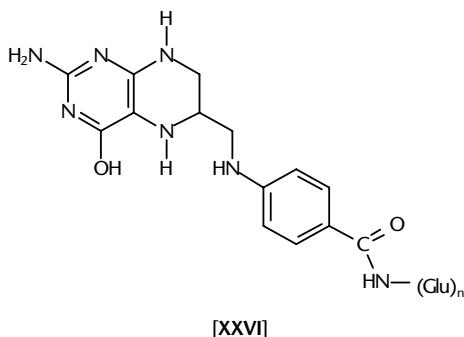
Lipoic acid [XXV], like the pantetheinyl coenzymes, serves in the transfer of active acyl groups, with the key difference being that lipoic acid concomitantly acts as a mediator in the transfer of electrons. The catalytically important functional group in lipoic acid is the disulfide, which can accept two electrons and two protons to be reduced to the dihydrolipoate state. It is in this reduced state that lipoic acid can accept activated acyl groups. In this way, lipoic acid couples the electron and acyl group transfers catalysed by α -ketoacid dehydrogenase multienzyme complexes.

One of these, the pyruvate dehydrogenase complex, is a key enzyme in glucose metabolism.



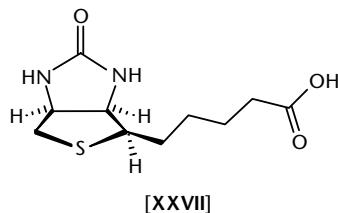
Folate coenzymes

Tetrahydrofolate (FH₄ [XXVI]) and related molecules serve as key coenzymes in the biosynthesis of purines and pyrimidines by supplying one-carbon units for the various synthetic steps. The one-carbon units supplied by *N*¹⁰-formyl-FH₄, *N*⁵,*N*¹⁰-methenyl-FH₄, *N*⁵,*N*¹⁰-methylene-FH₄ and *N*⁵-methyl-FH₄ are essentially the cell's supply of formate and formaldehyde, kept in a relatively innocuous form as an FH₄ adduct until needed. In addition to the critical purine and pyrimidine biosynthetic functions performed by the tetrahydrofolates, *N*⁵-methyltetrahydrofolate is the methyl group donor in the biosynthesis of methionine.



Biotin

Biotin [XXVII] plays a central role in enzyme-catalysed carboxylation reactions, most of which utilize bicarbonate and ATP as substrates. Biotin is covalently bound to the carboxylase enzymes with which it functions via an amide linkage between the biotin carboxyl and a lysyl ε-NH₂ group in the active site of the protein. The role of biotin in these carboxylase reactions is to act as mediator by accepting the activated carboxyl generated from bicarbonate and ATP and then to transfer this carboxyl to the appropriate substrate.



Organic cofactors

A number of enzymes have been identified that contain covalently modified amino acids which function as coenzymes in catalysis. These include amine oxidase, which contains a topa quinone cofactor (TPQ, a covalently modified tyrosyl residue; Janes *et al.*, 1990), and methylamine dehydrogenase, which contains a tryptophan tryptophylquinone (TTQ; McIntire *et al.*, 1991), as well as other examples. These quinoproteins are typically redox catalysts such as oxidases and dehydrogenases.

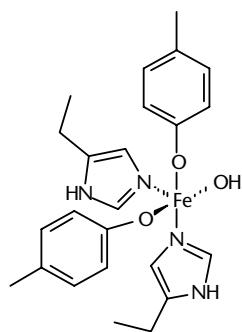
Enzymatic Reactions that Require Metal Ion Cofactors

Thousands of enzymatic reactions, perhaps as many as one third of all those known, require metal ion cofactors in functions ranging from Lewis acid catalysis to redox catalysis to electron transfer. Metals add functionality to enzymes by providing strongly electrophilic centres, and in some cases multiple readily available oxidation states that can facilitate redox and electron transfer reactions. Most of the first row transition metals (the only exceptions being scandium, titanium, and perhaps chromium) as well as molybdenum, tungsten and magnesium are known to function as cofactors in enzymatic catalysis. Typically, these metal ion cofactors are bound to the enzyme via coordination of amino acid side-chains, although in some cases the metal is bound to a nonprotein ligand, e.g. a haem group, and the metal–ligand complex is bound to the enzyme through the metal or the ligand or both. In most cases, the metal is reasonably tightly bound to the enzyme, such that the metalloenzyme can be isolated with the metal ion intact, allowing the observation of enzymatic activity in the absence of added metal ion. Enzymes with more loosely bound metal ion cofactors, commonly called metal-activated enzymes, require the presence of the appropriate metal ion in the buffer in order to observe maximal catalytic activity.

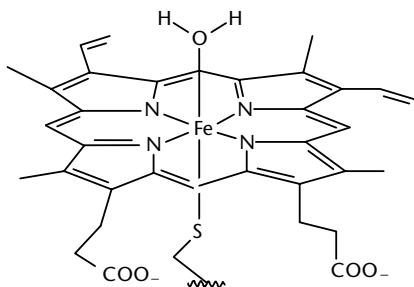
Iron

Iron is the most abundant transition metal in the Earth's crust, and thus it is perhaps not surprising that it is

ubiquitous in biological systems. Iron is a key player in some of the most central processes of biological systems, including oxygen transport and utilization, electron transfer, metabolism of nucleic acids and many other key biological molecules, degradation of biological pollutants, and many other reactions. A number of the enzymatic processes that require an iron cofactor are discussed below; these utilize both the accessibility of multiple redox states of iron (both Fe(II) and Fe(III) are readily accessible under physiological conditions) as well as the Lewis acidity of Fe(III) to aid in enzymatic catalysis. Iron cofactors can be bound directly to amino acid side-chains, for example in the intradiol catechol dioxygenases, where the iron is bound to two tyrosines, two histidines and one hydroxide to give a 5-coordinate environment [XXVIII] (Que and Ho, 1996). Iron is also commonly found bound to haem groups, for example in the ubiquitous cytochrome enzymes. In cytochrome P450, for example, iron is coordinated by the four pyrrole nitrogens of the protoporphyrin IX in a square planar arrangement, and is also coordinated in one of its axial positions by a cysteine sulfhydryl and in the other by water, to give an overall distorted octahedral coordination environment [XXIX] (Sono *et al.*, 1996).



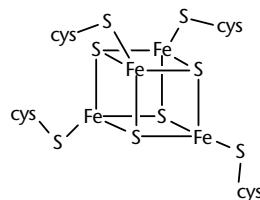
[XXVIII]



[XXIX]

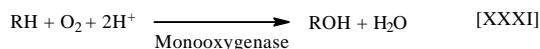
Some iron cofactors are found bound to a combination of amino acid side-chains and exogenous, nonsolvent, ligands. The most widespread examples of this are the iron–sulfur proteins, in which the iron atoms exist in clusters of two, three, four or more, coordinated by a

combination of bridging inorganic sulfide and amino acid side-chains, usually cysteines [XXX].



[XXX]

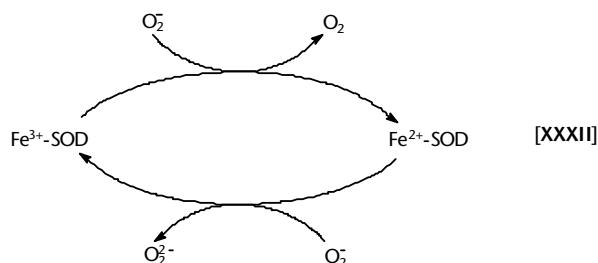
Metalloenzymes containing iron catalyse an enormous variety of reactions, most of which utilize the redox properties of iron and many of which involve reaction with oxygen and/or its reduced species, superoxide and peroxide. A common example of an iron-dependent enzymatic reaction is oxygenation, in which one or both atoms from molecular oxygen are incorporated into organic substrate (reaction [XXXI]). Oxygenases are central to many important metabolic processes, including the degradation of aromatic compounds, lipid metabolism, collagen formation, toxin degradation and alkane functionalization. Oxygenases also span the representative types of iron centres in proteins, with some containing mononuclear nonhaem iron centres, some binuclear iron centres, some haem iron centres, and some iron–sulfur clusters. In many of these enzymes, it is believed or has been clearly demonstrated that a primary role for the iron is to activate molecular oxygen for reaction with substrate. There is a strong thermodynamic driving force for the reaction of most organic molecules with dioxygen, yet such reactions do not occur spontaneously owing to the spin-forbidden nature of the reaction between the triplet dioxygen and singlet organic molecules. One way in which this spin restriction can be relaxed is via interaction of dioxygen with a metal centre such that the dioxygen is reduced to the superoxide or peroxide oxidation state; this type of ‘oxygen activation’ appears to be a primary role for iron in biological systems.



Iron also serves as a cofactor in oxidases, in which the oxidizing power of oxygen is utilized for a redox reaction, but oxygen is not incorporated into the product. One of the most interesting and functionally central oxidases is cytochrome *c* oxidase, which plays a central role in oxidative phosphorylation, the process by which the oxidizing power of oxygen is harnessed to synthesize ATP, the energy currency of the cell. Cytochrome *c* oxidase contains both copper and iron cofactors and is the site of reduction of oxygen to water. The ready accessibility of two oxidation states for both copper and iron is utilized by cytochrome oxidase in transferring electrons to oxygen.

Iron is also a central cofactor in the reactions catalysed by superoxide dismutase (SOD) [XXII] and catalase. Both

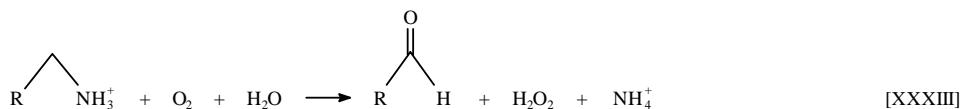
of these enzymes serve to protect the living system from the potential damaging effects of reactive reduced oxygen species. Iron has also been more recently shown to play a central role in the generation of catalytically essential amino acid radicals and in the initiation of radical mechanisms in certain enzymes (Stubbe and van der Donk, 1998). The best-characterized example of this type of iron metalloenzyme is ribonucleotide reductase, in which a binuclear iron(II) centre reacts with oxygen to produce a binuclear iron(III) centre and a tyrosyl radical, the latter being an absolute requirement for catalytic activity. Iron, in the form of an iron–sulfur cluster, is also centrally involved in the generation of the glycol radical in ribonucleotide reductase from anaerobic *E. coli* (Mulliez *et al.*, 1993) and the glycol radical of pyruvate formate-lyase (Broderick *et al.*, 1997); again, both of these radicals are catalytically essential. Iron, again in the form of iron–sulfur clusters, is also involved in initiation of radical chemistry in lysine 2,3-aminomutase (Lieder *et al.*, 1998).



Copper

Copper, like iron, has two oxidation states (I and II) that are readily available under physiological conditions, and therefore can participate in catalysis of redox reactions in much the same way that iron does. One example is in the superoxide dismutases, which catalyse the disproportionation of superoxide to peroxide and oxygen [XXXII]. An alternative to the Fe-SOD is the Cu/Zn-SOD, which contains copper and zinc in a binuclear site. In this case it is copper that binds superoxide and serves the redox role in the mechanism, while the role of zinc appears to be merely structural.

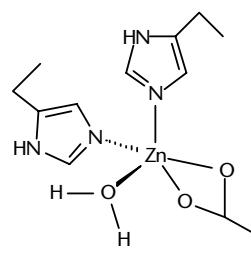
There are other analogies in the biological chemistry of copper and iron, the most notable being the prevalence of both in oxygenase and oxidase chemistry. Copper is a key cofactor in dopamine β -monooxygenase and peptidylglycine α -amidating enzyme, both of which catalyse mono-oxygenations (Klinman, 1996). Like iron in the iron oxygenases, copper is believed to activate dioxygen, via redox chemistry, for reaction with organic substrate.



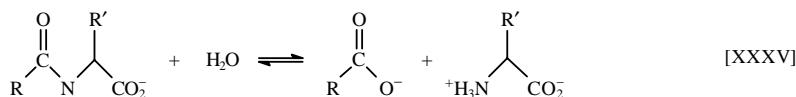
Copper is also a prevalent component of oxidases, including the cytochrome *c* oxidase mentioned above, as well as a variety of amine oxidases [XXXIII], and galactose oxidase. The amine oxidases also contain a covalent catalytically essential topaquinone cofactor, as discussed in an earlier section of this article. The similarities between iron and copper in biological systems go beyond their roles as enzyme cofactors to their roles in dioxygen transport and in electron transfer, roles that will not be discussed in this article. What is clear from studies to date on metalloenzymes is that the unique chemical properties of iron and copper, including their strongly electrophilic character and the availability of multiple redox states, make them uniquely suited as cofactors to a variety of critical biochemical reactions.

Zinc

Among the transition metals playing key roles in biological systems, zinc is second only to iron in biological prevalence. Unlike iron and copper, zinc has only one oxidation state (+2) accessible under physiological conditions, and therefore does not participate in catalysis of redox chemistry or electron transfer reactions. Zinc is, however, quite flexible in its preference for ligands. This flexibility is due in part to zinc's affinity for both the soft (polarizable) protein ligands, such as cysteine thiols, and the harder (less polarizable) ligands, such as carboxylate, histidine imidazole and water. In addition, zinc can easily adopt coordination numbers of 4, 5, or 6. These properties give zinc tremendous flexibility in binding to a variety of amino acid side-chains and substrates in a variety of geometries. The zinc coordination sphere in carboxypeptidase [XXXIV] contains two histidine imidazole ligands, two oxygens from the bidentate carboxylate of glutamate, and one water.



Zinc functions widely in Lewis acid catalysis and is also found to play important structural roles in enzymes and proteins. The types of enzymatic reactions in which zinc is



found to play a role include peptidases and amidases (reaction [XXXV]), phosphatases, phospholipase, phosphotriesterase, deaminases and alcohol dehydrogenase, among others (Lipscomb and Sträter, 1996). One mechanism by which zinc is believed to facilitate some of these reactions is through binding and thereby lowering the pK_a of water, generating a localized high concentration of metal-bound hydroxide in the active site, which can act as a nucleophile in the hydrolytic reactions.

Other metals

Although we have touched on the most common metal ion cofactors to enzymatic reactions, a number of others have been identified. Nickel has been found to be a required cofactor for urease and certain hydrogenases. Both vanadium and molybdenum have been identified as cofactors in nitrogenases and oxidases, and molybdenum has also been found to be a required cofactor for certain oxygen atom transfer reactions. A four-manganese cluster is the site of water binding and oxidation to O_2 in the photosynthetic oxygen-evolving complex. It is intriguing to note that this photosynthetic oxygen evolution requires a tyrosyl radical in addition to the manganese cluster, and this tyrosyl radical has been proposed to function in abstraction of hydrogen atoms from water (Hoganson and Babcock, 1997). Manganese is also a cofactor in oxidase chemistry, while magnesium has been shown to be required in certain hydrolases and isomerases. Tungsten has been found to be a cofactor in certain dehydrogenases.

Summary

Enzymes are responsible for an enormous variety of reactions in living systems, from the relatively simple bond hydrolysis catalysed by carboxypeptidase to the incredibly complex generation of O_2 from H_2O by the photosynthetic oxygen-evolving complex. With the diverse types of reactions that are required for life, and the limited number of amino acid side-chains to confer catalytic properties on enzymes, it is perhaps not surprising that many enzymatic reactions are dependent on nonprotein components to aid in catalysis. The coenzymes and cofactors utilized by enzymes add diverse functionality to enzymes, including new functional groups, redox capabilities, electrophilic centres, and sites for coordination of substrates.

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