Dalton Perspectives

Inorganic Reaction Mechanisms: the Bioinorganic–Organometallic Interface

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Bioinorganic and organometallic chemistry are often considered to represent the extreme ends of the spectrum of inorganic chemistry. This division is a consequence primarily of the structural aspects associated with each area, the type of ligands involved, a perception of the types of oxidation states of the metals in the two systems and the methodology employed to study the materials. However, the apparent barrier between these two disciplines starts to disappear when we consider reactivity and mechanistic chemistry. After all, a substitution reaction is a substitution reaction, whether it's in a cobalt(III) complex, such as $[CoCl(NH_3)_5]^{2+}$, a manganese(0) complex such as $[Mn(\eta^5\text{-}C_5H_5)(CO)_2(thf)]$ (thf = tetrahydrofuran) or a zinc(II) centre surrounded by a polypeptide, as in carboxypeptidase. The same set of basic mechanistic principles apply to the reactivity irrespective of the system.

This article will illustrate how studies designed to understand the reactivity of metalloenzymes can lead to new chemistry, or give insights into contemporary chemical issues which are far removed from any biological problem.

The Development of Inorganic Reaction Mechanisms

By the 1950s the mechanistic basis of organic chemistry was well advanced and thus it was a natural consequence that the principles established there should be applied to the study of the reactions of inorganic complexes.[1] Prior to this period isolated suggestions about the mechanisms of inorganic reactions had been made, but it is only from the middle of the twentieth century that a methodical, systematic approach was undertaken. The development of a Periodic Table-wide comprehensive picture of reaction mechanisms is a gargantuan task because of the large variety of oxidation states, co-ordination numbers and geometries associated with the elements. In the early days the development of the principles of inorganic reaction mechanisms was dominated by studies on classical co-ordination compounds. In particular, 'Werner type' octahedral cobalt(III) amine complexes and square-planar platinum(II) complexes, with an emphasis on substitution and redox reactions. Subsequently, the field flourished so that the substitution and electron-transfer reactivity patterns of many metals in a variety of different co-ordination environments are now established. Included in this expansion came studies on organometallic systems, which exhibited reactions not observed with classical co-ordination compounds.

Certainly the basic mechanisms of all the fundamental elementary reactions including substitution, electron transfer, insertion, oxidative addition and reductive elimination are established. The purist would probably argue that there is still a great deal to be done even in defining the mechanisms of substitution or redox reactions. This is a point of view with which I have little quarrel. What I do advocate, though, is that we look beyond the approach that has been adopted so far. If we assess the role of reaction mechanisms critically, it must be concluded that understanding the mechanism of any one particular reaction is of only limited value. At the very least, understanding the mechanism of a reaction must complement synthetic and structural chemistry. At best the results of a mechanistic study not only define how a reaction proceeds at the atomic level, but can also be employed to help in the preparation of new complexes, rationalise the previously unexplained behaviour of analogous complexes, or even open up a new, and previously unexplored, area of chemistry. At worst, a mechanistic study only adds another rate constant (or several numbers if the activation parameters are also determined), associated with a reaction type we already understand in great detail! There has been an overemphasis in the past to discuss inorganic reaction mechanisms in terms of a particular oxidation state of a metal, an electron configuration, or a particular geometry. This approach owes much to the developmental years where researchers were defining the reactions of octahedral cobalt(III) complexes (say). It is too limiting: we need to look for more general principles, those which are of such general utility that there is no hesitation in applying them to more complex, multistep processes as are found in the action of homogeneous catalysts and metalloenzymes. It is just this type of approach upon which I will elaborate.

Mechanisms: Bioinorganic and Organometallic Chemistry

In the last couple of decades there has been a move in inorganic reaction mechanisms towards studying bioinorganic systems.[2] This is an area where we should carefully assess what the inorganic mechanist can usefully contribute. Undoubtedly some excellent work is being done in this area but does not always address a biologically relevant problem.

There are two areas in which mechanistic studies can contribute meaningfully to our understanding of bioinorganic systems.

(1) Studies on isolated, purified metalloproteins: defining the structure–function relationship of the protein in terms of our understanding of the structure and reactivity of simple complexes. For example, understanding: (*i*) how the polypeptide backbone augments the reactivity of the active site and (*ii*) why particular metals (or groups of metals) with a specific set of ligands are employed by the biological system.

(2) Using simple metal complexes to establish a *chemical precedent* for the proposed mechanisms of the elementary reactions of metalloenzymes. This type of study is often referred to as involving 'model systems'. This is misleading and leads to the criticism that the metal site under investigation bears no relation to that in the enzyme. Undoubtedly, based on a structural comparison, this criticism is justified, but that is not what these studies purport to do. What is being 'modelled' is the *reactivity* (or part of the reactivity) of the enzyme.

Consideration of the types of mechanisms being studied in (2) shows that we are looking at the reactions of ligands, and the manner in which they can be activated or transformed. The activation and transformation of co-ordinated molecules is also a major concern in organometallic and co-ordination chem-

istry, and catalysis. It follows then that the mechanistic principles established for the reactions of ligands will pervade many areas of chemistry. Consequently, although understanding the reactivity of substrates of metalloproteins is a meritorious goal in its own right, the application of the knowledge we gain from this research can result in the development of new chemistry in areas far removed from metalloenzymes; chemistry which might otherwise have been overlooked.

Defining the Mechanisms of Catalysts: Functional Group Chemistry

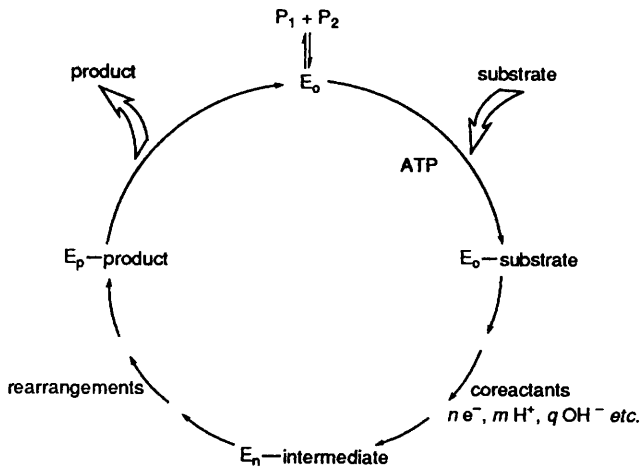
There are three stages by which any catalyst, including metalloenzymes, converts reactants into products: binding of the substrate; transformation of the substrate into product and finally the release of the product. The complexity of a generalised enzyme is shown in Scheme 1. Thus the catalysis consists of at least three elementary reactions corresponding to each of the stages listed above. However, this is just the start of the complexities. For example, it is common for the transformation stage to consist of several elementary reactions and, additionally in some cases, the enzyme may consist of several proteins, all of which must interact to produce the enzyme. Thus the manner in which the proteins interact must also be defined.

Considering the complexity of any catalyst system it is rarely, if ever, possible to define the intimate mechanism of the action of the catalyst at the atomic level by studies on the enzyme itself. There are three limitations in defining the detailed mechanism of any catalyst: (1) the large number of steps involved, and the catalytic nature of the process, means that it is often not possible to look at any one step in isolation from the rest; (2) the rate-limiting step of the catalysis limits the kinetic information; any steps occurring after the rate-limiting step are kinetically hidden; (3) a large number of species may be present in solution, in equilibrium with one another, but only some of these species are involved directly in the catalytic cycle; with all the other complications it is often difficult to establish which is the 'active' species.

The problems inherent in mechanistic studies on catalysts as they turn over are well illustrated by studies on Wilkinson's catalyst, $[RhCl(PPh_3)_3]$, which hydrogenates alkenes.[3] Early attempts to study the mechanism of this catalyst led to a variety of rate laws, all established by simulating the rate data using several variables. Given the number of steps involved and the number of independently adjustable parameters, it is hardly surprising that satisfactory fits can be obtained to several different rate laws.

It is possible, at least on paper, to break up any catalytic cycle into a series of elementary reactions. Anyone can arrive at a detailed mechanism for each of these elementary steps based on gross observations such as product distributions or the results of isotopic labelling studies on the catalyst, as it turns over. However, the mechanism of the elementary step must fulfil two prerequisites. First, it must be chemically reasonable, and secondly should be demonstrable in simple chemical complexes. At its best the reactivity is being defined on a complex which is structurally analogous to the active site in the catalyst. However, this is not always feasible and really the main criterion is a site which has similar electronic characteristics to those of the active site of the catalyst, for the elementary reaction under consideration. The model system must fulfil certain prerequisites: (*a*) the structure of the reactant and the product(s) must be established; (*b*) the co-ordination sphere of the complex should be robust, the only reactive site being the one pertinent to the elementary reaction under investigation; (*c*) the reaction being studied must be stoichiometric and as simple as possible, in order that the maximum mechanistic information can be obtained.

It is using this sort of approach that the detailed mechanisms of catalysts can be elucidated. Ultimately, by modelling each elementary reaction of the catalyst, we can present an overall



Scheme 1 General picture of the action of an enzyme, illustrating the complexity of the system including: the multiprotein nature of the enzyme ($P_1 + P_2$); binding of the substrate; transformation of the substrate including intermediates and release of the product. E_o, E_n and E_p represent different states of the enzyme

picture of the catalytic action. As an example, Scheme 2 shows the catalytic cycle for the conversion of dinitrogen into ammonia.[4,5] This cycle is based on the chemistry defined on molybdenum (the physiological metal) and tungsten complexes, such as *trans*-$[M(N_2)_2(dppe)_2]$, (dppe = $Ph_2PCH_2CH_2PPh_2$) and illustrates the possible reaction pathways that the enzyme nitrogenase could adopt.

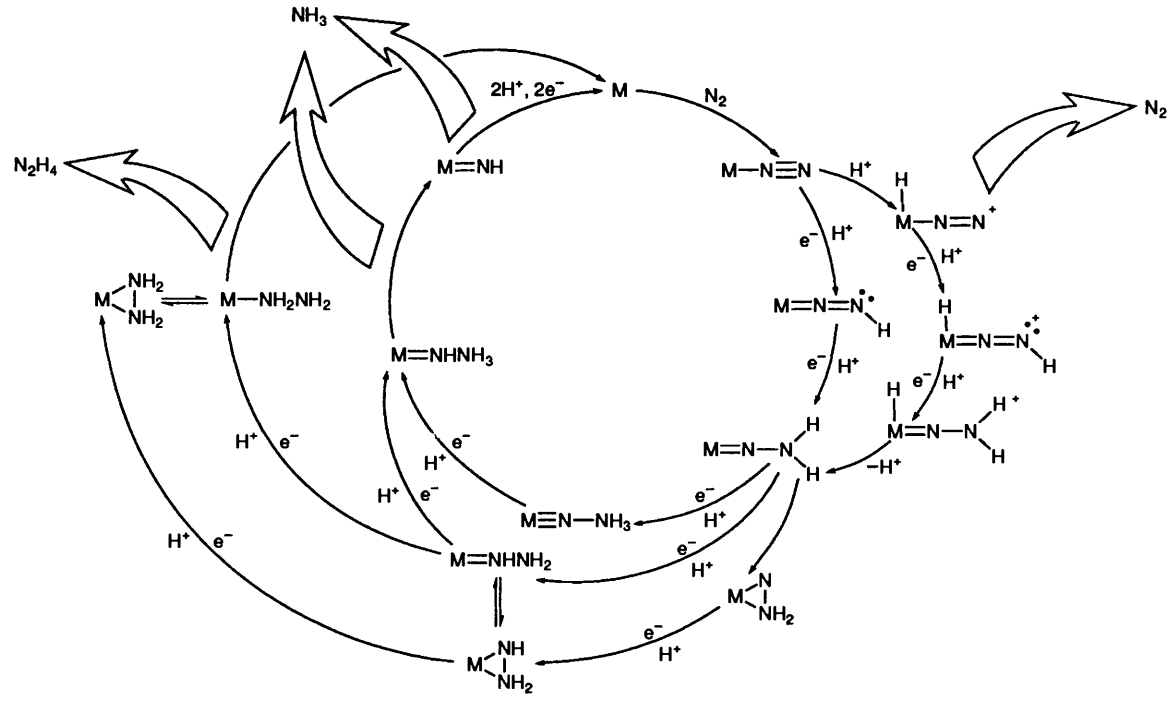
Clearly, the very detailed picture of the reactivity that emerges is *all* the possible ways in which the reactants are converted into products, not just the pathway that the enzyme employs. It is, of course, impossible to define the enzyme's preferred route from studies on these simple chemical systems. What is defined is all the pathways by which dinitrogen can be converted into ammonia or hydrazine at a single metal site. *In order to understand the reactivity of any catalyst at the atomic level it is just as important to understand the pathways that it does not use as it is to know the pathways that the catalyst does adopt.* What are established are chemical precedents for the transformations of the substrate. Scheme 2 then illustrates what is really the *functional group chemistry* of the co-ordinated dinitrogen molecule towards protons.

In the remainder of this Perspective I will discuss selected mechanistic studies on relatively simple complexes where the initial goal of the research was to understand the reactivity of various metalloenzymes, but which subsequently gave rise to insights into more diverse areas of chemistry.

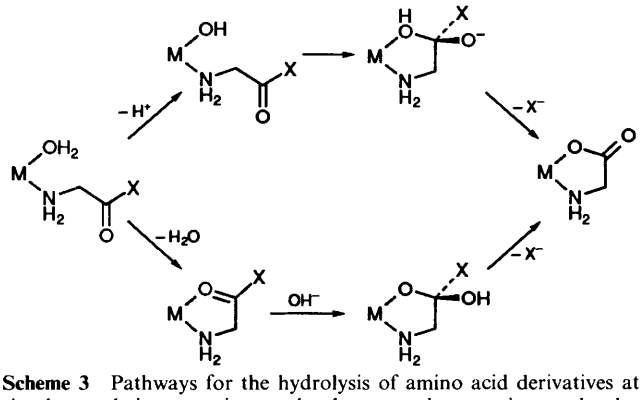
Action of Carboxypeptidase and Intramolecular Nucleophilic Attack

The enzyme carboxypeptidase[6] is a hydrolytic, pancreatic enzyme which specifically cleaves the C-terminus amino acid of polypeptides. It has a molecular weight of *ca.* 34 600, and its crystal structure has been determined. The active site of the enzyme has been identified as a hydrophobic cleft at the bottom of which is a zinc atom. The zinc is in a +2 oxidation state, has a distorted geometry, and is co-ordinated to the polypeptide backbone by three amino acid residues: two histidines (His-69 and His-196) and a bidentate glutamate (Glu-72). The fourth co-ordination site is occupied by a water molecule.

The zinc centre in carboxypeptidase is a hard metal ion, and it is this characteristic which is fundamental in understanding the action of the enzyme. It has been known for many years[7] that hard metal ions such as Cu^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, *etc.* are capable of hydrolysing a wide range of amino acid derivatives, such as, peptides, amides, phosphate esters,



Scheme 2 Pathways for the conversion of dinitrogen into ammonia or hydrazine at a single metal site. All species shown have been structurally characterised and the mechanisms of the interconversions studied in stoichiometric reactions



Scheme 3 Pathways for the hydrolysis of amino acid derivatives at simple metal sites: top, intramolecular route; bottom, intermolecular route

sulfonate esters, acetals and esters. The mechanism originally proposed for this hydrolysis is shown in the bottom line of Scheme 3. The mechanism described here is that intuitively expected of the enzyme. The metal ion is the site where the polypeptide binds, and is activated. Being hard it withdraws electron density from the substrate, thus rendering the carbonyl carbon more susceptible to nucleophilic attack by the free hydroxide ion.

It is difficult to define the intimate details of the mechanism of hydrolysis of amino acid derivatives at the metal ions listed above because their substitution lability makes it impossible to be sure of the exact nature of the co-ordination sphere. However, using the robust cobalt(III) amine[8] complex, β-*cis*-$[Co(OH_2)(glycine\ ester)(NH_2CH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2)]^{3+}$ in which the co-ordination sphere of the cobalt remains intact and of defined stereochemistry throughout the reaction, it is possible to show, by isotope labelling studies, that the major hydrolysis pathway involves *intramolecular* attack of co-ordinated hydroxide on the carbonyl carbon, as shown on the top line of Scheme 3. This mechanistic result is unexpected. Hydroxide bound to a hard metal site is intuitively expected to be a weaker nucleophile than free hydroxide ion because of the electron-withdrawing effect of the hard metal. The prevalence of the intramolecular hydrolysis route is due to the correct stereochemistry and proximity of the co-ordinated hydroxide to the carbonyl carbon.

It is not clear which of these two pathways operates in carboxypeptidase and enzyme mechanisms based on both these routes have been proposed for the hydrolysis of peptides as shown in Scheme 4.

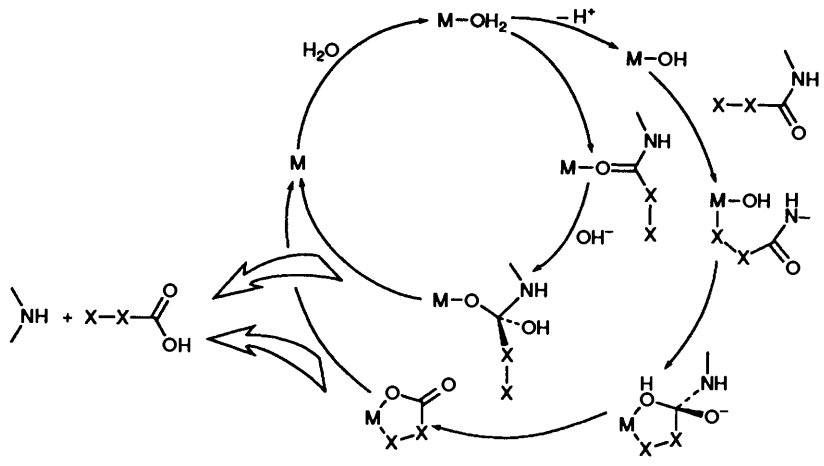
The general principle of intramolecular nucleophilic attack by co-ordinated hydroxide has been elaborated upon outside the biological sphere[9] as illustrated in Scheme 5. Thus a variety of different reactions of synthetic utility can be accomplished by this type of process including hydrolysis of amino acid derivatives, transesterification and hydrolysis of nitriles.

Other co-ordinated nucleophiles can undergo similar reactions.[10] In particular, amido-groups (generated by deprotonation of co-ordinated amines) can attack suitably positioned carbonyl carbon atoms in α-ketocarboxylates, α-aminocarbonyls, phosphate esters, disulfides and nitriles, as shown in Scheme 6. Finally, if co-ordinated amido-groups can act as intramolecular nucleophiles, they can also act as nucleophiles to external molecules.[11] This allows a further framework to be built on the existing ligands and thus result in the synthesis of more elaborate organic molecules including macrocyclic ligands, as shown in Scheme 7.

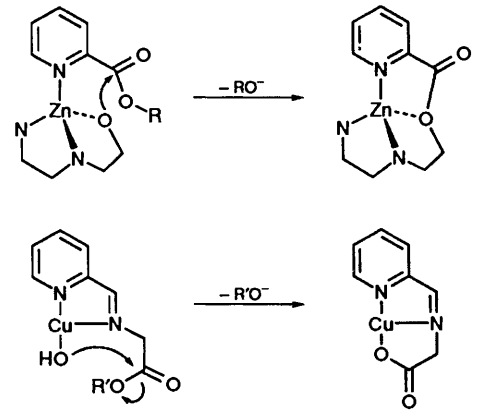
This chemistry is now a long way from its origins, based on defining the mechanism of nucleophilic attack at co-ordinated amino acid derivatives in order to understand the way in which carboxypeptidase works at the atomic level. Yet the basic mechanistic principle of intramolecular nucleophilic attack applies to a range of different hard metal ions and has resulted in a useful synthetic method to prepare multidentate or macrocyclic ligands, and organic molecules (after the metal has been removed).

Nitrogenases and the Protonation of Unsaturated Hydrocarbons

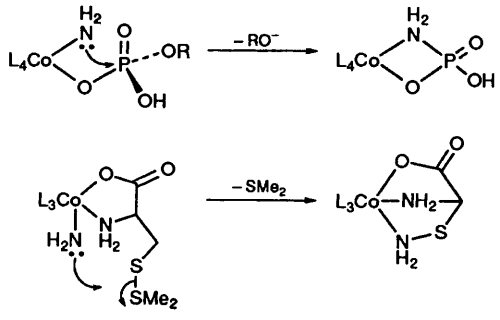
The physiological substrate of the nitrogenases, dinitrogen, has already been introduced in Scheme 2. There are three nitro-



Scheme 4 Possible pathways for cleavage of the peptide link in the action of carboxypeptidase



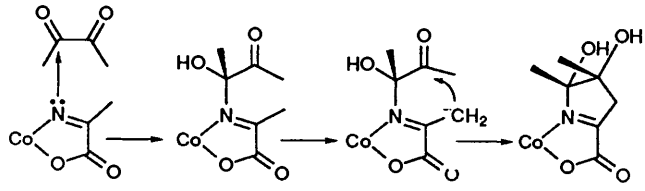
Scheme 5 Examples of intramolecular attack of oxygen-based nucleophiles; R = aryl, R′ = alkyl

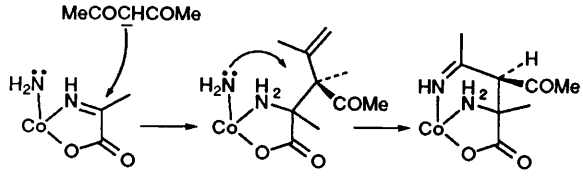


Scheme 6 Examples of intramolecular attack of amido-groups; R = aryl

genases, distinguished by their metal content and their product specificities. One contains iron and molybdenum, another iron and vanadium and a third which apparently contains only iron. Each enzyme consists of two metalloproteins, as shown in Scheme 8.

For the molybdenum nitrogenase the first protein, common to all the enzymes, is a Fe_4S_4 protein (molecular weight *ca.* 60 000) which transfers electrons to the larger protein. The larger protein (molecular weight *ca.* 230 000) contains two different types of metal clusters: the so-called P clusters which are believed to act as electron reservoirs before the electrons are transferred to the other cluster; the cofactor. The cofactor contains the molybdenum and is also believed to be the substrate binding site. It is an iron–sulfur-based cluster, as shown in Scheme 8, in which a molybdenum atom is located at one end and is further bound to a homocitrate (2-hydroxybutane-1,2,4-tricarboxylate) molecule in a bidentate fashion.





Scheme 7 Examples of nucleophilic attack of co-ordinated amido-groups on non-bound molecules

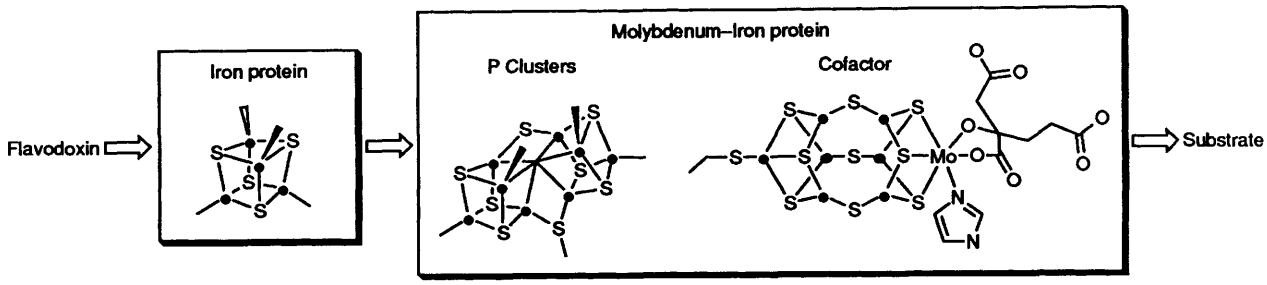
The cluster is bound to the polypeptide backbone only by the imidazole ring of His-442 (at molybdenum) and the sulfur of Cys-275 at the extreme tetrahedral iron atom. The cofactor from the vanadium enzyme is believed to be structurally analogous to the molybdenum cluster except for the presence of a vanadium atom in place of molybdenum. It is not obvious from this structure at which metal the substrates bind: molybdenum, tetrahedral iron or three-co-ordinate iron.

In terms of reactivity the nitrogenases are particularly diverse (and hence challenging to anyone trying to model their reactions), because of their ability to transform many substrates *in vitro* including: nitrous oxide, azide ion, cyanide, isocyanides, cyanamide and unsaturated hydrocarbons (alkynes and cyclopropene). Understanding the transformation of these 'alternative' substrates at simple metal complexes can give valuable, indirect, information about the nature of the substrate binding site.

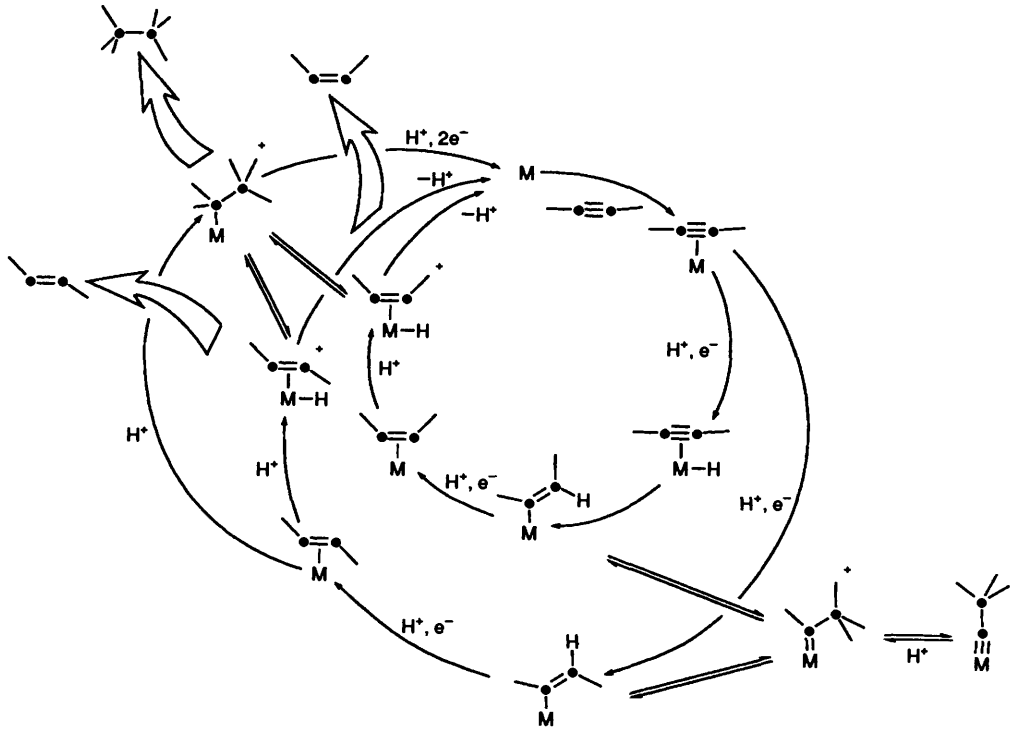
One of these 'alternative' substrates, which is particularly important, is acetylene. The molybdenum nitrogenase reduces acetylene to ethylene, and this is used as a field test for the presence of nitrogenase in the soil. The formation of ethane is characteristic of the presence of the vanadium nitrogenase.[12] There are two distinct problems associated with the reduction of acetylene which need resolution at the atomic level. First, the nitrogenases reduce acetylene stereospecifically to *cis*-CHDCHD in the presence of D_2O as shown in equation (1).

$$C_2H_2 + 2D^+ + 2e^- \longrightarrow \textit{cis}\text{-CHDCHD} \qquad (1)$$

Secondly, the factors which discriminate between the evolution of ethylene and the formation of ethane need to be defined if we



Scheme 8 The electron-transport chain, and the structures of the metal clusters in molybdenum nitrogenase



Scheme 9 Pathways for the transformation of alkynes to alkenes or alkanes at a single metal site

are to understand how the molybdenum enzyme only produces ethylene whereas the vanadium nitrogenase can also produce some ethane as described by equation (2). It is clear from

$$C_2H_2 + 4H^+ + 4e^- \longrightarrow C_2H_6 \qquad (2)$$

equations (1) and (2) that the mode of activation involves the sequential addition of electrons and protons. In modelling this type of reaction it is common to use a relatively low-oxidation-state complex; that is the electrons necessary to complement the proton additions to the ligand are effectively stored in the metal.

The overall picture for the transformation of alkynes at a single metal site, at least as far as we understand it to date, is shown in Scheme 9. Clearly a picture as detailed as this is not the result of a single study but rather from three independent studies on the stoichiometric reactions (3)–(5). In the systems

$$[V(\eta^5\text{-}C_5H_5)_2(\eta^2\text{-PhCCPh})] + 2HCl \longrightarrow [V(\eta^5\text{-}C_5H_5)_2Cl_2] + \textit{cis}\text{-PhCHCHPh (85\%)} + \textit{trans}\text{-PhCHCHPh (15\%)} \qquad (3)$$

$$\textit{trans}\text{-}[Mo(\eta^2\text{-RCCH})_2(dppe)_2] + HCl \longrightarrow \textit{trans}\text{-}[Mo(CCH_2R)Cl(dppe)_2] + RCCH \qquad (4)$$

$$\textit{trans}\text{-}[Mo(\eta^2\text{-}C_2H_4)_2(dppe)_2] + 2HCl \longrightarrow \textit{trans}\text{-}[MoCl_2(dppe)_2] + C_2H_4 + C_2H_6 \qquad (5)$$

described by equations (3) and (4) the factors involved in the protonation of co-ordinated alkynes are being probed, sites of protonation and stereospecificity, *etc.*, whereas in equation (5) the factors discriminating between formation of ethane and evolution of ethylene can be defined.

The study on reaction (3)[13] demonstrates that, even when bound to a highly symmetrical metal site, protonation of a symmetrical alkyne can result in predominant formation of the *cis*-alkene. This stereoselectivity is due to initial, rapid protonation at the metal, followed by intramolecular migration of the hydride on to the alkyne thus producing the *cis*-vinyl species. This corresponds to the pathway in the inner circle of Scheme 9. Provided the carbon–carbon double bond is retained, the stereochemistry of the subsequently formed alkene is defined by that of the vinyl species and hence by the initial site of protonation. A small amount of the *trans*-alkene is also produced, presumably as a consequence of the direct, but slow, protonation of the co-ordinated alkyne at the face remote from the metal.

The study on the bis(alkyne) system[14] shown in equation (4) further confirms the initial stages in the protonation of co-ordinated alkyne described above. However, it also illustrates a common reaction pathway for these electron-rich, low-oxidation-state systems, that is preferential protonation of the vinyl species at the remote carbon atom to produce an alkylidene, *trans*-$[Mo(CHCH_2R)Cl(dppe)_2]^+$ (R = alkyl or aryl), which ultimately loses a proton to give the corresponding alkylidyne,

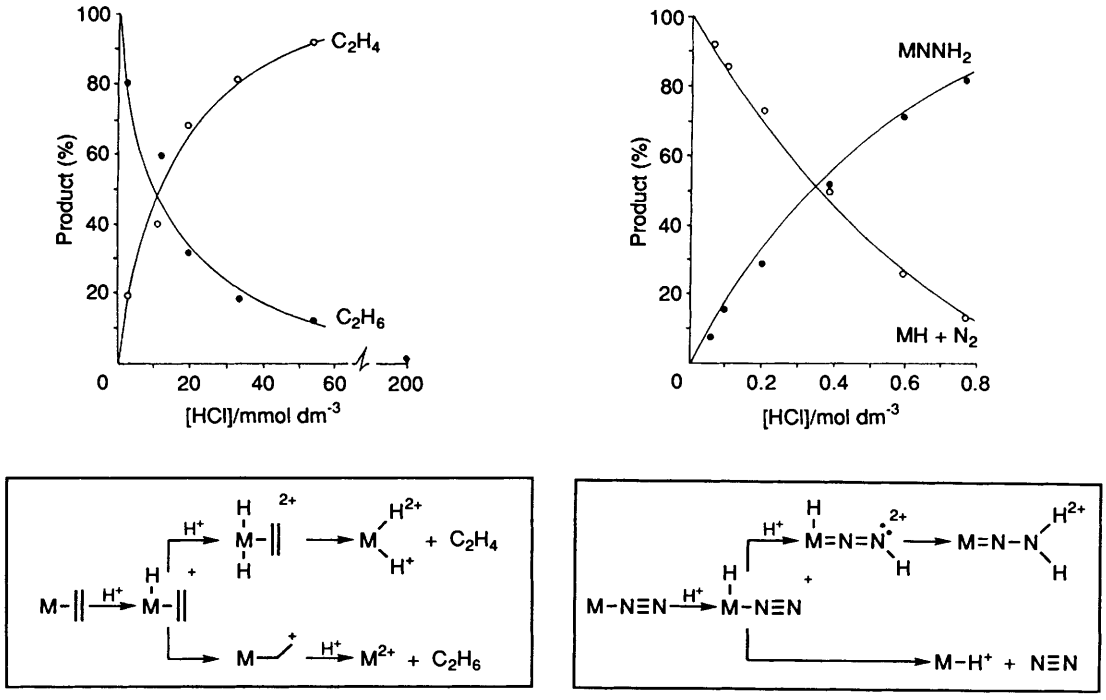


Fig. 1 Left: hydrocarbon product distribution from the protonation of *trans*-[Mo(η^2-C_2H_4)$_2$(dppe)$_2$], showing that ethane is produced preferentially at low acid concentrations whereas ethylene is formed at high concentrations. The curves drawn are those defined by the kinetics and the elementary rate and equilibrium constants derived therefrom. Right: the product distribution from the protonation of *trans*-[Mo(N$_2$)$_2$(Et$_2$PCH$_2$CH$_2$PEt$_2$)$_2$] showing that the hydrazido-complex is produced at high acid concentrations, but dinitrogen is evolved at low concentrations

trans-[Mo(CCH$_2$R)Cl(dppe)$_2$]; the driving force for this reaction is the attainment of the closed-shell, eighteen-electron configuration. This pathway is shown in the bottom right-hand side of Scheme 9.

There are two important points which need clarification. First, protonation of the vinyl species at the remote carbon atom necessarily results in the formation of a carbon–carbon single bond. Rapid free rotation about this single bond is a pathway for the equilibration of the *cis*- and *trans*-vinyl species, resulting in the loss of stereospecificity for the reaction. Secondly, protonation of the remote carbon atom in the vinyl species results in an increase in the formal oxidation state of the metal by two units, whereas protonation of the bound carbon atom, producing the alkene, does not change the formal oxidation state of the metal. Clearly, the stereospecificity exhibited by nitrogenase towards acetylene dictates that the enzyme does not proceed *via* an alkylidene species and it may be that a controlling factor is the ability, or not, of the enzyme site to undergo a change in oxidation state. It is worth mentioning, in this context, that a much poorer stereospecificity is observed in the reaction of nitrogenase with propyne. One explanation of this loss of stereospecificity is that the reaction with propyne occurs in part by this alkylidene pathway.

The study on the reaction shown in equation (5)[15] was able to define the factors which discriminate between the formation of alkane and evolution of alkene by the simple addition of electrons and protons. These pathways are summarised on the left-hand side of Scheme 9. Equation (5) is slightly misleading since this is the stoichiometry only at low concentrations of acid. At higher concentrations the carbon mass balance is retained, but the amount of ethylene increases whilst the amount of ethane decreases proportionately until at very high concentrations of acid only ethylene is produced and the limiting stoichiometry is that shown in equation (6). Detailed

trans-[Mo(η^2-C_2H_4)$_2$(dppe)$_2$] + 2HCl ⟶
trans-[MoH$_2$Cl$_2$(dppe)$_2$] + 2C_2H_4 (6)

mechanistic studies show that at low acid concentrations protonation at the alkene results in the formation of an ethyl complex which is the precursor to ethane, whilst at higher concentrations the slower protonation of the metal becomes important and results in labilisation of the bound ethylene. The hydrocarbon product distribution, as determined by GLC, can be simulated from the values of the elementary rate constants and equilibrium constants determined from the kinetic analysis as shown in Fig. 1. The interesting observation here is the counter-intuitive result that the alkane is produced at low acid concentrations. That is, the hydrocarbon requiring the addition of protons to be formed is produced preferentially at low rather than at the expected high concentrations of acid. For comparison, also shown in Fig. 1 is the intuitively expected behaviour observed for the protonation of dinitrogen in *trans*-[Mo(N$_2$)$_2$(Et$_2$PCH$_2$CH$_2$PEt$_2$)$_2$] by HCl.[16] In this system, initial protonation occurs at the metal to give [MoH(N$_2$)$_2$-(Et$_2$PCH$_2$CH$_2$PEt$_2$)$_2$]$^+$ and at low acid concentrations dinitrogen is released. Diprotonation of the dinitrogen ligand to form the hydrazido(2−)-species [Mo(NNH$_2$)Cl(Et$_2$PCH$_2$CH$_2$P-Et$_2$)$_2$]$^+$ only occurs at high concentrations of acid.

It is the unexpected result of the product distribution of the hydrocarbons outlined above upon which we can build. Protonation of [MoH(η^3-C_3H_5)(dppe)$_2$], at low concentrations of HCl, produces propene[17] as shown in equation (7).

[MoH(η^3-C_3H_5)(dppe)$_2$] + 2HCl ⟶
[MoH$_2$Cl$_2$(dppe)$_2$] + MeCHCH$_2$ (7)

However, at higher concentrations of HCl propyne is formed and the proportion of propene decreases whilst maintaining a constant carbon mass balance. At high concentrations of HCl the limiting stoichiometry is given by equation (8). The

[MoH(η^3-C_3H_5)(dppe)$_2$] + 2HCl ⟶
[MoH$_2$Cl$_2$(dppe)$_2$] + MeCCH + H$_2$ (8)

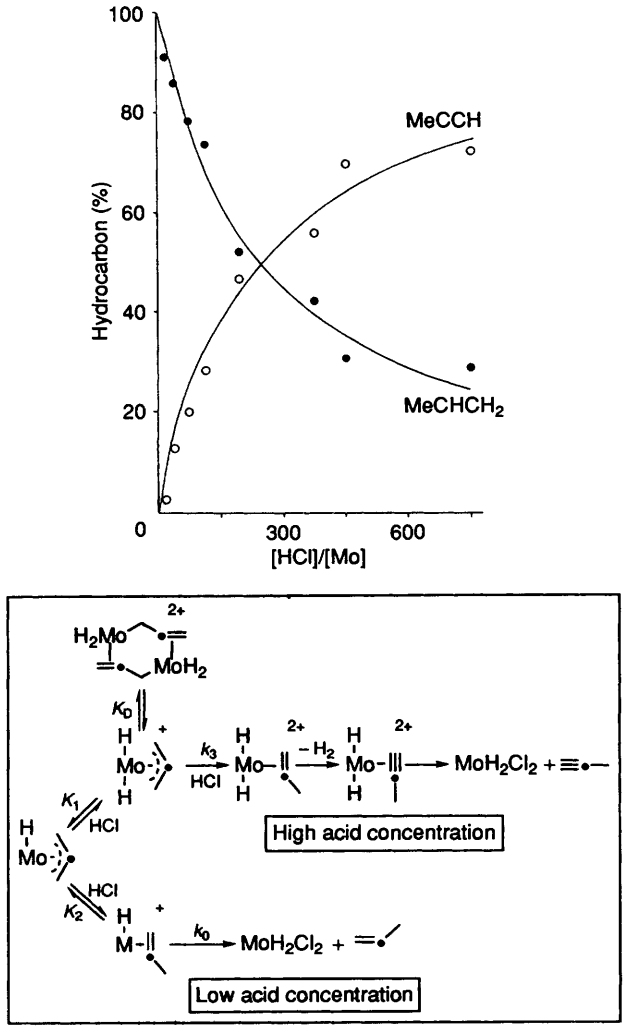
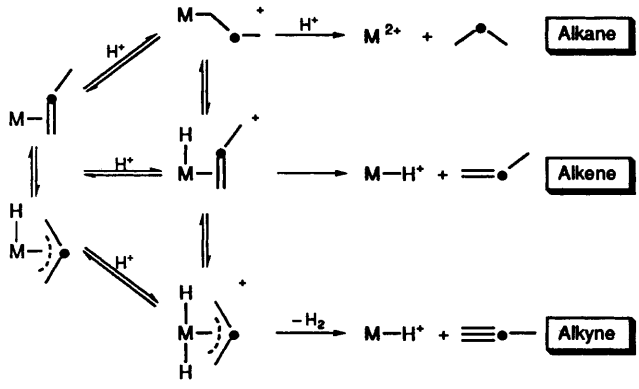


Fig. 2 Top: hydrocarbon product distribution for the protonation of $[MoH(\eta^3\text{-}C_3H_5)(dppe)_2]$ showing the preferential formation of propyne at high acid concentrations, whilst propene is the exclusive product at low concentrations. Curves drawn are those defined by the kinetic analysis and the derived elementary rate and equilibrium constants. Bottom: the mechanism of protonation of $[MoH(\eta^3\text{-}C_3H_5)(dppe)_2]$

hydrocarbon product distribution for the reaction of HCl with $[MoH(\eta^3\text{-}C_3H_5)(dppe)_2]$ over the range of acid is shown in Fig. 2. That the more unsaturated hydrocarbon is produced at higher acid concentrations is reminiscent of the behaviour described above for the ethylene complex, *but now we actually form an alkyne by protonation of an allyl species derived from an alkene complex*!

Detailed mechanistic studies, including low-temperature detection of intermediates by NMR spectroscopy, show that the mechanism for the formation of the two hydrocarbons is as shown at the bottom of Fig. 2. Again the unexpected product distribution observed in the protonation of $[MoH(\eta^3\text{-}C_3H_5)(dppe)_2]$ has its origins in the same effects observed in the reactions of *trans*-$[Mo(\eta^2\text{-}C_2H_4)_2(dppe)_2]$: competitive protonation of hydrocarbon ligand and metal. At low concentrations of HCl rapid protonation of the allyl ligand results in the evolution of propene *via* $[MoH(\eta^2\text{-}MeCHCH_2)(dppe)_2]^+$. In addition, competitive, rapid protonation of the metal produces $[MoH_2(\eta^3\text{-}C_3H_5)(dppe)_2]^+$, which at higher acid concentrations becomes further protonated (probably on the allyl group) to produce $[MoH_2(\eta^2\text{-}MeCHCH_2)(dppe)_2]^{2+}$, and then rapidly loses dihydrogen to produce the co-ordinatively unsaturated, five-co-ordinate, formally fourteen-electron intermediate, $[Mo(\eta^2\text{-}MeCHCH_2)(dppe)_2]^{2+}$ and commits the system to producing propyne. This fourteen-electron intermediate is so unsaturated that it abstracts hydrogen atoms from the co-ordinated alkene thus producing $[MoH_2(\eta^2\text{-}MeCCH)(dppe)_2]^{2+}$, which subsequently releases propyne.



Scheme 10 General scheme showing how the protonation of alkene complexes can give rise to alkane, alkene or alkyne. The reversibility of the initial protonation steps means that the isomerisation of alkenes could also be accomplished by this type of reaction, but to date this has not been observed

This work on the protonation of co-ordinated, unsaturated hydrocarbons is now a long way from studies aimed at understanding the product specificity of the various nitrogenases, but the mechanistic principles established in that study have been exploited to develop a complete picture of how we can control the hydrocarbon released by protonation of an alkene complex as summarised in Scheme 10.

Hydrogenases, Nitrogenases and Dihydrogen Ligands

The hydrogenases are a class of metalloenzymes which perform the reaction shown in equation (9). Hydrogenases *in vivo* are

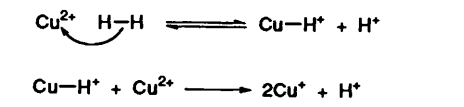
$$H_2 \rightleftharpoons 2H^+ + 2e^- \qquad (9)$$

probably unidirectional, but *in vitro* examples are known which are either uni- or bi-directional.[18] There are three types of hydrogenases distinguished by the elements that they contain: [Fe]-, [Ni-Fe]- and [Ni-Fe-Se]-hydrogenases. The functions of many hydrogenases are still not entirely clear but include: (*i*) delivery of electrons from dihydrogen to the membrane-bound electron-transport chain and (*ii*) to trap dihydrogen evolved in other enzyme reactions, for instance the dihydrogen produced as a by-product of dinitrogen fixation (see below).

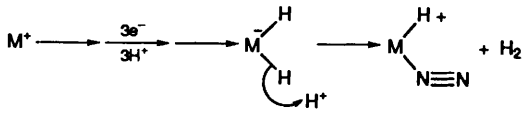
The periplasmic [Fe]-hydrogenases from sulfate-reducing bacteria from *Desulfovibrio* species have been isolated in a purified state. From *Desulfovibrio vulgaris* the hydrogenase consists of two subunits. The α subunit has a molecular weight of *ca.* 46 000 and has three Fe_4S_4 clusters bound through cysteine amino acid side chains. The β subunit is smaller with a molecular weight of *ca.* 9600 and probably has a predominantly electron-transfer function.

The [Ni-Fe]-hydrogenase from *Desulfovibrio gigas* also consists of two subunits. The α one has a molecular weight of *ca.* 56 000–68 000 and it is this which contains the nickel. The smaller β subunit has a molecular weight of *ca.* 28 000–35 000 and has only an electron-transfer function using two Fe_4S_4 clusters. The metals in the α subunit are probably in one Fe_3S_4 cluster and a single nickel atom, which is close to, or possibly even contained within, the cluster. Extended X-ray absorption fine structure (EXAFS) indicates that the co-ordination sphere of the nickel comprises 3 ± 1 light atoms (N or O) and 2 ± 1 S atoms.

Apart from the physiological reactions shown in equation (9), hydrogenases are also capable of catalysing H/D exchange as



Scheme 11 Mechanism for the oxidation of dihydrogen to protons



Scheme 12 Representation of the elementary steps in the binding of dinitrogen at the active site of the molybdenum nitrogenase, involving the displacement of dihydrogen

shown in equation (10) and the equilibration of *ortho*- and *para*-dihydrogen.

$$D_2 + H^+ \rightleftharpoons HD + D^+ \qquad (10)$$

To understand the reactivity of both uni- and bi-directional hydrogenases we must model reaction (9) in both directions independently, and look for suitable chemical precedents in the reactions of simple compounds. Considering first the activation of dihydrogen, three ways in which a metal can facilitate this process have been identified:[19] heterolytic cleavage; homolytic cleavage and oxidative addition. The heterolytic cleavage of dihydrogen is accomplished by a variety of simple metal complexes such as those of Cu^{II}, Ag^{I}, Hg^{I}, Hg^{II}, Pd^{II}, Rh^{III}, Ru^{II} and Ru^{III}. The mechanism of this reaction was established in the 1950s for copper acetate, as shown in Scheme 11, and remains essentially unchanged to this day. The homolytic cleavage of dihydrogen by two molecules of $[Co(CN)_5]^{3-}$ or Ag^{I} has also been identified. A mechanism which, in effect, bridges the forward and reverse reactions of equation (9) is the cleavage of dihydrogen by an oxidative-addition reaction typified by equation (11). Mechanistically, the cleavage of dihydrogen in

$$\textit{trans}\text{-}[IrCl(CO)(PPh_3)_2] + H_2 \longrightarrow \textit{cis, trans}\text{-}[IrH_2Cl(CO)(PPh_3)_2] \qquad (11)$$

this type of reaction must be considered to involve the transient interaction of an intact dihydrogen with the co-ordinatively unsaturated metal centre prior to the cleavage step. However, it was only with the isolation and structural identification of the first dihydrogen complex[20] that it was appreciated that relatively long-lived intermediates containing dihydrogen ligands may well be detected. In the last few years there have been many reports of dihydrogen complexes at a variety of different sites (including hydrogenases and nitrogenases), both as isolable complexes and transient intermediates.

The reactivity of the molybdenum nitrogenase implicates the involvement of an intermediate dihydrogen species at the substrate binding site.[21] In the absence of a substrate, nitrogenase acts as a hydrogenase and evolves dihydrogen by reduction of protons [*i.e.* the reverse of equation (9)]. This evolution of dihydrogen cannot be suppressed entirely, even at extreme pressures of dinitrogen. The limiting stoichiometry of the molybdenum nitrogenase is that described by equation (12).

$$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2 \qquad (12)$$

This obligatory evolution of dihydrogen, together with the observation that dihydrogen is an inhibitor of dinitrogen reduction, led to the proposal that dinitrogen binds to the active site of nitrogenase by displacing dihydrogen. The dihydrogen originates from a hydrido-species, which is formed by protons binding to the reduced active site, as shown in Scheme 12. However, pre-steady-state kinetic analysis and the isotopic composition of the liberated gas obtained from labelling studies on the enzyme indicate that the evolution of the dihydrogen from this hydridic site is catalysed by acid and, in particular, that this protonation step involves direct attack at the hydride ligand, but with no interaction between the proton and the metal.

At the time this mechanism was proposed it was without precedent in simple chemical systems.[4,22] Using the tetrahydride $[MoH_4(dppe)_2]$ it was shown that dinitrogen (and a variety of other small molecules such as nitriles, isocyanides, alkynes, carbon dioxide and sulfur dioxide, hydrogen sulfide, water, azide ion, halide ion and nitric oxide) could be bound rapidly, and in some cases activated, at a hydridic site in a process which is accelerated by acid. The unexpected mechanistic feature in the protonation reactions of $[MoH_4(dppe)_2]$ is that this tetrahydride binds *two protons* before dihydrogen is evolved, as shown in Scheme 13. The intermediate, '$[MoH_6(dppe)_2]^{2+}$', which is the precursor to dihydrogen evolution, must be a dihydrogen species, since otherwise the maximum oxidation state of molybdenum is exceeded.

The question remains, 'how is a dihydrogen ligand formed in the protonation of this and other polyhydridic sites?' Two mechanisms are possible. First, initial protonation of the metal followed by coupling two hydride ligands and secondly, direct protonation of a hydride ligand. Although dihydrogen ligands are formed in the protonation reactions of $[MoH_4(dppe)_2]$ it is not possible to define the intimate mechanism of their formation using this system since there are too many steps to be able to 'home in' on just the dihydrogen ligand formation. In principle it is possible to distinguish between the two limiting mechanisms by determining the relative proportions of HD and H_2 in the reaction of $[MoH_4(dppe)_2]$ with D^+; in the direct protonation pathway HD would be the exclusive gaseous product. However, intramolecular exchange and rapid intermolecular proton exchange (involving both hydrides and phenyl rings of the phosphine ligands) complicates the analysis.

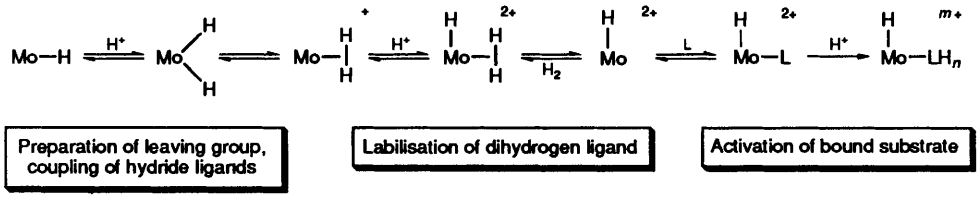
In order to define the intimate mechanism of protonation of hydrido-complexes it is clear that we must move to a simpler system. Stoichiometrically, the simplest possible process that we can study is the single protonation of a hydrido-complex, in which the nett result of the reaction is that a proton is added to the metal as shown in the generalised equation (13). If the

$$[MH_nL_y] + H^+ \longrightarrow [MH_{n+1}L_y]^+ \qquad (13)$$

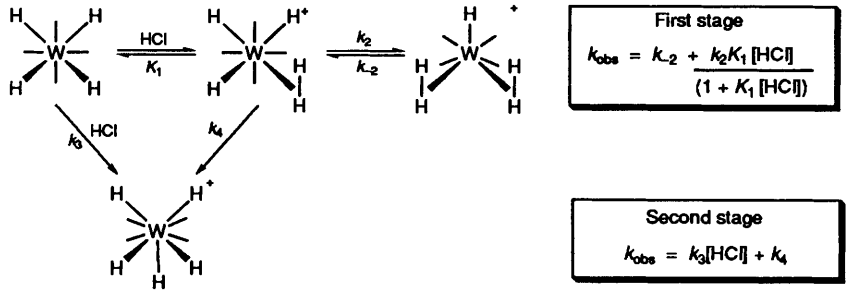
mechanism of this reaction is a simple direct protonation of the metal then the associated kinetics is very simple: a first-order dependence on both the concentration of the acid and the complex. This is the kinetic behaviour observed in the reaction[23] of $[WH_4(dppe)_2]$ with HCl to form $[WH_5(dppe)_2]^+$. However, studies on the analogous reaction of $[WH_4(PMePh_2)_4]$ to form $[WH_5(PMePh_2)_4]^+$ reveal a more complex kinetic behaviour,[24] reflecting a more complicated mechanism as shown in Scheme 14.

The rate law for this reaction demonstrates that there are two pathways by which the metal centre is protonated. The simpler pathway (k_3) involves direct protonation of the metal {as observed for $[WH_4(dppe)_2]$}, but this pathway is slow. The major pathway is associated with a rate law which describes a mechanism involving an initial, rapidly established, equilibrium protonation followed by a relatively slow intramolecular rearrangement step. Owing to the stoichiometric simplicity of the reaction, and that the only potentially protonatable sites on $[WH_4(PMePh_2)_4]$ are the metal and the hydride ligands, we can ascribe the initial protonation step to attack at a hydride ligand to form the dihydrogen species as shown in Scheme 14. This dihydrogen ligand then undergoes an intramolecular oxidative cleavage to generate the $[WH_5(PMePh_2)_4]^+$ product.

This was the first unambiguous demonstration that hydride ligands were susceptible to direct attack by protons, despite much speculation that such mechanisms did operate, not only in nitrogenases and hydrogenases, but also in metal complexes.



Scheme 13 Mechanism for the rapid binding of a variety of small molecules at a polyhydridic site, involving protonation and a dihydrogen ligand intermediate



Scheme 14 Mechanisms and rate equations for the protonation of $[WH_4(PMePh_2)_4]$ to give $[WH_5(PMePh_2)_4]^+$, showing direct attack at the metal and another pathway involving protonation of a hydride ligand. Phosphine ligands omitted for clarity

The success of the approach is due to the use of rapid reaction techniques, in effect following the progress of the reaction as it occurs. The kinetics observed for the direct protonation of a hydride ligand followed by an intramolecular cleavage step is sufficiently diagnostic to be used as a general method for establishing the mechanism of protonation of other hydride complexes provided those complexes contain no other ligands capable of being protonated. In addition, the quantification of the elementary rate and equilibrium constants associated with this pathway allows detailed analysis of the fundamental reactions associated with this type of complex such as the acidity of the dihydrogen ligand, *etc.*

Again we see that the establishment of chemical precedent for the elementary reactions of metalloenzymes can have a more general applicability in chemistry.

Conclusion

I hope to have shown in this Perspective that studying the reaction mechanisms of relatively simple transition-metal complexes can define what are feasible elementary reactions for metalloenzymes. In addition, more importantly, that these studies can lead to an understanding of reactivity beyond that of the biological problem, and even to the development of new areas of chemistry. I have tried to illustrate this with work on three very different metalloenzymes where the impact of the inorganic mechanistic work spreads across the areas of bioinorganic, co-ordination, organometallic and synthetic organic chemistry. Clearly the inorganic reaction mechanist must not become so polarised towards one area of chemistry that the relevance of the results to other, more diverse areas, is overlooked.

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