Coordination Compounds in Biology

The Chemistry of Vitamin B_{12} and Model Compounds

K Hussain Reddy

Vitamin B_{12} is an important coordination compound in biology. It is an interesting biomolecule in the sense that no other vitamin contains a metal ion. This is the only naturally occurring organometallic compound found in biology. An intriguing aspect of vitamin B_{12} is the great stability of the metal-carbon bond. A great deal of new and interesting inorganic chemistry has been uncovered while studying systems pertinent to B_{12}. In this article some salient features of this unique molecule (B_{12}) and its model compounds are presented.

K Hussain Reddy is Reader in Chemistry at Sri Krishnadevaraya University where he has been teaching since 1983. He teaches bioinorganic chemistry and structural inorganic chemistry. His research interests are in the chemistry of model compounds of biochemical relevance. He did post-doctoral research in the University of California at San Diego during 1989–90.

Keywords
Vitamin B_{12}, Pernicious anaemia, mercury poisoning.

Vitamin B_{12} is one of the naturally occurring coordination compounds in biology. Some of the other important examples are chlorophyll, haemoglobin, myoglobin and cytochromes. The common feature in these biomolecules is that a metal ion is enclosed in a macrocyclic ligand. Although vitamin B_{12} is certainly the most complex non-polymeric compound found in nature, it is devoid of protein structure making its biological role relatively easy to understand.

Vitamin B_{12} is known to be present in plants, animals and also in bacteria. It plays an important role in the metabolism of nucleic acids and in protein synthesis. It is of critical importance in the reaction by which residues from carbohydrates, fats and proteins are used to produce energy in living cells. In humans, deficiency of vitamin B_{12} leads to pernicious anaemia. In this article, the structure and reactivity of vitamin B_{12} and its analogues are described. Attempts to model the complex biomolecule using simple coordinating compounds are discussed.

> In humans, deficiency of vitamin B_{12} leads to pernicious anaemia.

Structure of Vitamin B_{12}

The active component of liver extract was first separated and finally

Box 1. Pernicious Anaemia

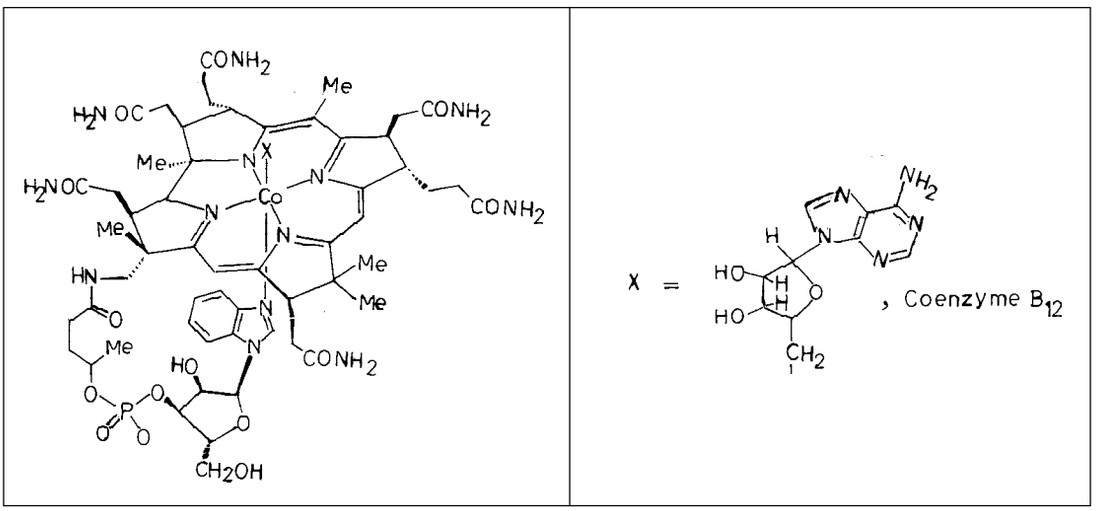
Pernicious anaemia (relatively uncommon in tropical countries) is usually accompanied in mammals by the increased excretion of methylmalonic acid in urine. It occurs mainly between 45–65 years and affects females more frequently than males. One of the first signs of deficiency is the failure to form red blood cells, hence the term anaemia. But the disease is not treated successfully by the methods that work for common iron deficiency, which explains the term 'pernicious' (serious) anemia. Today it is effectively controlled with hydroxocobalamin. It should be given parenterally in a dosage of 1000μg twice during the first week, then 1000 μg weekly for further 6 doses. The patient must have regular parenteral doses of hydroxocobalamin indefinitely (1000 μg intramuscularly every 3 months) for life.

crystallized in 1948. In 1965, Dorothy Hodgkin determined the structure (*Figure* 1) crystallographically[1]. The molecule is an octahedral cobalt (III) complex with a 15-membered 4-nitrogen ring ligand, called corrin, occupying the equatorial plane. All the side chains of corrin are made of acetamide and/or propionamide groups. One of them is an isopropanol phosphate residue attached to a ribose and finally terminated by 5, 6-dimethyl benzimidazole, which binds to the Co(III) ion.

[1] For more on the life and contributions of Dorothy Hodgkin, see *Resonance*, Vol. 3, No. 6, 1998.

Figure 1. Structural glossary of vitamin B_{12} derivatives;
X = CN, Cyanocobalamin;
X = H_2O, Aquocobalamin (B_{12});
X = CH_3, Methylcobalamin;
X = 5′-Deoxyadenosyl, coenzyme B_{12}.

The corrin ring resembles a porphyrin ring at first glance but is not fully conjugated and therefore quite different chemically. The structures of corrin and porphyrin are given in *Figure* 2. Besides the



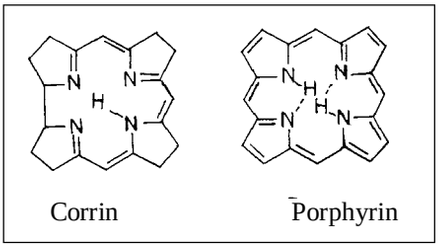


Figure 2. Structural comparison of corrin and porphyrin.

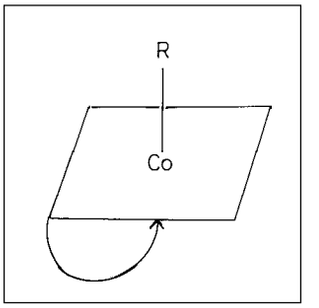
equatorial ligand, there are two axial ligands in most B_{12} derivatives.

Derivatives of B_{12}

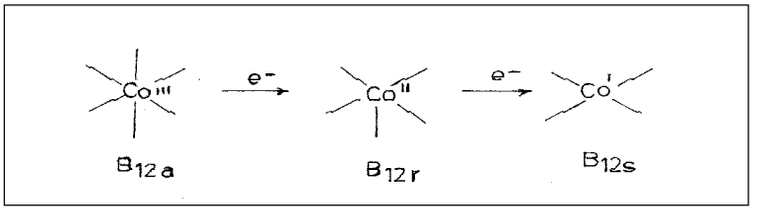
The various derivatives of B_{12} result most commonly from changes in the axial ligands bound to cobalt. Often it is convenient to draw a greatly abbreviated structure for a B_{12} molecule using a rectangle for corrin ring (*Figure* 3) and a curve with arrow at the end to pendant axial base viz. α-5, 6-dimethyl benzimidazole nucleotide, which acts as the ligand from below the basal plane. When this group is the lower ligand, the molecules are usually referred to as cobalamins. Thus, if R = CN^-, the molecule is cyanocobalamin. Cyanocobalamin is also properly called vitamin B_{12}. The presence of cyanide is an artefact of the isolation procedure and is of no biological significance. The cyanide complex is not active as the coenzyme. Other common cobalamins are methylcobalamin (R = CH_3), aquocobalamin (R = H_2O), hydroxycobalamin (R = OH^-), and 5′-deoxyadenosyl cobalamin (R = 5′-deoxyadenosine). This latter compound is also often referred to as coenzyme B_{12}.

Upon hydrolysis the benzimidazole side chain can be cleaved at the phosphate to give a class of derivatives referred to as cobinamides. In cobinamides unless otherwise specified, a molecule of water replaces the benzimidazole as the lower axial ligand. Just as with the cobalamins, the name of cobinamide derivatives depends on the nature of the upper axial ligand, for example, methyl cobinamide, aquocobinamide. In some cases another ligand (usually cyanide) occupies the lower axial position. In such a situation one would refer to the molecule as dicyanocobinamide or aquocyano-cobinamide for R = CN^- or H_2O, respectively.

Figure 3. Abbreviated structure of vitamin B_{12} derivative.



Scheme 1. Oxidation states of vitamin B_{12}.



Finally, Co (III) can be reduced chemically or electrolytically to give derivatives with Co in lower oxidate states. The one electron reduction product is referred to as vitamin B_{12r} and the two electron reduction product is referred to as vitamin B_{12s} (r stands for reduced and s for super-reduced). The oxidation states are given in *Scheme* 1.

The most common reducing agents used to produce B_{12s} include sodium borohydride, zinc dust in ammonium chloride and chromous ions (pH 9-10). It is possible to generate the intermediate B_{12r} species chemically with zinc in acetic acid or ascorbic acid or chromous ions (pH 5).

B_{12r} is a low spin d^7 complex. It contains one unpaired electron (in the $3d_z^2$ orbital) and is the only paramagnetic B_{12} derivative. It is a 5-coordinate complex. The B_{12s} form is a low spin d^8 complex. It is diamagnetic, a potent nucleophile and an important intermediate in the synthesis of alkyl cobalamin derivatives. The coenzyme B_{12} is believed to be formed via nucleophilic attack on adenosine triphosphate (ATP). In the presence of diazomethane, the B_{12s} form is converted to methylcobalamin. Therefore an alkyl radical, a carbocation and a carbanion can all be produced (*in vitro*) in B_{12} chemistry.

The coenzyme B_{12} is believed to be formed via nucleophilic attack on adenosine triphosphate (ATP).

$$\text{R - Co(III)} \rightarrow \text{R} + \text{Co(II)} \quad (B_{12r})$$
$$\text{R - Co(III)} \rightarrow \text{R}^+ + \text{Co(I)} \quad (B_{12s})$$
$$\text{R - Co(III)} \rightarrow \text{R}^- + \text{Co(III)} \quad (B_{12a})$$

In both bacteria and in liver, coenzyme B_{12} is most abundant, but smaller amounts of methylcobalamin also exist. Bacteria present in intestine synthesise these derivatives and supply to our body.

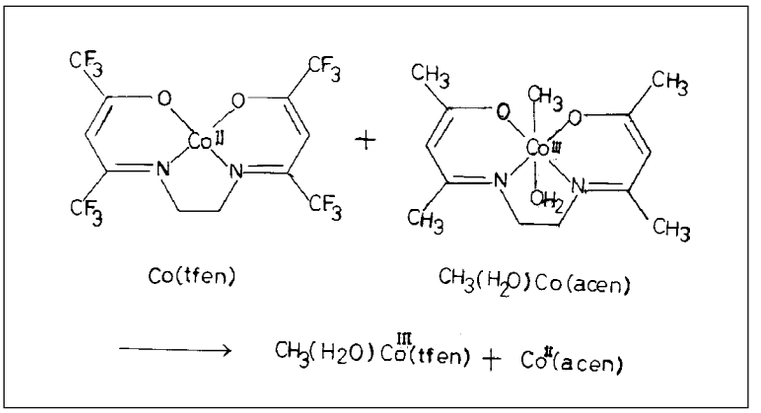
Nature of the Carbon–Cobalt Bond

The successful determination of the molecular structure of coenzyme B_{12} provided an unexpected surprise in that it contained an apparently stable carbon–cobalt bond. This is the only naturally occurring organometallic compound. An intriguing aspect of the B_{12} system is the great stability of the carbon–cobalt bond. Although alkyl–cobalt bond in alkyl cobalamins is surprisingly stable, it can be cleaved and transferred into a number of other chemical species. During the reaction involving model systems in *Scheme* 2, the initial Co(II) species is oxidized to Co(III), while the original alkyl Co(III) species is reduced to Co(II). The initial Co(II) species usually has an equatorial ligand with more electron withdrawing groups. Therefore, this is an organometallic example of electron transfer reaction mediated by group transfer. Some important reactions of methylcobalamin and aquocobalamin are presented in *Scheme* 3.

Biochemical Function (*in vivo* Studies)

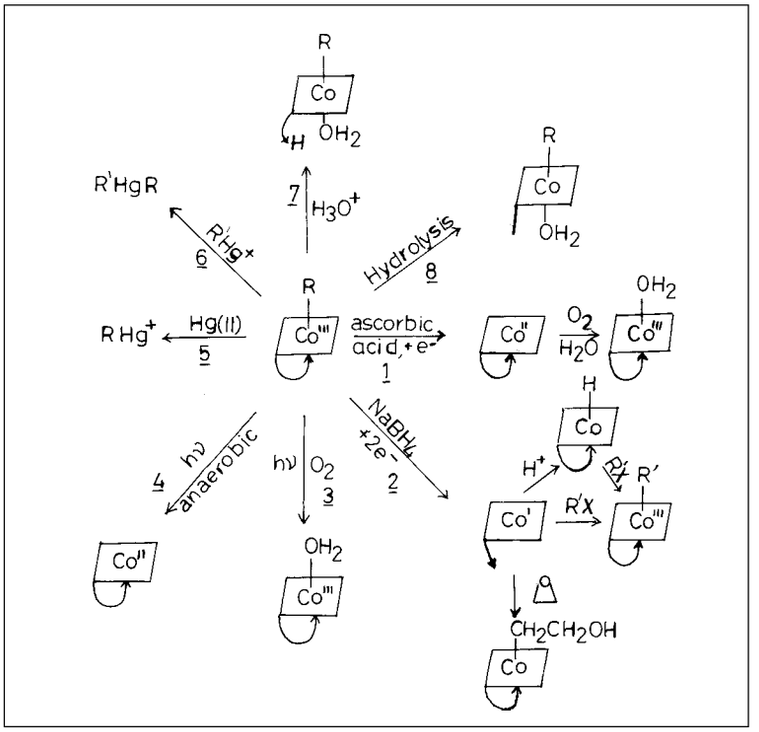
B_{12} functions in biological systems as a coenzyme. That is, it binds to an appropriate nonactive enzyme (the apoenzyme) to form the

Scheme 2. Alkyl transfer reaction:
Co(tfen)=N,N′-ethylene bis(4,4,4-trifluoro-1-methyl-3-oxobutylidene-iminato)cobalt(II) complex.
$CH_3(H_2O)$Co(acen)=N, N′-ethylene bis (1-methyl-3-oxobutylidene-iminato) methyl (aquo) cobalt (III) complex.



Box 2.

Alkyl transfer reactions involving cobalt alkyls have received considerable amount of attention due to a similar alkylation that occurs with mercury. Concern about environmental pollution by mercury virtually originated from the incidence of Minamata disease during 1953–1960 in Japan which resulted in many deaths and babies with genetic defects. Undoubtedly a major factor contributing to the attention focused on this reaction is the current concern about environmental mercury pollution. It has been demonstrated that methylation of mercury in B_{12} rich organisms is responsible for this environmental problem.



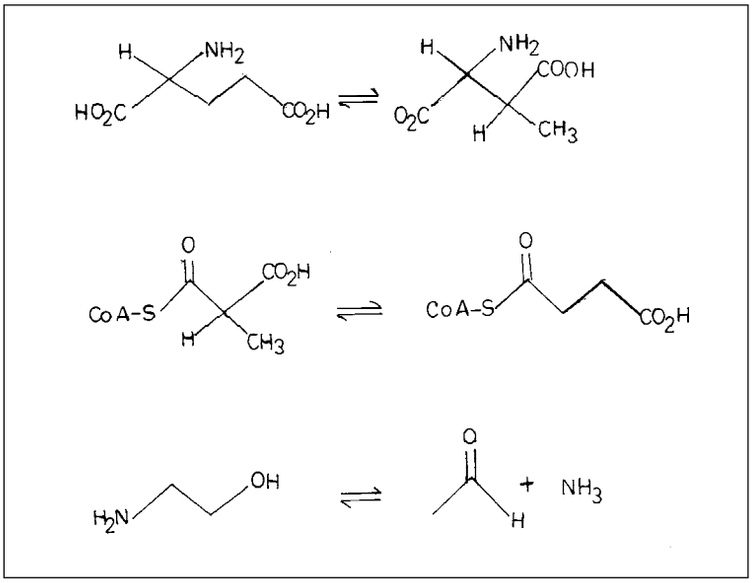
Scheme 3. Reactions of vitamin B_{12} derivatives [in reactions 1 and 2, R = H_2O and in the rest R = CH_3].

biologically active 'enzyme-coenzyme complex'. B_{12} becomes part of the active site of the catalytically functional unit. Generally, B_{12} dependent enzymes are classified into two different categories, namely, (a) those using coenzyme B_{12}(5′-deoxy-adenosyl) as the cofactor and (b) those using methylcobalamin as cofactor.

The enzymes using coenzyme B_{12} as cofactor carry out a catalytic reaction which involves transfer of a hydrogen atom. Therefore these enzymes are sometimes referred to as hydrogen transfer enzymes. The second categories of B_{12} dependent enzymes, which use methylcobalamin as cofactor, are involved in the metabolism of one-carbon fragments.

The enzymes using coenzyme B_{12} as cofactor carry out a catalytic reaction which involves transfer of a hydrogen atom.

The important coenzyme B_{12} dependent enzymes are glutamate mutase, methylmalonyl CoA isomerase, dioldehydrase, ethanolamine ammonia lyase and ribonucleotide reductase. Methionine synthetase, methane synthetase and acetate synthetase depend on the activity of

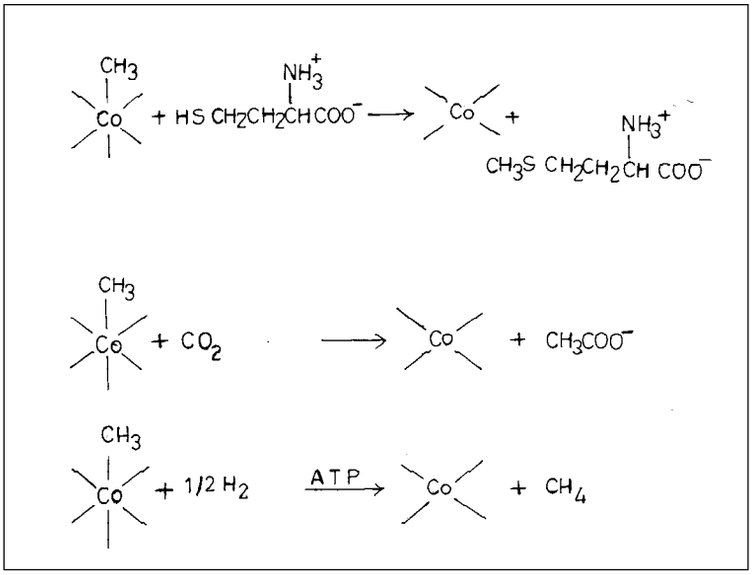


Scheme 4. Different reactions that require co-enzyme B_{12}.

methylcobalamin. Some important reactions are given in *Schemes* 4 and 5.

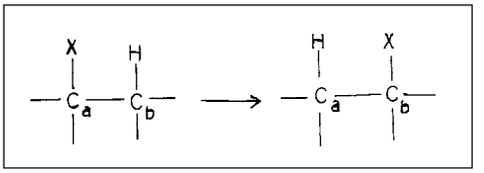
Scheme 6 can be used to demonstrate the type of reaction carried out by hydrogen transfer enzymes in the presence of coenzyme B_{12}.

Here, a hydrogen atom is abstracted from carbon-b of a substrate



Scheme 5. Different reactions in which methyl group is transferred.

Scheme 6. Hydrogen transfer mechanism.



molecule. Some group X is transferred to the carbon atom from which the hydrogen is abstracted and a hydrogen atom replaces the X group at carbon-a. This yields an isomer of the original substrate. Therefore, the reactions carried out by enzymes depend on the activity of coenzyme B_{12} and are known as isomerase reactions. Methylcobalamin transfer methyl groups from one site to the other. Methionine is one of the essential amino acids. The terminal step in the biosynthesis of methionine involves methylation of the sulphur atom of homocysteine. The methylation reaction can be summarized in the following way (*Scheme* 7).

The reactions carried out by enzymes depend on the activity of coenzyme B_{12} and are known as isomerase reactions.

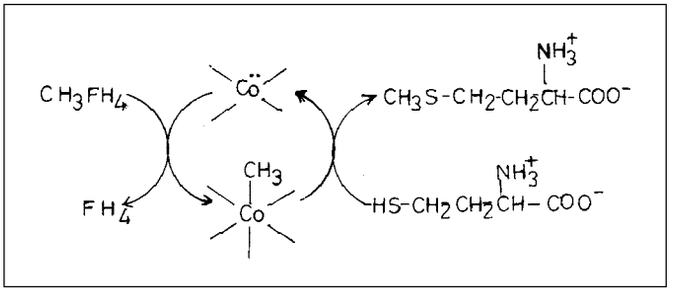
Thus, the reactions catalyzed by B_{12} derivatives are:

(i) The transfer of methyl groups, for example in the formation of methionine from homocysteine and the formation of methyl mercury compounds.

(ii) The rearrangement reactions or isomerase reactions which involve the 1,2-shift, for example, the inter-conversion of succ-inyl coenzyme A and *l*-methyl malonyl coenzyme-A fall in this category.

(iii) The reduction reactions, for example, the reduction of the CHOH- group of ribonucleotide triphosphate to $-CH_2$ group.

Scheme 7. Methyl reaction mechanism.



Model Compounds (*in vitro* Studies)

Study of model compounds involves the design, synthesis, structure determination, physical measurements and reactions of simple coordination compounds. Through studies on model compounds some insight into the workings of the natural system is sought. An additional objective is to mimic in a simple system the catalytic function of a metalloenzyme for industrial or biomedical or synthetic purposes. Current attempts to mimic catalytic properties of vitamin B_{12} by certain cobalt complexes exemplify this aspect.

The organometallic chemistry of cobalt in vitamin B_{12} and its derivatives is unusual and interesting. This may be parallelled quite remarkably in many cases by simple model compounds, some of which are shown in *Figure* 4. The most commonly mentioned B_{12} model system is bis (dimethylglyoximato) cobalt complex. These complexes are often referred to as cobaloximes. The common feature of the different models is that each possesses a very strong equatorial ligand field. Even porphyrins have been used as models for vitamins B_{12}. Cobalt porphyrins can be converted into organic derivatives by demetallation reaction, but they cannot be reduced to

The organometallic chemistry of cobalt in vitamin B_{12} and its derivatives is unusual and interesting.

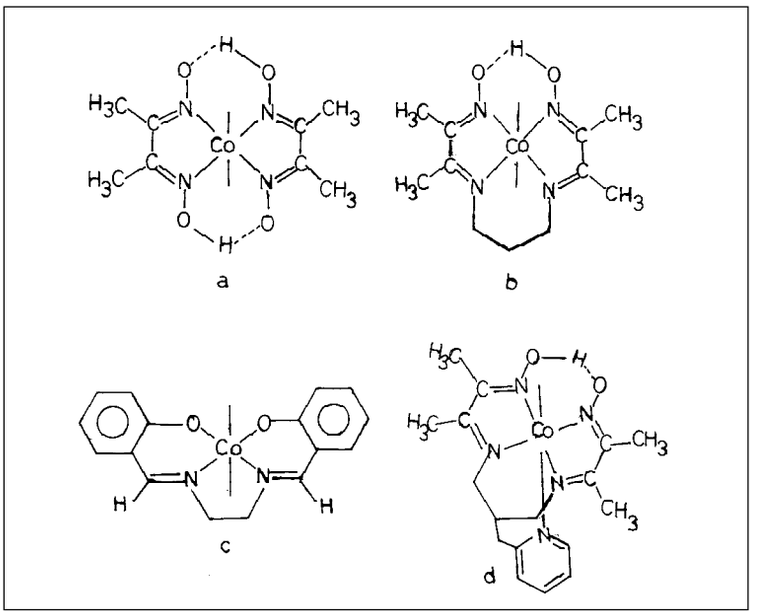


Figure 4. Some important model compounds of vitamin B_{12}.
a)Bis(dimethylglyoxime) cobalt, $Co(dmg)_2$
b)Diacetylmonoximeimino-diacetylmonoximato imino propane-1,3-cobalt, [Co(OO)(DOH)pn]
c) N, N′-ethylene bis (salicylideneiminato) cobalt, Co(Salen)
d) Analogue of (b) with pendant 2-pyridyl methyl group

It appears that the close similarity between cobalamins and cobaloximes is due to the presence of an in-plane ligand of similar strength and is independent of the axial ligands.

the cobalt(I) state in aqueous solution. The reduction is usually carried out with a Grignard reagent in non-aqueous solution.

Similarities between Vitamin B_{12} and Model Compounds

Model compounds, for example, cobaloximes show very many of the reactions of the cobalt atoms in corrins. They too add on axial groups and form stable organo derivatives readily, and also they can be reduced to Co(I) species. The comparison between cobaloximes and B_{12s} has contributed to an understanding of the latter. It appears that the close similarity between cobalamins and cobaloximes is due to the presence of an in-plane ligand of similar strength and is independent of the axial ligands. This is supported by spectroscopic and theoretical studies. The crystal structure of a substituted alkyl cobaloxime shows that the Co-N (in plane) and Co-C bond lengths are very similar to those found for the coenzyme.

Methylcobaloximes (and some other model compounds) will similarly methylate homocysteine, although the reaction is not reversible. However, demethylation is possible, provided it is first converted to the *S*-adenosyl derivative.

Both vitamin B_{12} coenzymes and cobaloximes also catalyse reduction reactions involving the synthesis of *N*-methyl groups from formaldehyde and amines in the presence of a reducing agent.

$$HCHO + NH_2C_6H_5 \rightarrow C_6H_5NHCH_2OH \rightarrow C_6H_5NHCH_3 + H_2O$$

Differences between Vitamin B_{12} and Model Compounds

Although inorganic model complexes exhibit nearly the same coordination chemistry as B_{12} itself, some differences do exist. For example, some of the model compounds can be alkylated simultaneously in both axial positions while B_{12} cannot be alkylated similarly due to the large size of the corrin ligand. The second example is the inability of B_{12} to dimerize while model compounds can exist as dimers. The above differences in behaviour are due to the great deal of steric hinderance in the coenzyme not duplicated by the cobaloximes. This is important in Co-C homolysis, a vital factor for the catalytic behaviour of vitamin B_{12}.

Conclusions

The reactions of model compounds to mimic the functions of vitamin B_{12}, thus far succeeded in matching the chemical properties and structural features of vitamin B_{12}. However, more work remains to be done in reproducing the catalytic functions and on the applications of model compounds to natural systems. The subject continues to be a vibrant topic of research in bio-inorganic chemistry.

Suggested Reading

[1] **R W Hay, *Bioinorganic Chemistry*, Ellis Harwood, Chichester, New York, 1984.**

[2] **M N Hughes, *The Inorganic Chemistry of Biological Process*, John Wiley, Chichester, New York, 1988.**

[3] **J M Pratt, *Inorganic Chemistry of Vitamin B_{12}*, Academic Press, 1972.**

[4] **G N Schrauzer, in *Advances in Chemistry series*, No. 100, Ed. R F Gould, American Chemical Society, Washington DC, 1971.**

[5] **K Hussain Reddy, *Biorganic Chemistry*, New Age International, Delhi, 2003.**

References

[1] **P G Lenhert and D C Hodgkin, *Nature*, Vol.192, 937 , 1961.**

[2] **D C Hodgkin, *Proc. Roy. Soc.* , Vol. A288, 294, 1965.**

[3] **J M Wood, F S Kennedy and C G Rosen, *Nature*, Vol. 220, 173, 1968.**

[4] **G N Schrauzer and J P Lee, *J. Am. Chem. Soc*, Vol.90, 6541, 1968.**

[5] **G N Schrauzer, J P Lee and J W Sibert, *J. Am. Chem. Soc.* Vol. 92, 2997 1970.**

[6] **P G Lanhert, *Chem. Comm*., 980, 1967.**

Address for Correspondence
K Hussain Reddy
Department of Chemistry
Sri Krishnadevaraya University
Anantapur 515 003
Andhra Pradesh, India.