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Nature's design and use of catalysts based on Co and the macrocyclic corrin ligand: 4 × 10^9 years of coordination chemistry

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Abstract. The Co corrinoids (derivatives of vitamin B_{12}) offer an unrivalled opportunity to assess the separate contributions of the metal, the macrocyclic ring and the protein towards enzymatic activity. We summarise current ideas and evidence on: reasons for the selection of Co, the corrin ring and the Ado ligand; the role of the protein; and the possible function of Co corrinoids in the pre–DNA era.

INTRODUCTION

Nature's most famous macrocyclic ligands are the tetrapyrroles which include the Fe porphyrins, Mg chlorophylls, Co corrinoids (B_{12} derivatives) and the Ni–containing F430. They were designed as high–tech materials for catalysing chemical interconversions or converting light into chemical energy. The metalloenzymes represent a partnership between the metal (which provides the active site) + ligand (which together form the co–factor) + protein. A large "menu" of potential reaction mechanisms is offered by the metal ion, a partial selection then made using the steric and electronic properties of the ligand, and the desired reaction fine–tuned by the protein to fulfil the required metabolic function. We focus here on the Co corrinoids which, when compared against the Fe porphyrins as the "standard", pose a series of intriguing questions.

B_{12} or cyanocobalamin is a diamagnetic six–coordinate Co(III) complex with the structure shown in fig. 1. It is the parent of a large family of naturally–occurring Co corrinoids which all share the same complex, crowded and distorted corrin ring with the substituents shown in figs. 1–2, but may differ in the end–groups of the side–chains (carboxylic acid or amide, presence or absence of a nucleotide on side–chain f). Corrinnoids with exactly the same side–chains (including the nucleotide and its base) as B_{12}, but differing only in the nature of the upper (β) ligand, are termed cobalamins (Cbl's); those which lack the nucleotide are called cobinamides (Cbi's). The corrins differ from all other tetrapyrroles in the existence of a direct link between rings A and D, which destroys the planarity of the ring and creates a smaller central hole for the metal, and heavy methylation around the periphery, which fixes the outer ring in its reduced form (H atoms would be readily removed by oxidation). For a general introduction to the chemical, biochemical and medical aspects of B_{12} see refs. 1–3, together with more recent reviews on biosynthesis (ref. 4), electrochemistry (ref. 5) and reactions involving the Co–C bond (ref. 6).

Co is a rare metal in the environment; so why the special need for Co? The biosynthesis of porphyrins is almost ubiquitous and Co porphyrins of the isobacteriochlorin series (but of no known function) are found in certain bacteria (refs. 7,8); so why the need for the much more complex corrin ligand? Perhaps most intriguing of all, the Co corrinoids appear to have become functionally important before the Fe porphyrins and Mg chlorophylls (refs. 4,9), perhaps even before DNA (ref. 10); why were the Co corrinoids so important at such an early stage of evolution? Eschenmoser has pointed out in a thought–provoking review (ref. 11) that certain seemingly unlikely structural features of porphyrins and corrins can be reproduced in vitro, including the ring contraction of a corphinoid (with rings A and D linked by –CMeOH–) to the smaller corrinoid (rings A and D linked directly); this occurs fairly readily when the macrocycle is coordinated to Co or Ni which have smaller ionic radii than Fe. He speculated that some simple corrinoid could have arisen prebiotically and, conversely, that this potential for "self–assembly" of the basic corrin structure accounted for its selection as a co–factor. The latest evidence suggests that photosynthetic organisms

appeared more than 3.8 x 10^9 year ago (ref. 12). We may therefore be dealing with at least 4 x 10^9 years of corrinoid coordination chemistry.

Our group has been particularly interested in understanding the factors which determine the properties of the metal–ligand bond, including the electronic state of the metal ion and the influence of the other ligands (cis– and trans–effects), and how the protein then modifies and controls specific equilibrium or rate constants, with particular reference to the Co corrinoids and Fe porphyrins. After summarising the B_{12}–dependent enzymatic reactions, we discuss in turn current evidence concerning the roles of the Co, the corrin ligand and the protein and possible functions for the Co corrinoids in the earlier stages of evolution.

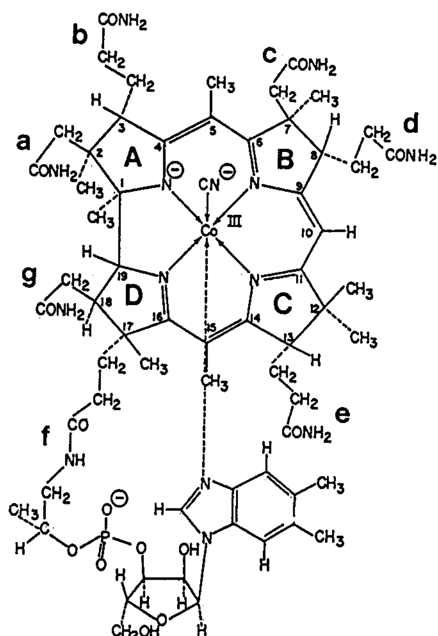


Fig. 1. The molecular structure of vitamin B_{12} (cyanocobalamin) with rings A–D, side–chains a–g and ring carbon atoms 1–19 labelled. The positive charges of the Co(III) ion are balanced by negative charges on the cyanide, corrin ring and phosphate.

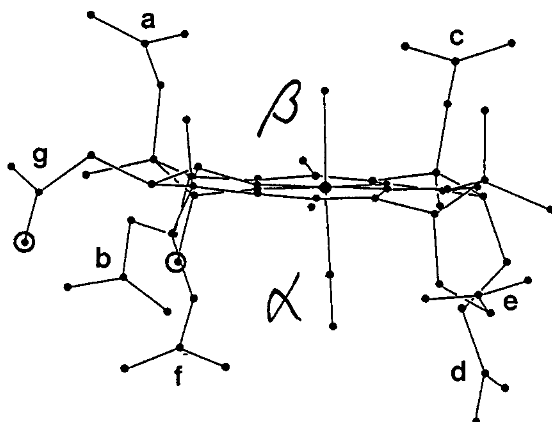


Fig. 2. Projection of the atomic positions in cobyric acid (where side–chain f terminates in $-CO_2^-$ and the α and β ligands are CN^- and H_2O), viewed roughly along the C5–C15 axis. Sides α and β (lower and upper, carrying propionamides and acetamides respectively) and side–chains a–g are labelled, the amide O of side–chain g and the methyl C20 on C1 are circled. Adapted from ref. 54.

B_{12}-DEPENDENT ENZYMATIC REACTIONS

Co corrinoids occur as air–stable six–coordinate Co(III), five–coordinate Co(II) and the highly reactive 4–coordinate, square planar Co(I). They also form alkyl–Co derivatives, which may be either five– or six–coordinate and which can formally be treated as Co(III) + coordinated carbanion R^-, even though the electron density corresponds more closely to a covalent bond between Co(II) and the radical R. The Co–C bond is unusually stable towards both air and water in the dark, but very sensitive to light, and the corrinoids are present in vivo mainly with the simple methyl or the complex deoxyadenosyl (Ado) ligand (see fig. 3). The Co–C bond can readily be formed in vitro by alkylation (e.g. with MeI) of the Co(I) corrinoids, which are some of the most reactive nucleophiles known. All currently known B_{12}–dependent enzymatic reactions fall into two main groups:

(A) The methyl transferases catalyse the transfer of methyl groups from the N atom of tetrahydrofolic acid (or, in certain bacteria, from the O of MeOH) to the S of a thiol such as homocysteine (to form methionine, as occurs in man). These reactions involve the alternate formation of Co(I), formed by an initial reduction of the co–factor, and Co–Me corrinoids. It is also now established that methane can be metabolised anaerobically (refs. 13,14), and we have suggested that the unknown co–factor may be a Co corrinoid (ref. 15).

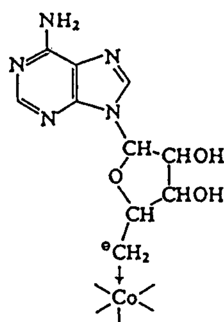


Fig. 3. The 5′–deoxyadenosyl (Ado) Ligand.

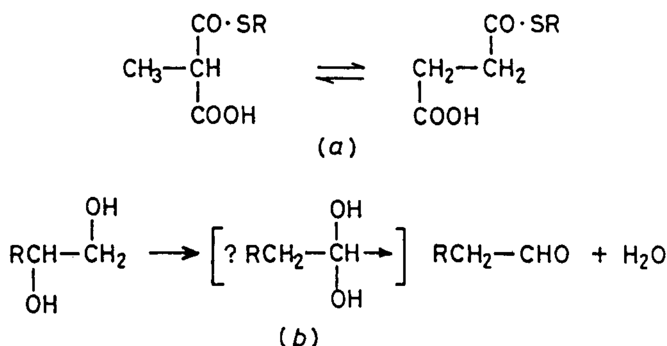


Fig. 4. Examples of B_{12}–dependent enzymatic rearrangements (a) between methylmalonyl and succinyl derivatives (RSH = coenzyme A) and (b) of diols to aldehydes (R = H, CH_3, CH_2OH).

(B) The isomerases or mutases catalyse the isomerisation of various substrates, exemplified by the reversible C–skeleton isomerisation shown in fig. 4a (which occurs in man) and the irreversible rearrangement of diols into aldehydes in fig. 4b; they all involve the interchange of H with C, N or O between neighbouring C atoms without exchange of H with the solvent. The B_{12}–dependent ribonucleotide reductases (RNred's) of anaerobic bacteria form a sub–group which catalyses reduction of the –CHOH– of ribose to $-CH_2-$ of 2–deoxyribose; by analogy with the diol rearrangement, the reaction may involve an initial rearrangement of the –CHOH·CHOH– to $-CH_2\cdot CO-$, followed by reduction to $-CH_2\cdot CHOH-$. All these enzymes require an Ado–Co corrinoid as "coenzyme". It is generally agreed that the first step involves reversible homolytic fission of the Co–C bond according to (1), where R=Ado, to give Co(II) and the free (though still protein–bound) radical. The Ado radical then abstracts an H atom from the substrate, followed by rearrangement of the substrate radical, return of the H atom and re–formation of the Co–C bond.

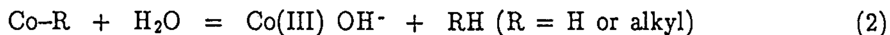
$$\text{Co–R} = \text{Co(II)} + \text{R}\cdot \qquad (1)$$

All these reactions involve the making and breaking of Co–C bonds. The question "Why Co?" becomes "Why should Co form more stable M–C bonds than other available metals?" and is joined by the question "Why the complex Ado ligand?". It seems reasonable to assume that those reactions which depend on the complex Ado ligand represent a later stage of evolution and that an answer to the question "Why corrin?" should be sought among the methyl transferases and not among the isomerases.

ROLE OF THE METAL: WHY Co?

The presence of a Co–C bond in Ado–Cl was established by X–ray analysis in 1961 (ref. 16); it challenged prevailing ideas that such M–C and M–H bonds were inherently unstable towards hydrolysis according to reaction (2) and that, if observed in aqueous solution, this was due merely to kinetic and not thermodynamic stability. Over the next two decades a large number of alkyl–Co(III) complexes with other ligands were prepared, five– as well as six–coordinate, and qualitative comparisons suggested that alkyl– and hydrido–Co(III) complexes, together with the few known alkyl–Cr(III) complexes, were significantly more stable (e.g. towards H_2O) than those of other first–row transition metal ions. A corrinoid with the heavier analogue Rh could be reduced and methylated just like Co (ref. 17), but the Fe corrinoid could only be reduced to Fe(I) and not methylated (ref. 18). The thermodynamic stability of Me– and Ado–Cbl towards reaction (1) was established in 1977 by observation of the very rapid reformation of the Co–C bond after flash photolysis (ref. 19). Subsequent work has provided quantitative data on Co–C bond dissociation enthalpies, which fall with increasing steric hindrance from 37 ± 3 Kcal/mole for Me–Cbl (ref. 20) to ca 20 Kcal/mole for neopentyl– and isopropyl–Cbl (ref. 21); for summaries see refs. 6, 22 and 23. We have established the thermodynamic stability of the Co–C and Co–H bonds towards hydrolysis by determining values of the equilibrium constants $K = [\text{Co–X}]/[\text{Co–OH}_2][X^-]$ (values here given as log K), first for H^- in $[Co(CN)_5H]^{3-} \geq 29$ (ref. 24) and then for the

Cbi's with $X^- = (NC)_2CH^-$ 11.5 (ref. 25), acetylide $HC_2^- \geq 23$ (ref. 26) and probably H^- ca 41 (ref. 27), cf. also CN^- 16.6 (ref. 25).



$$Co\text{–}R + H_2O = Co(III)\ OH^- + RH\ (R = H \text{ or alkyl}) \qquad (2)$$

It has been appreciated since at least 1954 (ref. 28) that d orbitals are too contracted to allow good overlap with the ligand orbitals and that the formation of a partially covalent M–C or M–H bond requires the use of the outer s and p orbitals. In addressing the question "why Co?" in 1982 (ref. 29), we treated M–C bonds as if formed from a C–centred radical and, e.g. Co(II), rather than a carbanion and Co(III). We suggested that for octahedral and square pyramidal complexes of the first row with the more usual ligands the lowest 3d to 4s/4p promotion energies (and hence the best overlap for M–C/H bond formation) would be found for the d^4 Cr(II) and low–spin d^7 Co(II) ions, giving rise to products formally considered as $Cr(III)R^-$ and $Co(III)R^-$ complexes, and that the d^8 Ni(II) might also form reasonably stable M–C bonds. Since Cr(II), not to mention Cr(I), would not readily be accessible with biologically available ligands, Co was nature's choice for organometallic chemistry.

A better insight into the nature of M–H and of M–C bonds and verification of the importance of outer s and p orbitals has come from recent work in a very different field. Studies of the electronic states and bond dissociation energies (BDE's) of binary MH molecules and MH^+ ions and the simple MCH_3 and MCH_3^+ in the gas phase (for summary see ref. 30) have revealed a good inverse correlation between the BDE's and the d–s promotion energies, while calculations suggest that such bonds may have up to ca 75% s character. These studies not only support the approach of trying to rationalise nature's choice of Co in terms of d–s/p promotion energies, but will clearly prompt a fresh look at many aspects of metal–ligand bonding in general.

ROLE OF THE MACROCYCLIC LIGAND: WHY CORRIN?

Effective manipulation of the Co–C bond requires access to low–spin Co(II) and Co(I) complexes, which are more easily formed with conjugated ligands (e.g. porphyrins, phthalocyanines, dimethylglyoxime, Schiff bases, as well as corrin) than with simple amines or heterocycles such as Lys or His. But why corrin rather than porphyrin or a more reduced form such as sirohydrochlorin (see ref. 31 for other porphyrins)? The corrin ring is less readily oxidised or reduced than the porphyrin ring, but does it offer further possibilities for promoting or inhibiting particular reactions? We focus here on one aspect of B_{12} chemistry, viz. the formation of dimers.

NMR studies reported in 1985 showed that corrinoids can form β–face–to–β–face dimers in aqueous solution with dimerisation constants ranging up to 1–2 x 10^4 M^{-1} for Me–Cbl in 2.5 M NaCl (ref. 32). These dimers are involved in the facile transfer of Me from a Me–Co(III) corrinoid to H_2O–Co(III), Co(II) or Co(I) corrinoids (ref. 33–35). Computer modelling indicated that steric hindrance between the side–chains would prevent the Co...Co distance being less than 5.8 Å in the transition state (ref. 35). An earlier X–ray structural determination of an iodide–bridged Co(II)–I^-–Co(II) dimer (refs. 2,36) had revealed a β–to–β structure with a Co...Co distance of 5.65 Å, in striking agreement with the NMR and computer modelling results. Examination of the corrin structure (see figs 1–2) shows that both axial ligand sites are surrounded by a fairly rigid hydrophobic cyclinder of "sentinel" methyl and methylene groups. There are four sentinel groups on the β side (acetamides on C2 and C7, methyls on C12 and C17), separated by four "valleys" (over C5, C10, C15 and C19–C1), but five on the α side (propionamides on C3, C8, C13 and C17, and the C20 methyl circled in fig. 2) separated by three valleys over C5, C10 and C15 and a much narrower valley over C18–C19. The structure of the iodide–bridged dimer shows that the Co–N bonds of the roughly parallel CoN_4 planes are rotated ca 45° to each other such that the 4 sentinel groups of the β face of one molecule fit snugly into the 4 valleys of the β face of the other. The β–to–β structure is clearly preferred; an α–to–α structure would be disfavoured by the additional sentinel group on the α side, but an α–to–β structure might be tolerated. A comparison of all published corrinoid structures shows that it can accommodate a certain degree of folding (2–22°) along the Co–C10 line (ref. 37). It is difficult to escape the conclusion that the nature and disposition of substituents on the corrin ring has evolved to promote the formation of a dimer designed to hold the two Co atoms at a particular distance (ca 5.7 Å in the β–to–β dimer).

Two reactions of Co(II)–Cbl are known which show kinetics not observed for analogous reactions with non–corrinoids and not readily interpreted on the basis of monomers alone; the rate of oxidation of Co(II)–Cbl by O_2 at O° shows a second–order dependence on the concentration of Co(II), but a zero–order dependence on O_2–concentration above 0.4 atm O_2 (ref. 38), while the rate of reaction of Co(II)–Cbl with MeI shows a second–order dependence on Co(II)–concentration and first–order dependence on MeI concentration (ref. 39). These unusual kinetics are now most easily interpreted in terms of an initial reversible formation of the dimer, followed by either a relatively rapid reaction with O_2 or a relatively slow reaction with MeI (ref. 6). They show the potential of the dimer for opening up additional mechanistic pathways.

$$2\,L.M^{II} + RH \rightleftharpoons L.M^{III}H\cdot + L.M^{III}R\cdot \qquad (3)$$

Two reactions of other d^7 Co(II) and Rh(II) complexes with H_2 and CH_4 are of particular interest. First, aqueous solutions of Co(II) cyanide complexes react reversibly with H_2 according to reaction (3) where L = $(CN)_5$ and RH = H_2; this reaction, discovered in 1942 (ref. 40), is probably the fastest known homogeneous reaction of H_2 at room temperature. We have shown that the reaction involves cation–containing dimers such as K_4^+, $[Co(CN)_5]_2^{3-}$ and does not liberate H^+, and have suggested that the cations play an essential role in holding the two Co atoms of the $[Co(CN)_5]^{3-}$ units in the correct relative disposition to facilitate the homolytic fission of H_2 (ref. 41). Secondly, benzene solutions of Rh(II) porphyrins react reversibly with CH_4 at $\geq$ 50° (also with H_2) according to equation (3) where L is the tetramesityl– or tetraxylyl–porphinato ligand and RH = CH_4. This reaction, discovered in 1990 (ref. 42), is one of the few known reactions of CH_4 with metal complexes and occurs under the mildest conditions yet reported; the evidence points to a linear ($Rh\cdot\cdot CH_3\cdot\cdot H\cdot\cdot Rh$) transition state. We have already suggested (ref. 15) that the unknown co–factor for the anaerobic activation of methane by certain bacteria may be a Co corrinoid. Putting together the ready formation of dimeric Co corrinoids with the remarkable activity of other dimeric Co(II) and Rh(II) units towards H_2 and CH_4, we tentatively suggest that the anaerobic activation of CH_4 may involve reaction with a dimeric Co(II) corrinoid according to equation (3) where L = corrin and RH = CH_4. We are currently exploring this type of reaction.

Brief reference should also be made to the mechanism of action of side–chain f with its nucleotide in controlling binding to the protein Intrinsic Factor as the first step in absorption from the gut (ref. 43) and to the possible role of side–chain g, whose amide O atom appears to interact with the C20 methyl (see circled atoms in fig. 2), in modulating the properties of the Co ion and providing an explanation for some extraordinary oscillating reactions of Co corrinoids (refs. 15,44). The corrin ligand with its various side–chains and substituents obviously offers greater scope than the porphyrin for control by the protein, but much remains to be discovered. Other possibilities for modulating the cis–effect of the conjugated ligand include loss of a proton from the allylic C3, C8 or C13 (by distorting side–chain b, d or e towards the equatorial position) or protonation of the conjugated ring itself (by other modes of distortion).

The two key features of the corrin structure are the direct A–D link and the fully reduced and heavily substituted exocyclic ring. Eschenmoser has postulated (ref. 11) that "self–assembly" of the A–D link was a natural consequence of the relatively small ionic radius of the Co ion. We suggest that selective pressure for evolution of the exocyclic ring as we now know it could have been the increased scope offered both for exploiting new mechanistic pathways based on the dimer and for better manipulation of the reactivity of the Co ion through the cis–effect.

ROLE OF THE PROTEIN: HOW TO LABILISE THE Co–C BOND?

Little can yet be said about the role of the protein in the apparently simpler methyl transferases. No–one has yet been able to transfer a methyl group to a protein–free Co(I) corrinoid from MeOH, tetrahydrofolic acid or any other N atom. The protein clearly serves to "activate" the substrate and/or cofactor in some way. Much can be said, by contrast, about the isomerases and the analogous first step in the RNred's.

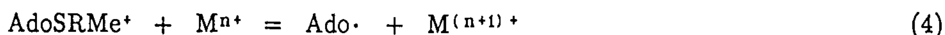
When e.s.r. evidence for homolytic fission of the Co–C bond began to accumulate from around 1970, it became clear that the role of the protein was to labilise the Co–C bond for

use during enzymatic activity. We suggested in 1975 (ref. 45) that the protein used steric distortion of the Co coordination sphere (most probably of the Co–C–C bond–angle), caused by a substrate–induced conformation change, to displace equilibrium (1) to the right; Abeles (ref. 46) and Babior (ref. 47) subsequently emphasised the activation or weakening of the Co–C bond by the protein alone in the absence of substrate. Extensive studies of the steric effects of increasingly bulky alkyl ligands (from Me to Pr^i, cyclohexyl, neopentyl, etc) on Co–C bond dissociation energies and rates of fission, equilibrium constants for coordination of the second ligand, and uv–vis spectra have provided a comprehensive picture of the behaviour of protein–free alkyl– and Ado–corrinoids, against which to assess the behaviour of the protein–bound Ado–corrinoids and pin–point the role of the protein; for summaries see refs. 6 and 48. We now know: that the protein must increase the rate of Co–C bond fission observed in the protein–free form by a massive overall factor of $\geq 10^{11}$ to reach that observed in the enzymatic reaction; that this is achieved in two stages of ca 10^6 each, first when the coenzyme is bound by the protein and then when the substrate is bound; and that, since the increase in the rate of Co–C bond fission cannot be accompanied by a parallel increase in the rate of the reverse reaction which is already diffusion–controlled, this represents a thermodynamic effect (changing the overall equilibrium constant) and not a kinetic effect (increasing both rate constants). Simple homolytic fission at room temperature has been modelled with neopentyl–Cbl(Co–CH_2CMe_3). It is generally agreed that labilisation involves steric distortion around the coordinated C_α atom, but whether by "push" from a distorted corrin (refs. 46,47,49) and/or "pull" from a displaced adenine (refs. 29, 48) must await crystallographic data on the enzymes.

The B_{12}–dependent isomerases are the first family of any metalloenzymes where we can claim to understand the main role of the protein, at least in outline. In contrast to most enzymes described in text–books, the protein in the isomerases (and, by extension, the RNred's) activates the <u>coenzyme</u> (not the substrate) by changing an <u>equilibrium</u> (not a rate) constant and changes it by a colossal factor of $\geq 10^{11}$. They demonstrate that enzymes can indeed exploit carbon–centred radicals, previously considered too reactive to be handled by enzymes.

B_{12} IN EVOLUTION

The replacement of RNA by DNA for carrying the genetic code was probably the key step at or near the base of the whole evolutionary tree as we now know it (except for some viruses). Eschenmoser's discussion (ref. 11) of the synthetic aspects of the corrin structure, including the possibility of "self–assembly", invites a closer look at the possible functions of the Co corrinoids during the pre–DNA era. Work over the last decade has, in fact, altered our perceptions of both the methyl transfer and isomerase groups of reactions. There has been active interest in the metabolism of very primitive anaerobes such as the acetogenic and methanogenic bacteria (see, for example, ref. 50). They share unusual metabolic pathways, exotic co–factors and relatively high concentrations of Co corrinoids. Acetate plays a central role in both energy–production and cell carbon synthesis. The key steps in acetate synthesis (ref. 51) involve Co corrinoids and a Ni/Fe/S–containing carbon monoxide dehydrogenase (CODH); the Co transfers a methyl to the CODH which, in the presence of CO and the thiol coenzyme A (RSH), catalyses the insertion of CO between Me^+ and RS^- to give CH_3COSR (acetyl–CoA). It is difficult to believe that such an apparently essential metabolic reaction and its co–factors were not in place long before the arrival of DNA as an inessential refinement (cf. also ref. 10).



$$AdoSRMe^+ + M^{n+} = Ado\cdot + M^{(n+1)+} \qquad (4)$$

One example each has recently been reported of an isomerase (ref. 52) and of an anaerobic RNred (ref. 53) where the co–factor is not B_{12} but S–adenosyl–methionine or SAM (the sulphonium ion $HOOC.CHNH_2.CH_2.CH_2.SMeAdo^+$, denoted here by $AdoSRMe^+$). It has been suggested (ref. 53) that use of the Ado radical is the common denominator, formed in this case by the generalised redox reaction (4). Such a close parallel in both structure and function between two co–factors is probably unique. This suggests that Ado–Co corrinoids could simply have appropriated pathways pioneered by SAM through use of the common adenosine–binding site which, in turn, could explain the presence of such an extraordinary ligand in the corrinoids. Nature has, in fact, developed, two further classes of RNred based on Mn or Fe, as in the human enzyme, together with a tyrosine radical; these are found only in aerobic organisms. All four classes involve free radicals and are metalloenzymes (ref. 53).

It would appear that one of the key chemical innovations required for the DNA revolution was the controlled application of free radical chemistry (to reduce the ribose ring) and that

this was achieved by a metalloenzyme (either SAM + metal or B_{12}). It remains to be seen whether or not B_{12} replaced SAM as the main co-factor before or after final consolidation of the DNA revolution. It is possible that the more primitive SAM-dependent enzyme has been retained because it avoids dependence on external supplies of Co corrinoids or inorganic Co; E. coli, for example, can switch from an Fe-dependent to a SAM-dependent enzyme under anaerobic conditions (ref. 53).

Present evidence suggests three key reactions where exploitation of the coordination chemistry of Co corrinoids could have made a significant contribution at earlier stages of evolution, viz. (1) synthesis of acetate (for energy-production and cell carbon synthesis), (2) synthesis of deoxyribonucleotides (as building-blocks for DNA), and possibly (3) the anaerobic "fixation" of methane. Co corrinoids were almost certainly involved in (1) before the advent of DNA and may well have been involved in the DNA revolution through (2). The co-factor for (3) is not yet known, but a Co corrinoid seems a reasonable guess; the development of enzymes (with whatever co-factor) to exploit methane as a source of reduced carbon compounds could have made a significant impact on the pace of evolution. In spite of the large gaps in our present knowledge (no X-ray structures of any B_{12}-dependent enzymes, no model for methyl transfer from N or O to Co), the Co corrinoids have already demonstrated how one can separate the different contributions of the metal, the macrocycle and the protein and can usefully speculate about their functions, as well as their synthesis (ref. 11), ca 4 x 10^9 years ago.

REFERENCES

1. D.Dolphin ed., B_{12}, vols. 1-2, Wiley, New York (1982).
2. J.M. Pratt, Inorganic Chemistry of Vitamin B_{12}, Academic Press, London (1972).
3. Z. Schneider and A. Stroinski, Comprehensive B_{12}, de Gruyter, Berlin (1987).
4. A.I. Scott, Pure Appl. Chem., 62, 1269-1276 (1990).
5. D. Lexa and J. Savéant, Acc. Chem. Res., 16, 235-243 (1983).
6. J.M. Pratt, Metal Ions Biol. Systems, 29, 229-286 (1992).
7. J.J.G. Moura, I. Moura, M. Bruschi, J. Le Gall and A.V. Xavier, Biochem. Biophys. Res. Comm., 92, 962-970 (1980); E.C. Hatchikian, Biochem. Biophys. Res. Comm., 103, 521-530 (1981).
8. A.R. Battersby and Z. Sheng, J. Chem. Soc., Chem. Commun., 1393-4 (1982).
9. K. Decker, K. Jungerman and R.K. Thauer, Angew. Chem. Int. Ed., 9, 138-158 (1970).
10. S.A. Benner, A.D. Ellington and A. Tauer, Proc. Natl. Acad. Sci. USA, 86, 7054-7058 (1989).
11. A. Eschenmoser, Angew. Chem. Int. Ed., 27, 5-39 (1988).
12. M. Schidlowski, Nature, 333, 313-318 (1988).
13. R.S. Hanson, Adv. Appl. Microbiol., 26, 3-39 (1980).
14. I.J. Higgins, D.J. Best, R.C. Hammond and D. Scott, Microbiol. Revs., 45, 556-590 (1981).
15. J.M. Pratt, in The Biological Alkylation of Heavy Elements, P.J. Craig and F. Glockling eds, Royal Society of Chemistry, 1988, 46-61.
16. P.G. Lenhert and D.C. Hodgkin, Nature, 192, 937-938 (1961); P.G. Lenhert, Proc. Roy. Soc., A303, 45-84 (1968).
17. V.B. Koppenhagen, B. Elsenhans, F. Wagner and J.J. Pfiffner, J. Biol. Chem., 249, 6532-6540 (1974).
18. R. Bieganowski and W. Friedrich, Z. Naturforsch., 36C, 9-15 (1981).
19. J.F. Endicott and G.J. Ferraudi, J. Am. Chem. Soc., 99, 243-5 (1977); J.F. Endicott and T.L. Netzel, J. Am. Chem. Soc., 101, 4000-4002 (1979).
20. B.D. Martin and R.G. Finke, J. Am. Chem. Soc., 112, 2419-2420 (1990).
21. G.N. Schrauzer and J.H. Grate, J. Am. Chem. Soc., 103, 541-546 (1981).
22. J. Halpern, Polyhedron, 7, 1483-1490 (1988).
23. L. Randaccio, N.B. Pahor, E. Zangrando and L.G. Marzilli, Chem. Soc. Revs., 18, 225-250 (1989).
24. M.B. Mooiman and J.M. Pratt, S. Afr. J. Chem., 35, 171-173 (1982).
25. E.A. Betterton, S.M. Chemaly and J.M. Pratt, J. Chem. Soc., Dalton Trans., 1619-1622 (1983).
26. D.A. Baldwin, E.A. Betterton and J.M. Pratt, J. Chem. Soc., Dalton Trans., 225-229 (1983).
27. S.M. Chemaly and J.M. Pratt, J. Chem. Soc., Dalton Trans., 595-599 (1984).
28. D.P. Craig, A. Maccoll, R.S. Nyholm, L.E. Orgel and L.E. Sutton, J. Chem. Soc., 332-353 (1954).

29. J.M. Pratt, in ref. 1, Vol. 1, 325–392 (1982).
30. P.B. Armentrout and R. Georgiadis, Polyhedron, 7, 1573–1581 (1988); C.A. Tsipis, Coord. Chem. Rev., 108, 163–311 (1991).
31. H.C. Uzar, A.R. Battersby, T.A. Carpenter and F.J. Leeper, J. Chem. Soc. Perkin Trans. I, 1689–1696 (1987).
32. J.J. Pignatello and Y. Fanchiang, J. Chem. Soc. Dalton Trans., 1381–1386 (1985).
33. Y. Fanchiang, G.T. Bratt and H.P.C. Hogenkamp, Proc. Natl. Acad. Sci. USA, 81, 2698–2702 (1984).
34. B. Kräutler, M. Hughes and C. Caderas, Helv. Chim. Acta, 69, 1571–1575 (1986).
35. B. Kräutler, Helv. Chim. Acta, 70, 1268–1278 (1987).
36. J.P. Glusker in ref. 1, Vol. 1, 23–106 (1982).
37. V.B. Pett, M.N. Liebman, P. Murray-Rust, K. Prasad and J.P. Glusker, J. Am. Chem. Soc., 109, 3207–3215 (1987).
38. E.W. Abel, J.M. Pratt, R. Whelan and P.J. Wilkinson, S. Afr. J. Chem., 30, 1–12 (1977).
39. H. Blaser and J. Halpern, J. Am. Chem. Soc., 102, 1684–1689 (1980).
40. M. Iguchi, J. Chem. Soc. Jpn., 63, 634–643 (1942); CA, 41, 2975d (1947).
41. M.B. Mooiman and J.M. Pratt, J. Mol. Cat., 27, 367–371 (1984).
42. A.E. Sherry and B.B. Wayland, J. Am. Chem. Soc., 112, 1259–1261 (1990); B.B. Wayland, S. Ba and A.E. Sherry, J. Am. Chem. Soc., 113, 5305–5311 (1991).
43. E.R. Andrews, J.M. Pratt and K.L. Brown, FEBS Lett., 281, 90–92 (1991).
44. S.M. Chemaly, R.A. Hasty and J.M. Pratt, J. Chem. Soc., Dalton Trans., 2223–2227 (1983).
45. J.M. Pratt in Techniques and Topics in Bioinorganic Chemistry, C.A. McAuliffe ed., Macmillan, London, 1975, 109–204.
46. R.H. Abeles and D. Dolphin, Acc. Chem. Res., 9, 114–120 (1976).
47. J.S. Krouwer, B. Holmquist, R.S. Kipnes and B.M. Babior, Biochem. Biophys. Acta, 612, 153–159 (1980).
48. J.M. Pratt, Chem. Soc. Revs., 14, 161–170 (1985).
49. J.H. Grate and G.N. Schrauzer, J. Am. Chem. Soc., 101, 4601–4611 (1979).
50. J.G. Zeikus, R. Kerby and J.A. Krzycki, Science, 227, 1167–1173 (1985).
51. W. Lu, S.R. Harder and S.W. Ragsdale, J. Biol. Chem., 265, 3124–3133 (1990).
52. J. Baraniak, M.L. Moss and P.A. Frey, J. Biol. Chem., 264, 1357–1360 (1989).
53. R. Eliasson, M. Fontecave, H. Jörnvall, M. Krook, E. Pontis and P. Reichard, Proc. Natl. Acad. Sci. USA, 87, 3314–3318 (1990).
54. D.C. Hodgkin, Proc. Roy. Soc., A288, 294–305 (1965).