The B_{12}-dependent Isomerase Enzymes; How the Protein Controls the Active Site

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1 Introduction

Metalloenzymes and metalloproteins can be considered as a partnership between the metal ion or complex, which provides the active site, and the protein, which enhances and controls the activity of the metal through, for example, changes in conformation which are somehow coupled to changes on the metal. The role of the protein and the mechanism of coupling are now understood in the case of the non-enzymatic haemoglobins; see the review by Perutz.[1] The aim of this article is to summarize present knowledge concerning the B_{12}-dependent isomerases, which are the first family of redox-active metalloenzymes where we can probably claim to understand the role of the protein and, at least in outline, the mechanism of coupling; the peroxidases may provide the second such family, together with an instructive contrast in the mechanism of coupling (see below). Good sources of background information are available on the B_{12}-dependent enzymes[2] and on the co-ordination chemistry,[3] redox potentials,[4] and organometallic chemistry[2,3] of the B_{12} (Co corrinoid) complexes.

2 The B_{12} Coenzymes and the B_{12}-dependent Isomerase Enzymes

The structure of vitamin B_{12} (cyanocobalamin) was determined by Hodgkin and co-workers;[5,6] the molecular structure is shown in Figure 1. It is a diamagnetic, six-co-ordinate complex containing the d^6 Co^{III} ion. The naturally occurring Co corrinoids comprise a family of complexes which all possess the same conjugated 'corrin' ring as in B_{12}, but may differ in the nature of the side-chains and nucleotide base as well as other axial ligands; those that possess the same side-chains as B_{12} itself are called 'cobalamins'. The B_{12}-dependent isomerases catalyse the isomerization of various organic substrates, as exemplified by the reversible C-skeleton rearrangement shown in Figure 2*a* (one of the B_{12}-dependent reactions occurring in man) and the irreversible rearrangement of diols to aldehydes shown

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[1] M. F. Perutz, *Ann. Rev. Biochem.*, 1979, **48**, 327.
[2] 'B_{12}' (2 vols.), ed. D. Dolphin, Wiley, New York, 1982.
[3] J. M. Pratt, 'Inorganic Chemistry of Vitamin B_{12}', Academic Press, London, 1972.
[4] D. Lexa and J. Savéant, *Acc. Chem. Res.*, 1983, **16**, 235.
[5] D. C. Hodgkin, J. Lindsey, R. A. Sparks, K. N. Trueblood, and J. G. White, *Proc. R. Soc. London, Ser. A.*, 1962, **266**, 494.
[6] C. Brink-Shoemaker, D. W. J. Cruickshank, D. C. Hodgkin, M. J. Kamper, and D. Pilling, *Proc. R. Soc. London, Ser. A*, 1964, **278**, 1.

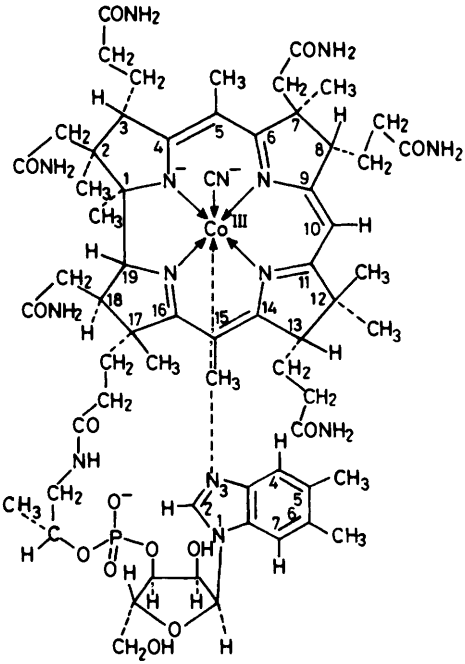

Figure 1 *Molecular structure of* B_{12} (Reproduced by permission from *ref.* 3)

in Figure 2*b*. All these enzymatic reactions involve the interchange of H with C, N, or O between neighbouring atoms; each enzyme is reasonably, but not completely, specific for one substrate. The corrinoid is present in a so-called 'coenzyme' form, in which the upper (β) axial co-ordination site is occupied by the extraordinary ligand 5′-deoxyadenosyl (hereafter denoted by **R**) which is shown in Figure 3, while the lower (α) ligand may be the heterocyclic base (denoted by B) present in the cobalamins or another base, or may even be absent (to leave a five-co-ordinate Co-atom). The structure of the red six-co-ordinate cobalamin coenzyme has been determined by Hodgkin and Lenhert;[7] the atomic positions are shown in Figure 4. Evidence that the yellow alkyl-corrinoids (including the acidified form of the coenzyme) are five-co-ordinate is indirect,[8] but supported by the very high rate of reaction ($k_2 \geqslant 10^8$ dm^3 mol^{-1} s^{-1}) of methylcobinamide with cyanide.[9] Both the normal red and acidified yellow forms of the coenzyme are diamagnetic[10] and can formally be regarded as complexes of Co^{III} with a carbanion. Other families of B_{12}-dependent enzymes are known; here we consider only the isomerases. The

[7] (*a*) P. G. Lenhert and D. C. Hodgkin, *Nature*, 1961, **192**, 937; (*b*) D. C. Hodgkin, *Proc. R. Soc. London, Ser. A*, 1965, **288**, 294; (*c*) P. G. Lenhert, *Proc. R. Soc. London, Ser. A.*, 1968, **303**, 45.
[8] J. M. Pratt, in *ref.* 2., Vol. 1, p. 325.
[9] D. A. Baldwin, E. A. Betterton, and J. M. Pratt, *S. Afr. J. Chem.*, 1982, **35**, 173.
[10] J. A. Hill, J. M. Pratt, and R. J. P. Williams, *J. Chem. Soc.*, 1964, 5149.

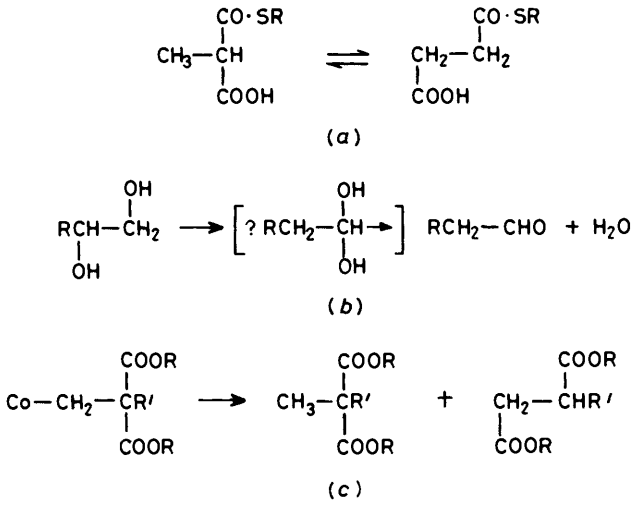

Figure 2 *Examples of B_{12}-dependent enzymatic rearrangements,* (a) (*RSH = coenzyme A*) *and* (b) (R = H, Me, $HOCH_2$), *and protein-free model reactions,* (c) (R = Me, Et; R′ = H, Me)

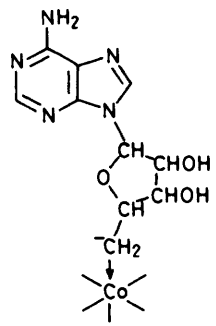

Figure 3 *The 5′-deoxyadenosyl ligand present in the* B_{12} *coenzymes* (Reproduced by permission from *ref.* 3)

mammalian enzymes accept only the cobalamin coenzyme, although a range of coenzymes is found in bacterial enzymes; here we discuss only the cobalamin coenzyme.

The cobalamin coenzyme is bound by the apoenzyme (protein) with no significant change in its absorption spectrum; this suggests that no change has occurred in the axial ligands, although the possibility that B is replaced by another nitrogenous base (lysine, histidine) cannot be rigorously excluded. The cobalamin coenzyme is, therefore, the only metallo-coenzyme (excluding other B_{12} coenzymes) whose chemistry can be directly compared in the presence and absence

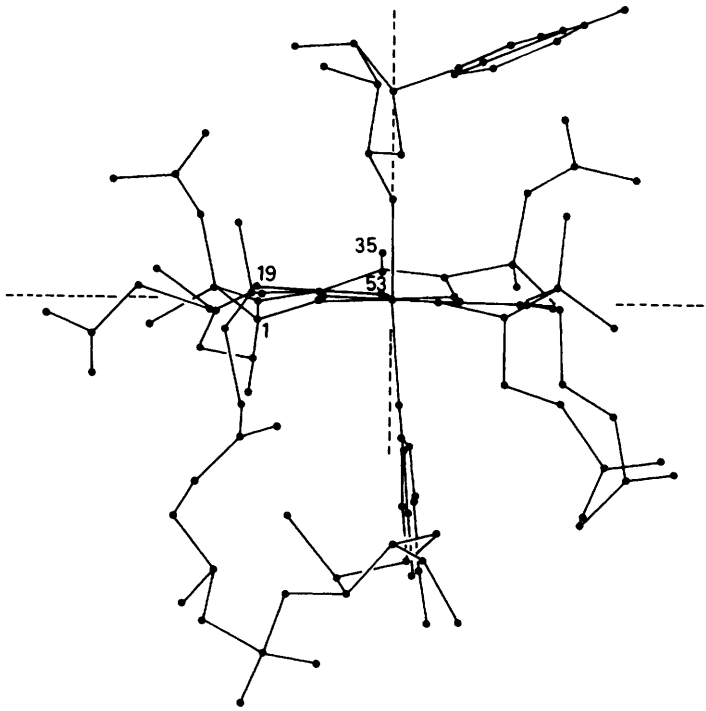

Figure 4 *Structure of the cobalamin coenzyme* (Reproduced by permission from *ref.* 7*b*)

of protein; in addition, the physical properties of the Co corrinoids (especially their u.v.–visible spectra) provide an excellent probe for following changes at the active site during the enzymatic reaction. The enzymes require no additional cofactors for the C-skeleton rearrangements and only an alkali-metal cation for the diols; their reactions involve only the single substrate. In spite of the lack of any *X*-ray data on these enzymes, they provide the ideal opportunity for investigating the basis of the partnership between a metal complex and its protein.

3 The Main Steps in the Enzymatic Reaction

It is now generally agreed,[2] though not proven (see below), that the first step involves homolytic fission of the Co–C bond according to reaction 1 (axial ligands only given) and that the (protein-bound) radical **R** derived from the ligand then abstracts a hydrogen atom from the substrate (SH) according to reaction 2. The Co^{II} complex (B_{12r}) is assumed to be five-co-ordinate[3] and this is supported by the very high rate of reaction with methyl radicals to form the Co–Me complex;[11] but

[11] J. F. Endicott and G. J. Ferraudi, *J. Am. Chem. Soc.*, 1977, **99**, 243.

an *X*-ray determination of the structure would be desirable. There is, however, no agreement on the nature of the subsequent steps, *i.e.* on the form in which the substrate undergoes rearrangement; candidates include radicals, fully or partially formed carbocations (by electron transfer to Co^{II}), and organo-cobalt derivatives.[2] The product is formed, and the coenzyme re-formed, by the reverse of these steps.

$$\mathbf{B\text{–}Co\text{–}R} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \mathbf{B\text{–}Co^{II}} + \mathbf{R}\cdot \qquad (1)$$

$$\mathbf{R}\cdot + \mathbf{SH} \rightleftharpoons \mathbf{RH} + \mathbf{S}\cdot \qquad (2)$$

In the absence of protein the Co–C bond of the coenzyme is surprisingly stable in neutral aqueous solution, although decomposed by light, by heating in acid, and by reagents such as cyanide; we shall see below that in the presence of protein and substrate the Co–C bond can be labilized by a factor of $\geqslant 10^{11}$! The abstraction of a hydrogen atom according to reaction 2 is, of course, well known but C-skeleton rearrangements of the type shown in Figure 2*a* were unprecedented in organic chemistry until 1975 when they were modelled (see Figure 2*c*) with various protein-free alkyl-cobalt complexes (cobalamins, cobaloximes), in which the radical was generated by photolysis or thermolysis of the Co–C bond;[12–13] it was subsequently shown that the radical does not undergo rearrangement except in the presence of the Co^{II} ion.[14] The protein therefore plays a major role in step (1) but a relatively minor role in the subsequent steps, perhaps confined to preventing the various species from diffusing away from each other.

This review focuses exclusively on step (1) of the enzymatic reaction, where the main theme is the use (by the protein) of steric distortion to labilize the Co–C bond. It should be mentioned that many groups throughout the world are interested in mechanistic studies on the B_{12}-dependent isomerases and relevant protein-free models (*e.g.* those of B. T. Golding, H. A. O. Hill, A. W. Johnson, M. D. Johnson, and R. J. P. Williams in the U.K., Arigoni in Switzerland, Rétey in Germany, Yurkevich in Russia, Fukui and Toraya in Japan, Dolphin in Canada, Abeles, Babior, Barker, Halpern, Hogenkamp, Marzilli, Schrauzer and Stadtman in the U.S.), but that most of this work is aimed at unravelling the nature of the steps *after* fission of the Co-C bond; reference 2 provides a good introduction to this area.

The greater instability of the Co–C bond to a secondary, compared to a primary, alkyl ligand was first noted (with Co–Pr^i) in 1968.[15] This led to the suggestion that the enzyme might distort the coenzyme such that the state of the Co–C bond approached that of the transition state for heterolytic fission to give the carbonium ion.[16] The first e.s.r. evidence for homolytic fission of the Co–C bond in the

[12] P. Dowd, M. Shapiro, and K. Kang, *J. Am. Chem. Soc.*, 1975, **97**, 4754.
[13] G. Bidlingmaier, H. Flohr, U. M. Kempe, T. Krebs, and J. Rétey, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 822.
[14] J. Rétey, in 'Vitamin B_{12}', ed. B. Zagalak and W. Friedrich, de Gruyter, Berlin, 1979, p. 439.
[15] R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 1968, 2419.
[16] H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *Chem. Brit.*, 1969, **5**, 156.

isomerases and related ribonucleotide reductases was reported in 1969—1971,[17-19] and it was suggested that the protein somehow served to stabilize Co^{II} and the organic radical.[19] It was later suggested more specifically that the protein used steric distortion of the cobalt co-ordination sphere (most probably of the Co–C–C bond angle), caused by a substrate-induced change in conformation, to displace equilibrium (1) to the right.[20] Others have stressed distortion of the corrin ring,[21] which may lead to pressure on the adenine group of the ligand[22] or on the C-atoms closer to the cobalt.[23] Systematic studies over the last five years have provided a fairly comprehensive picture of steric effects in protein-free alkyl-corrinoids, against which one can assess the behaviour of the protein-bound coenzyme.

4 Steric Distortion in Protein-free Alkyl-corrinoids

The effects of increasing distortion around the co-ordinated carbon atom (C_α) have been studied systematically[23-25] by varying the alkyl ligand in the series $-CH_3$ to $-CHMe_2$ (the $Co-CMe_3$ complex is too unstable to study), $-CH_2CH_3$ to $-CH_2CMe_3$, and cyclopropyl to cyclohexyl (here denoted by C_3–C_6) with particular reference to equilibrium (1) and to the equilibrium between the red six-co-ordinate alkylcobalamin and its yellow five-co-ordinate form (with B remaining unco-ordinated at the end of the side-chain). Neopentylcobalamin ($B–Co–CH_2CMe_3$) undergoes reversible homolytic fission at room temperature and provides an unambiguous protein-free model for homolytic fission in the coenzyme.[23,25c] Alkyl-corrinoids with H on the C_β-atom usually decompose by overall β-elimination (to give Co^I and an olefin),[8,23] but recent evidence indicates that β-elimination occurs *via* an initial homolytic fission to give the caged (Co^{II} + radical) pair.[26]

The results form a very simple pattern, in which all three series of alkyl ligands can be placed in virtually a single order of increasing steric compression, *viz.* Me ~ C_3 ~ **R** (the ligand in the coenzymes) < Et ~ Pr^n < Bu^i ~ C_4 < Pr^i, neopentyl, C_5, C_6.[8,26] If we start with methyl-cobalamin and increase the degree of steric distortion around C_α, then we observe an increase in the lability of the Co–C bond in the six-co-ordinate form and an increasing stability of the five- over the six-co-ordinate form. If we next study the five-co-ordinate forms alone (by protonating B in the cobalamins or by removing the side-chain to form the 'cobinamides'), we find a parallel increase in lability with increasing distortion, although the absolute rates are lower (by about 10^3) than in the six-co-ordinate forms; in spite of increasing conversion into the more inert five-co-ordinate form, the net result of

[17] B. M. Babior and D. C. Gould, *Biochem. Biophys. Res. Commun.*, 1969, **34**, 441.
[18] J. A. Hamilton and R. L. Blakley, *Biochim. Biophys. Acta*, 1969, **184**, 224.
[19] M. A. Foster, H. A. O. Hill, and R. J. P. Williams, *Biochem. Soc. Symp.*, 1971, **31**, 187.
[20] J. M. Pratt, in 'Techniques and Topics in Bioinorganic Chemistry', ed. C. A. McAuliffe, Macmillan, London, 1975, p. 109.
[21] R. H. Abeles and D. Dolphin, *Acc. Chem. Res.*, 1976, **9**, 114.
[22] J. S. Krouwer, B. Holmquist, R. S. Kipnes, and B. M. Babior, *Biochim. Biophys. Acta*, 1980, **612**, 153.
[23] J. H. Grate and G. N. Schrauzer, *J. Am. Chem. Soc.*, 1979, **101**, 4601.
[24] G. N. Schrauzer and J. H. Grate, *J. Am. Chem. Soc.*, 1981, **103**, 541.
[25] S. M. Chemaly and J. M. Pratt, *J. Chem. Soc., Dalton Trans.*, 1980, (*a*) 2259, (*b*) 2267, (*c*) 2274.
[26] J. M. Pratt, *J. Mol. Catal.*, 1984, **23**, 187.

increasing distortion in the cobalamins is always a marked increase in lability, *e.g.* by a factor of $\geqslant 10^7$ on replacing –Et ($t_{\frac{1}{2}} \sim 6$ months) by $-CHEt_2$ ($t_{\frac{1}{2}} \sim 3$ s).[23] It has been shown[27] that at 95 °C, $t_{\frac{1}{2}}$ for the coenzyme (180 min) is greater than that for ethylcobalamin (9 min), as expected from the steric order; by assuming that the ratio of rates is not too temperature-sensitive, one can derive a 'working' $t_{\frac{1}{2}}$ of *ca.* 10 years for the coenzyme at room temperature. Increasing distortion also produces a regular change in the spectrum of the five-co-ordinate forms, such that the maximum shifts from 458 nm in the five-co-ordinate form of the coenzyme to ~440nm in the strained complexes;[8] the spectrum serves as a molecular strain-gauge! Finally, we can use the alkyl-cobinamides to study the co-ordination of imidazole and show that the same pattern (of equilibria and Co–C bond lability) is observed, irrespective of whether the heterocyclic base is imidazole or the much bulkier ligand B present in the cobalamin side-chain.[8,26] These studies on alkyl-corrinoids in solution are complemented by structural determinations of various alkyl-cobaloximes of formula $[R–Co(dmgH)_2py]$, where $dmgH^-$ is the dimethylglyoximate monoanion, which reveal remarkable distortions; *cf.* the lengthening of the Co–C bond from 1.998 (R = Me) to 2.085 Å (R = Pr^i) and the surprisingly large 'tetrahedral' Co–C–C bond-angle of 130° when R = $-CH_2CMe_3$.[28]

5 Co–C Bond Fission in the Protein-bound Coenzyme

The main evidence relating to the first step in the enzymatic reaction can be summarized as follows. (*i*) The use of coenzyme analogues has shown that none of the O-atoms in the ribose moiety are essential for activity,[29–32] *i.e.* one can at this stage treat the ligand as a simple alkyl ligand. (*ii*) The u.v.—visible spectrum of the enzyme in the resting state (max. *ca.* 520 nm) is virtually identical to that of the protein-free coenzyme, but the addition of substrate causes partial and reversible conversion into Co^{II} (max. *ca.* 475 nm), probably with the base B still co-ordinated,[19] and often the appearance of an anomalous band at *ca.* 440 nm.[19,33–36] Since protein-free corrinoids do not exhibit a prominent band *ca.* 440 nm either in aqueous solution or in organic solvents such as CH_2Cl_2,[37] this probably indicates the presence of some additional species. (*iii*) Several isomerases show an unusual and characteristic e.s.r. signal in the presence of substrate (*i.e.* when frozen during the enzymatic steady-state), which indicates the presence of both paramagnetic Co^{II} and an organic radical with overlap between their atomic orbitals at a distance of

[27] J. Aron, J. M. Pratt, and M. S. Shaikjee, unpublished work.
[28] L. Randaccio, N. Bresciani-Pahor, P. J. Toscano, and L. G. Marzilli, *J. Am. Chem. Soc.*, 1981, **103**, 6347.
[29] H. P. C. Hogenkamp and T. G. Oikawa, *J. Biol. Chem*, 1964, **239**, 1911.
[30] S. S. Kerwar, T. A. Smith, and R. H. Abeles, *J. Biol. Chem.*, 1970, **245**, 1169.
[31] T. Toraya, K. Ushio, S. Fukui, and H. P. C. Hogenkamp, *J. Biol. Chem.*, 1977, **252**, 963.
[32] H. P. C. Hogenkamp, in 'Vitamin B_{12}', ed. B. Zagalak and W. Friedrich, de Gruyter, Berlin, 1979, p. 489.
[33] B. M. Babior, T. H. Moss, W. H. Orme-Johnson, and H. Beinert, *J. Biol. Chem.*, 1974, **249**, 4537.
[34] Y. Tamao and R. L. Blakley, *Biochemistry*, 1973, **12**, 24.
[35] T. Toraya, E. Krodel, A. S. Mildvan, and R. H. Abeles, *Biochemistry*, 1979, **18**, 417.
[36] M. R. Holloway, H. A. White, K. N. Joblin, A. W. Johnson, M. F. Lappert, and O. C. Wallis, in 'Vitamin B_{12}', ed. B. Zagalak and W. Friedrich, de Gruyter, Berlin, 1979, p. 471.
[37] Y. Murakami, Y. Hisaeda, and A. Kajihara, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3642.

$\geqslant 10$ Å![38–40] In both cases where the radical has been identified, it is derived from the substrate or a substrate analogue.[33,41] Even though the occurrence of step (1) is accepted by almost all workers in the B_{12} field, failure to detect the radical derived from the ligand leaves a slight question mark hanging over any discussion of step (1) and of the role of the protein. There is a real need to find a substrate analogue (perhaps a diol in which the C–H bonds are replaced by C–F bonds) which can trigger step (1) of the reaction without itself reacting further with the radical produced.

The 'working' value of $t_{\frac{1}{2}} \sim 10$ years for homolytic fission of the Co–C bond in the protein-free coenzyme at room temperature can be combined[26] with the value of $k_r \sim 2 \times 10^9$ dm^3 mol^{-1} s^{-1} (obtained by picosecond flash photolysis)[11,42] to give a value of $K_{eq} = ([B\text{–}Co^{II}][R\cdot])/[B\text{–}Co\text{–}R] = k_f/k_r \approx 10^{-18}$ dm^3 mol^{-1} for the equilibrium constant of reaction (1); the Co–C bond is clearly very stable towards homolytic fission. By contrast, turn-over numbers of up to 370 s^{-1} (*i.e.* $t_{\frac{1}{2}} \leqslant 2.5$ ms) have been reported for one enzyme[43] and similar values may be expected for others; this corresponds to labilization of the Co–C bond by $\geqslant 10^{11}$ in the presence of substrate. In the same enzyme the Co–C bond is slowly decomposed by O_2 (presumably *via* an initial homolytic fission)[42] even in the absence of substrate with $t_{\frac{1}{2}} = 20$ min at 30 °C,[44] which corresponds to labilization by *ca.* 3×10^5. The sensitivity of O_2 can, however, be reduced by small changes in the ligand and side-chains of the coenzyme[35,38] and also appears to be lower in many other isomerases; one can therefore assume a working figure of $\leqslant 10^5$ for labilization by the protein alone.

The main role of the protein is therefore to displace equilibrium (1) to the right by a massive overall factor of $\geqslant 10^{11}$, which is achieved in two stages, *viz.* a smaller step of $\leqslant 10^5$ when the coenzyme is bound by the protein and a larger step of $\geqslant 10^6$ when the substrate is added; further discussion will be confined to the latter. Since k_r (in free solution) is virtually diffusion controlled, there is no scope for the protein to labilize the Co–C bond by increasing both the rate constants k_f and k_r without significantly altering the equilibrium constant K_{eq} (*i.e.* by a kinetic effect) and the protein must labilize the Co–C bond by changing K_{eq} (*i.e.* by a thermodynamic effect).[8,26]

Little is known about the associated changes in protein conformation, though in one enzyme the difference in conformation (between the forms with intact and broken Co–C bond) is sufficient to produce significant differences in their susceptibility to denaturing agents.[45] Co–C bond labilization therefore appears to be associated with the binding of substrate and with a change of conformation; it

[38] K. L. Schepler, W. R. Dunham, R. H. Sands, J. A. Fee, and R. H. Abeles, *Biochim. Biophys. Acta*, 1975, **397**, 510.
[39] G. R. Buettner and R. E. Coffman, *Biochim. Biophys. Acta*, 1977, **480**, 495.
[40] J. F. Boas, P. R. Hicks, J. R. Pillbrow, and T. D. Smith, *J. Chem. Soc., Faraday Trans. 2*, 1978, 417.
[41] J. E. Valinsky, R. H. Abeles, and A. S. Mildvan, *J. Biol. Chem.*, 1974, **249**, 2751.
[42] J. F. Endicott and T. L. Netzel, *J. Am. Chem. Soc.*, 1979, **101**, 4000.
[43] T. Toraya, T. Shirakashi, T. Kosuga, and S. Fukui, *Biochem. Biophys. Res. Commun.*, 1976, **69**, 475.
[44] O. W. Wagner, H. A. Lee, P. A. Frey, and R. H. Abeles, *J. Biol. Chem.*, 1966, **241**, 1715.
[45] B. M. Babior, in *ref.* 2, Vol. 2, 1982, p. 263.

can be re-written as in equation (3), where P_T and P_R represent the two conformations of the protein and the square brackets enclose the protein and protein-bound species. Equation (3) serves to emphasise that a large change in equilibrium (1) can readily be accommodated, even with a low binding constant for the substrate, provided it is accompanied by a large compensating change in free energy due to the change in protein conformation.[20]

$$[P_T{\cdot}B\text{–}Co\text{–}R] + SH \rightleftharpoons [P_R{\cdot}B\text{–}Co^{II} + R{\cdot}SH] \quad (3)$$

The protein must also ensure that the products of step (1) are not destroyed by, for example, irreversible reaction with O_2. Kinetic studies on the protein-free Co^{II} indicate that autoxidation can be suppressed simply by preventing interaction between two Co^{II} complexes and by excluding amino-acids such as cysteine and tyrosine, without the need to exclude O_2.[46] Free radicals, on the other hand, react avidly with O_2; protection is much more difficult and, as shown by decomposition even in the absence of substrate, only partly effective. Perhaps the protein provides a (?H-bonded) environment which reduces the 'solubility' of O_2 in the vicinity of the radicals.

6 Synthesis and Summary

The B_{12}-dependent isomerase enzymes exploit the organometallic chemistry of an unusual organocobalt corrinoid coenzyme to catalyse some highly unorthodox reactions involving the intermediate formation of radicals. These B_{12} coenzymes offer an apparently unique opportunity for directly comparing the properties of a metallocoenzyme in the presence and absence of protein. Studies on the protein-free corrinoids have shown that increasing steric distortion around the co-ordinated C_α-atom (*a*) can cause a dramatic labilization of the Co–C bond through the displacement of equilibrium (1), and (*b*) will eventually convert a relatively stable red six-co-ordinate form into a much more labile yellow five-co-ordinate form with a band at *ca.* 440 nm, while studies on the enzymes have shown that the addition of substrate (*i*) produces Co^{II} through the displacement of equilibrium (1) by a factor of $\geqslant 10^6$ and (*ii*) often causes the appearance of an additional band at *ca.* 440 nm which does not belong to Co^{II}. Point (*a*) alone provides experimental support for the postulate that the protein (on the binding of the substrate) displaces equilibrium (1) to the right by steric distortion around C_α. The coincidence between (*ii*) and (*b*) provides supporting evidence and, in addition, suggests that the single step of reaction (1) should be expanded into two distinct steps as follows.[26] The coenzyme is present in the resting state as the relatively unstrained red form with the protein in a strained conformation (P_T). The binding of the substrate is accompanied by a change to the relaxed conformation (P_R) which (by, for example, moving the adenine part of the ligand relative to the corrin ring) distorts the co-ordination sphere around C_α (and also strains most of the coenzyme–protein contacts) and converts the coenzyme into a strained yellow form with its absorption band at *ca.* 440 nm (first step). The strained form is then in labile

[46] E. W. Abel, J. M. Pratt, R. Whelan, and P. J. Wilkinson, *S. Afr. J. Chem.*, 1977, **30**, 1.

equilibrium with 'base-on' Co^{II} and the free radical with minimal change in the protein conformation (second step); the steady-state concentration of the strained intermediate (and whether it can be 'seen' or not) will obviously vary from one enzyme to another, depending on the relative rates of formation and removal. In other words, the protein acts as a 'molecular switch' to ensure that the dangerously active radical is generated only when the substrate is present, with the red form and strained yellow form of the coenzyme corresponding to the 'off' and 'on' positions respectively.

$$[N\text{–}Fe^{II}] + O_2 \rightleftharpoons [N\text{–}Fe^{II}\text{–}O_2] \qquad (4)$$

The B_{12}-dependent isomerases have helped to crystallize some of the emerging themes of metalloenzyme chemistry such as; metal–ligand specificity (why Co for organometallic chemistry or Mo for N_2-fixation?); sophisticated redox reactions (of a type hardly yet studied by the co-ordination chemist); and, perhaps most important of all, the mechanism of coupling between changes involving the protein and changes involving the metal, which form the basis of the partnership between metal and protein. Where no new bonds are formed between the protein and the metal complex during the reaction, steric and coulombic interactions provide the main method of coupling. There are interesting parallels between the isomerases and the non-enzymatic haemoglobins, both of which use steric effects. In the haemoglobins the Fe^{II} porphyrin functions by the reversible co-ordination of O_2 according to equation (4), where only the axial ligands are given and N represents the co-ordinated (proximal) histidine. In both the isomerases and haemoglobins[1] the protein controls a key equilibrium of the active site [*viz.* (1) and (4)] by using a change in conformation to link the equilibrium on the metal with some other equilibrium (binding of substrate and, for example, changes in the other haemoglobin sub-units) and couples the changes involving the metal and the protein through steric distortion of one of the axial ligands (5-deoxyadenosyl and histidine). Coulombic effects are less well known but are obviously involved in the proton-coupled reactions (reduction, co-ordination of monobasic anions and probably of HO_2^-) of the Fe^{III} ion in peroxidases; our understanding of these effects will be aided by the recent structural determination of a peroxidase[47] and by the availability of protein-free models for proton-coupled reduction.[48,49]

In the field of purely organic enzymes, stress is usually placed on the role of the protein in 'activating' the *substrate* and increasing a *rate* constant by lowering the energy of the transition state; the metalloenzymes and proteins, on the other hand, emphasize the ability of the protein (on interaction with substrate, another protein, *etc.*) to 'activate' the *metal* (*i.e.* the active site)[50] by changing an *equilibrium* constant.[20]

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