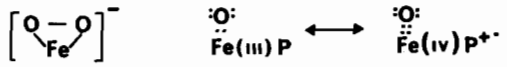
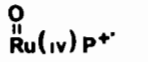


1 2 3

P-450 (Table I). We have extended this chemistry using ruthenium porphyrins [10] and find that a ruthenium (III) octaethylporphyrin and iodosylbenzene will catalyse epoxidation and hydroxylation in a similar fashion (Table I). In addition, a relatively stable complex, characterized as (4) has been isolated which exhibits an ESR signal at g = 2.0 confirming its porphyrin radical nature. This isolated complex performs the same oxidations as in the catalytic regime described in Table I. where the formation of,



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inter alia, cyclohexyl bromide from cyclohexene confirms the radical nature of the oxidation processes.

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B26

The Determination of Hydration Numbers of Metal Ions in Metalloenzymes by NMR

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Proton relaxation enhancement (PRE) was one of the first applications of NMR to biological systems [1]. It was successfully used to monitor changes in the hydration sphere of paramagnetic metal ions embedded in the active sites of enzymes, upon binding of substrates and inhibitors [2]. However, no unambiguous quantitative interpretation of PRE in terms of hydration numbers (q), exchange lifetimes (τ_M) and correlation times (τ_C) is available [3, 4].

The evaluation of these parameters on the basis of the frequency dependencies of the proton longitudinal relaxation times cannot be made in a unique manner mainly because of the invalidity of the relaxation equations at low magnetic fields, where, at least for Mn(II), the contribution of the zero field splitting cannot be ignored. Our approach was to evaluate all the parameters at one, high magnetic field strength, using the four relaxation times, T_{1p}^H, T_{2p}^H, T_{1p}^D and T_{2p}^D of the water protons and deuterons in the same solution. As a result, in addition to the three parameters, q, τ_M and τ_C, a contribution from the outer sphere relaxation could also be characterized.

For Mn(II) bovine carboxypeptidase and Mn(II) bovine carbonic anhydrase B, a hydration number of unity was obtained, and the exchange lifetimes $\tau_M = 1.0 \times 10^{-7}$ s and 0.75×10^{-7} s were found for the two enzymes respectively.

The same q and τ_M were obtained in independent measurements at different magnetic fields, and τ_M was found to be twice as long upon reducing the temperature from 20 °C to 0 °C, as expected.

The outer sphere relaxation could not be explained either by spin diffusion from the protein protons or by dipolar interaction between the Mn(II) ion and freely diffusing water molecules [5]. A good account of this contribution could be obtained by a mechanism involving water molecules which are bound outside the first hydration sphere and have an average exchange lifetime of about 3×10^{-10} s.

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B27

Role of the Protein in the B_{12}-dependent Enzymes: Steric Control of a Molecular Switch

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The initial step in the B_{12}-dependent isomerase and ribonucleotide reductase enzymes involves the reversible homolytic fission of the Co–C bond in

the B_{12} coenzyme to give Co^2 and the free radical R· according to (1); for recent reviews see [1] and, in particular, [2]. Since the Co–C bond of organocorrinoids is normally very stable at physiological pH in the dark, one of the main roles of the protein is to labilise this bond [2, 3]. The use of synthetic analogues has shown [4, 5] that none of the oxygen atoms in the ribose part of the organo-ligand of the coenzyme are essential for enzymatic activity, although the adenine part is probably essential for binding to the protein, *i.e.* the ligand can be considered as a simple alkyl ligand.

$$Co{-}R \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Co^2 + R\cdot \qquad (1)$$

Studies of the effect of increasing steric compression around the coordinated C atom of alkylcorrinoids (due to increasing the degree of substitution on the α or β atoms or the size of the alicylic ring) on the rate of Co–C bond fission, the coordination of ligands in the *trans*-position and the spectra of the alkyl-corrinoids (data summarised in ref. [2]), as well as the structures of the cobaloximes [R–Co-$(DH)_2$py] with R = Me [6], Et [7], Pr^i [8] and neopentyl [9], have revealed a simple pattern of steric interactions [2]. The main results of interest here are that increasing steric compression labilises the Co–C bond (by $> 10^7$ on changing from R = Me to $-CHEt_2$) and will convert the normal red six-coordinate alkylcobalamin to a yellow fine-coordinate form with its first adsorption band at ~440 nm.

Several B_{12}-dependent enzymes do, in fact, show a hitherto unexplained band at ~440 nm, but only during the enzyme–substrate steady-state [10–13]. This can now fairly confidently be identified as a distorted five-coordinate yellow form of the coenzyme (Co–R*), which is formed (see expanded scheme (2)) from the normal six-coordinate red form (B–Co–R) as the substrate (SH) binds (and presumably induces a change in protein conformation P to P^1) and which forms a labile equilibrium with Co^2 and R· [2].

$$[P{\cdot}B{-}Co{-}R] + SH_{free} \rightleftharpoons$$

$$[P'{\cdot}Co{-}R^*{\cdot}SH] \rightleftharpoons [P'{\cdot}Co^2{\cdot}R{\cdot}SH] \qquad (2)$$

Values of $k_r \sim 2 \times 10^9\ M^{-1}\ s^{-1}$ have been obtained [14, 15] by picosecond flash photolysis for methylcobalamin and the coenzyme, which are close to the diffusion-controlled limit. Since the equilibrium constant for reaction (1), $K_{eq} = k_f/k_r$, there is no scope for the protein to labilise the Co–C bond in the coenzyme by increasing both k_f and k_r without significantly affecting K_{eq} (*i.e.* by a kinetic effect); the protein therefore labilises the Co–C bond by changing K_{eq} (*i.e.* by a thermodynamic effect).

The evidence indicates (*cf.* the predictions in ref. [3]) that in these B_{12}-dependent enzymes the protein (1) uses steric distortion (*cf.* also the non-enzymatic haemoglobins [16]) (2) to change a key equilibrium (as distinct from rate) constant (*cf.* also the peroxidases [3, 17]), and (3) acts as a molecular switch (with the red and yellow forms of the coenzyme indicating the 'off' and 'on' positions respectively), triggered by the binding of the substrate, in order to generate the dangerously active free radical (R·) only when the substrate is present.

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