The results reflect the complexity of each system, the simultaneous presence of several species in them, the sensitivity of the complex formation equilibria on internal (structure of ligand) and external (matrix) factors. The equilibrium constants have been used for the calculation of the concentration distribution of species of different compositions.

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B6

A New Model of Coenzyme B_{12}?

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A survey of vitamin B_{12}-dependent rearrangements suggests that they involve homolytic splitting of the Co–C bond in 5′-deoxyadenosylcobalamin. Numerous attempts were made to simulate these processes using 5′-deoxyadenosylcobalamin in nonenzymatic systems as well as its most popular models, organocobaloximes. Nevertheless, striking features of the homolysis step, namely mild conditions, reversibility and controllability, have been neither imitated nor properly explained so far. In this connection, certain results of our studies with a new type of organocobalt chelate [1] may be of interest.

The cationic complexes in question involve the trivalent metal bound to an alkyl group, a chelating diamine and a mixed tridentate ligand derived from a Schiff base constituted by the same diamine and an *o*-hydroxycarbonyl compound at a 1:1 ratio. Conditions of their formation and its mechanism are considered; the spatial and electronic structure of the complexes is also studied. Some of the reactions related to modelling vitamin B_{12} (*e.g.* photolysis, reduction and oxidation) are discussed, with emphasis being put on the unusual behaviour of the complexes under the influence of acids [2].

Decomposing readily in acidic media, the alkylcobalt chelates under consideration give all the products of disproportionation and coupling of the alkyl groups (RH, R_{-H} and R_2), the yields of the RH alkanes being substantially higher than those of alkenes. Experiments with an isotope label (D_2O) revealed that the excess of the former was due to the abstraction of hydrogen atoms from chelating ligands by alkyl free radicals rather than to protolysis of the Co–C bond. These findings suggest homolytic cleavage of the organocobalt complexes under the action of protons.

The formation of alkyl free radicals in the course of decomposition was directly proved by the spin-trapping technique. Spin adducts of the radicals (*viz.* Me, Et and *c*-C_6H_{11}) with Bu^tNO and $PhCH{=}N(O)Bu^t$ were identified by ESR spectroscopy in phosphate buffer solutions. Furthermore, kinetic measurements with the latter trap at various pHs indicated that protons are involved in steps leading to the formation of alkyl free radicals. The intermediacy of protonated complexes still holding the Co–C bond was established by means of spin-trapping, NMR and spectrophotometric techniques.

The ability of the complexes in question to generate alkyl free radicals under mild conditions and at a conveniently regulated (pH-controlled) rate was used to imitate vitamin B_{12}-dependent dehydration of α-glycols. Some positive results give support to the speculation that protonation-deprotonation or related polar interactions may control the dissociation of the Co–C bond of 5′-deoxycobalamin in enzymatic systems, thus triggering the biological dehydratation of glycols as well as other vitamin B_{12}-dependent rearrangements.

The potential use of the complexes as sources of free radicals in living organisms is also discussed.

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B7

Iron–Carbon Bond Formation During Substrate Activation by Hemoproteins

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Recent results point to the existence of an important organometallic chemistry of certain hemoproteins, with the formation of iron–carbon bonds during substrate activation. Evidence for such iron–carbon bond formation comes both from spectroscopic studies on the hemoproteins themselves and from model studies on iron-porphyrins. These iron–carbon bonds are formed either upon reduction or oxidation of several substrates [1].

Reduction of benzylhalides, $ArCH_2X$, by microsomal cytochrome P450 leads to σ-alkyl complexes of this cytochrome involving a $Fe(III)–CH_2Ar$ bond. Reduction of halothane, $CF_3CHClBr$, leads also to a σ-alkyl cytochrome $P450–Fe(III)–CHClCF_3$

complex, whereas microsomal reduction of other polyhalogenated compounds such as CCl_4 or CCl_3-CN leads to iron–carbene complexes, cytochrome P450–Fe(II) ← CCl_2 (or CClCN). Similar reactions occur upon reduction of the same compounds by iron-porphyrins, the corresponding σ-alkyl or carbene complexes having been isolated and characterized [2]:

$$\text{Fe(II)} + \text{RR'CX}_2 \xrightarrow[-X^-]{+e^-} [\text{Fe(III)–CRR'X}] \xrightarrow[-X^-]{+e^-} [\text{Fe(II)} \leftarrow \text{CRR'}]$$

Cytochrome P450–iron(II)–carbene complexes are also formed upon metabolic oxidation of the methylene group of 1,3-benzodioxole derivatives, suggesting a mechanism for C–H bond activation by cytochrome P450 that involves the intermediate formation of an iron–carbon bond [3].

Very recently, it has been indicated that σ-alkyl (or -aryl) iron(III) complexes of cytochrome P450 [4] or hemoglobin [4, 5] are formed upon metabolic oxidation of various monosubstituted hydrazines by these hemoproteins. Most often, iron(II)–diazene complexes are involved as intermediates in this reaction:

$$\text{Fe(III)} + \text{RNHNH}_2 \xrightarrow[-2H^+]{-e^-} [\text{Fe(II)} \leftarrow \text{NH=NR}] \xrightarrow[-H^+]{-e^-} [\text{Fe}^{\text{III}}\text{–R}]$$

Similar diazene and σ-alkyl complexes are formed upon reaction of $RNHNH_2$ with iron–porphyrins; they have been isolated and characterized [6].

A general scheme for iron-carbon bond formation will be given; it involves a reductive or oxidative activation of the substrate leading to an organic free radical, and the combination of this radical with the hemoprotein–iron.

Most of these hemoprotein–σ-alkyl or –carbene complexes have also been detected *in vivo*; their biological implications will be discussed.

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B8

Protein-Induced Changes of the Electronic Structure of the Heme

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For an understanding of the various functions of heme proteins it is important to comprehend how a particular protein structure controls the geometric and electronic properties of the heme (and *vice versa*). The flexible structure of the heme group allows it to exist in various structurally distinct forms depending on the electronic structure of the central Fe ion and on the particular contacts and constraints exercised on the heme by the protein. In studying protein–porphyrin–iron interactions it is particularly useful to direct one's attention to the symmetry properties of the heme and their changes caused by these interactions. As an example we shall compare heme in solution with heme in myoglobin (Mb) for the $3d^5$ configuration of Fe. Information about the symmetry of the heme is provided by several spectroscopic methods; we shall discuss here mainly ^1H NMR measurements.

The hyperfine shifted magnetic resonances of protons belonging to the heme reflect the electronic properties of the central Fe ion and of the porphyrin [1, 2]. Data are available particularly on the 4 methyl resonances [3, 4], which appear as pairs and originate from methyls related by a twofold rotation axis and by a reflection plane respectively. The range of their chemical shifts, which are centered near –16 ppm (from TMS) in the ferric low spin and near –64 ppm in the ferric high spin states increases to the same extent as protein–heme interactions lower the heme symmetry. The actual symmetry of the heme iron may be approximated by C_{4v} symmetry with a triclinic perturbation. The range of the spread of the methyl resonances relative to the value of the magnetic susceptibility is larger for low spin than for high spin states. This indicates that the triclinic perturbation has a smaller influence on the electron distribution of the ferric high spin Fe than on that of the ferric low spin Fe.

The electronic structures of the Fe ion in hemes and heme proteins as inferred from Mössbauer, ESR and magnetic susceptibility measurements [5] serve as a basis for interpreting the NMR results: Ferric high spin Fe in $Mb(H_2O)$ has for C_{4v} symmetry a spherically symmetric $^6A_1$ ground state and low lying ^2E, $^4A_2$ and $^2B_2$ levels at about 1100, 1400 and 1700 cm^{-1}. A small triclinic perturbation splits the ^2E doublet into E_+ and E_- levels separated by about 300 cm^{-1} in a first approximation. The electron distribution of these two levels is rhombic C_{2v}. Only in a second approximation a triclinic perturbation mixes