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]:

$$Fe(II) + RR'CX_2 \xrightarrow[-X^-]{+e^-} [Fe(III)-CRR'X] \xrightarrow[-X^-]{+e^-} [Fe(II) \leftarrow CRR']$$

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:

$$Fe(III) + RNHNH_2 \xrightarrow[-2H^+]{-e^-} [Fe(II) \leftarrow NH{=}NR] \xrightarrow[-H^+]{-e^-} [Fe^{III}-R]$$

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.

1 D. Mansuy, *Reviews in Biochem. Toxicol., 3,* 283 (1981).
2 D. Mansuy, *Pure Appl. Chem., 52,* 681 (1980).
3 D. Mansuy, J.-C. Chottard, M. Lange and J.-P. Battioni, *J. Mol. Catalysis, 7,* 215 (1980).
4 P. Battioni, J.-P. Mahy, M. Delaforge and D. Mansuy, submitted.
5 O. August, K. L. Kunze and P. R. Ortiz de Montellano, *J. Biol. Chem., 257,* 6231 (1982).
6 P. Battioni, J.-P. Mahy, G. Gillet and D. Mansuy, *J. Am. Chem. Soc.,* (1983) submitted.

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

the $^2B_2$ level with the $^2E_\pm$ levels which yields a triclinic electron distribution. The $^2E_\pm$ levels admix to the $^6A_1$ ground state by spin–orbit coupling. The electron distribution is therefore predominantly spherically symmetric with a small rhombic component C_{2v}. Ferric low spin Fe in Mb(CN) has for C_{4v} symmetry a ^2E ground state and low lying $^2E_2$ and $^4A_2$ levels at about 300 and 800 cm^{-1}. A triclinic perturbation splits the ^2E doublet into $^2E_+$ and the new ground state $^2E_-$. The electron distribution is therefore always of rhombic symmetry C_{2v}.

It is intended to discuss also the symmetry properties of hemes and heme proteins for the $3d^6$ configuration of Fe.

1 K. Wüthrich, *Structure and Bonding, 8,* 53 (1970).
2 R. G. Shulman, S. H. Glarum and M. Karplus, *J. Mol. Biol., 57,* 93 (1971).
3 G. La Mar, in 'Magnetic Resonance in Biology', R. G. Shulman, ed., Acad. Press (1979).
4 A. Mayer *et al., J. Mol. Biol., 86,* 749 (1974).
5 H. Eicher, *Z. Naturforsch., 30c,* 701 (1975).

B9

Jack Bean Urease: the First Nickel Enzyme

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Jack bean urease is the first example of a nickel metalloenzyme [1–3]. Reassessment of the molecular properties of the enzyme shows that the native enzyme has a molecular weight (M_r) of 590,000 ± 30,000, and that under denaturing conditions, urease breaks down to identical subunits with M_r = 90,000–100,000 [4]. The molecular weight of subunits is 96,600, as determined by titration with radioactive inhibitors (acetohydroxamic acid [5] and phosphoramidate) [2, 4]. Thus the native enzyme consists of six identical subunits, and these are arranged in the form of a regular octahedron [6]. Each subunit contains one cystine disulfide bond and a total of fifteen cysteine residues [7].

Each subunit contains 2.0 ± 0.1 very tightly bound nickel ions [1, 2, 8–10]. After the electronic absorption spectrum of native urease has been corrected for effects of light scattering, the peaks associated with nickel ion (λ_{max}: ~407 nm, 745 nm, 1060 nm) are consistent with Ni(II) in an octahedral environment [6, 11]. β-Mercaptoethanol binds rapidly and reversibly to urease to produce marked reversible changes in the absorption spectrum of the enzyme [12]. New absorption peaks in the difference spectrum (324 nm, 1550 M^{-1} cm^{-1}; 380 nm, 890 M^{-1} cm^{-1}; 420sh nm, 460 M^{-1} cm^{-1}) are consistent with charge transfer transitions of a thiolate anion coordinated to Ni(II).

A detailed mechanism was developed in which urea is activated towards nucleophilic attack by virtue of O-coordination to Ni(II) ion [13], and has been subsequently successfully modelled [14].

The competitive inhibitors acetohydroxamic acid, phosphoramidate and fluoride [4, 12, 15], produce small, reversible changes near 400 nm in the absorption spectrum of urease, consistently with their direct coordination to Ni(II) ion.

These and other aspects of the chemistry of this system will be discussed.

1 N. E. Dixon, C. Gazzola, J. J. Watters, R. L. Blakeley and B. Zerner, *J. Am. Chem. Soc., 97,* 4130 (1975).
2 N. E. Dixon, C. Gazzola, R. L. Blakeley and B. Zerner, *J. Am. Chem. Soc., 97,* 4131 (1975).
3 R. L. Blakeley, E. C. Webb and B. Zerner, *Biochemistry, 8,* 1984 (1969).
4 N. E. Dixon, J. A. Hinds, A. K. Fihelly, C. Gazzola, D. J. Winzor, R. L. Blakeley and B. Zerner, *Can. J. Biochem., 58,* 1323 (1980).
5 K. Kobashi, J. Hase and K. Uehara, *Biochim. Biophys. Acta, 65,* 380 (1962).
6 R. L. Blakeley, N. E. Dixon and B. Zerner, *Proc. Aust. Biochem. Soc., 14,* 13 (1981).
7 P. W. Riddles, R. K. Andrews, R. L. Blakeley and B. Zerner, *Biochim. Biophys. Acta,* (1983), in press.
8 N. E. Dixon, C. Gazzola, R. L. Blakeley and B. Zerner, *Science, 191,* 1144 (1976).
9 N. E. Dixon, R. L. Blakeley and B. Zerner, *Can. J. Biochem., 58,* 469 (1980).
10 N. E. Dixon, C. Gazzola, C. J. Asher, D. S. W. Lee, R. L. Blakeley and B. Zerner, *Can. J. Biochem., 58,* 474 (1980).
11 R. L. Blakeley, N. E. Dixon and B. Zerner, *Biochim. Biophys. Acta,* (1983), in press.
12 N. E. Dixon, R. L. Blakeley and B. Zerner, *Can. J. Biochem., 58,* 481 (1980).
13 N. E. Dixon, P. W. Riddles, C. Gazzola, R. L. Blakeley and B. Zerner, *Can. J. Biochem., 58,* 1335 (1980).
14 R. L. Blakeley, A. Treston, R. K. Andrews and B. Zerner, *J. Am. Chem. Soc., 104,* 612 (1982).
15 R. L. Blakeley, J. A. Hinds, H. E. Kunze, E. C. Webb and B. Zerner, *Biochemistry, 8,* 1991 (1969).

B10

Ions and Ionophores

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It will be shown using typical examples that it is possible to account, by explicit computations, for the complexing preferences of ionophores for certain cations. In the case of valinomycine, the preference observed for complexing the alkali cations is in the order $Rb^+ \sim> K^+ > Cs^+ \gg Na^+$, and can be accounted for by making an energy balance between the ener-