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Multiple resonance studies of ligand exchange in metalloporphyrins

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The biological function of porphyrin proteins and enzymes can often be correlated with a variety of independent parameters. In the corrins and in heme proteins the axial ligands play an integral role in determining the activity of the metal in electron transport and in the binding and activation of substrates. Considerable effort has been devoted to the study of the effect of various ligands on several physical properties, such as electronic spectra, complex stability, and binding interactions with metals contained within the porphyrin ring[1]. In many of the reactions of heme enzymes and enzymes requiring vitamin B_{12}, removal of the axially coordinated ligand is suggested as the initial step, but little is known about the kinetics or mechanism of this reaction[2-4]. Even though the identity of the axial ligands bound to a metal in a heme protein under given conditions frequently remains unclear, histidine is often considered to be bound to the iron atom[5]. This suggests that the elucidation of the mechanisms of binding of imidazole and substituted imidazoles to metalloporphyrins should lead to a better understanding of the enzyme systems.

Multiple resonance NMR techniques readily allow study of the kinetics of imidazole metalloporphyrin systems as well as facile assignment of resonances and stereochemistry. The mechanism of tautomerization of the imidazole derivative of ruthenium carbonylmesoporphyrin-IX dimethyl ester is particularly interesting in view of the recent proposal of metal "shuttling" in intramolecular reactions, for which intermolecular exchange was discounted as an explanation for the observed interchange of nitrogen substituents[6].

There is a rapid tautomerism in free imidazole which allows the interconversion of the molecule with a proton bound to one nitrogen atom to that with a proton bound to the other nitrogen atom; hence, the protons on the 4- and 5-carbon of the imidazole become equivalent on the NMR time scale. Thus, in free imidazole one observes a triplet

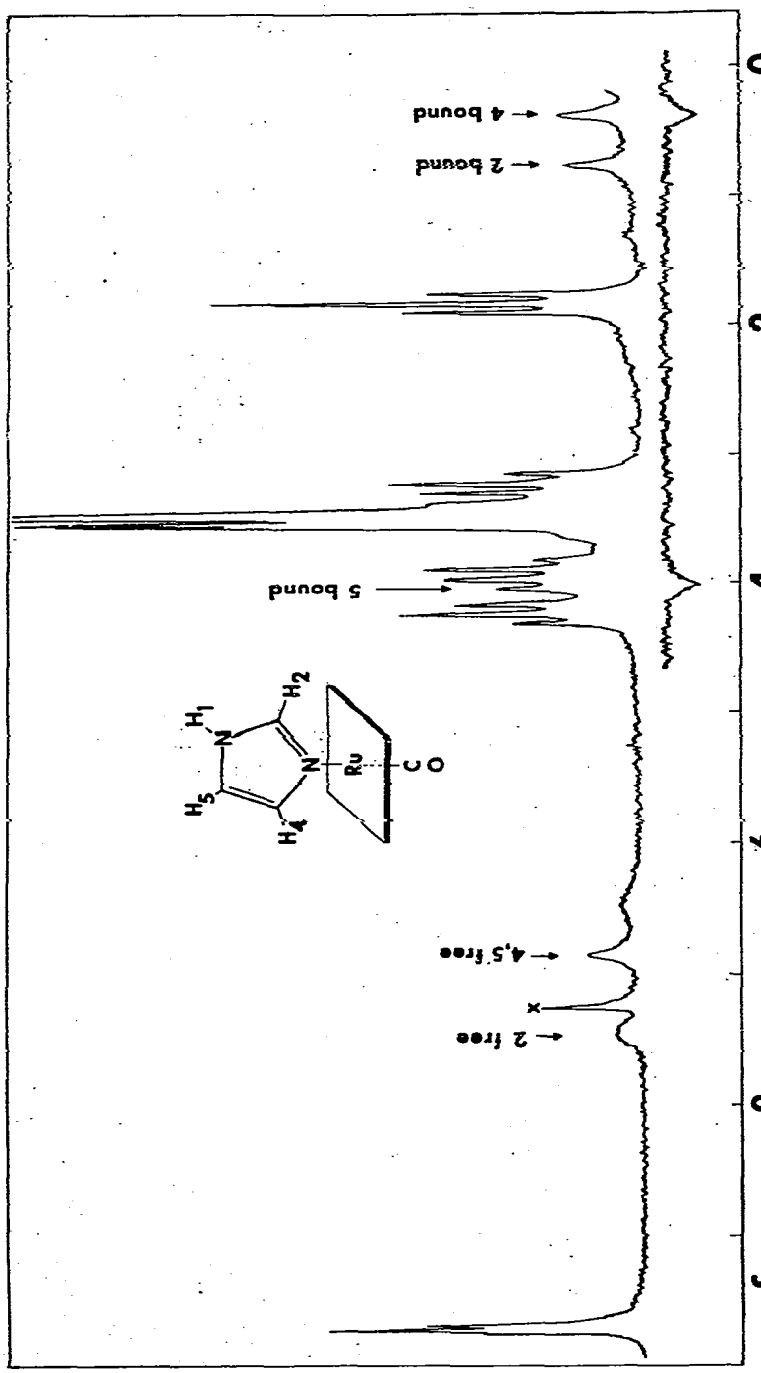

Fig.1. The PMR spectrum of a deuteriochloroform solution of ruthenium carbonylmesoporphyrin IX dimethyl ester in the presence of a 50% excess of imidazole at 4°C. The spectrum was taken in frequency sweep mode with TMS lock. Chemical shifts given in the text refer to a sample containing methylene chloride (see Table 1). The lower trace is a plot of intensity of resonance 4,5*b* as a function of the frequency of a second RF field.

(J = 1.2 Hz) of intensity one at δ 7.12 for the 2-proton and a doublet at δ 6.71 of intensity two for the 4- and 5-protons. The tautomerism of the bound imidazole, however, is much slower and only two resonances of intensity one are observed for these three protons, the third resonance being buried beneath the porphyrin resonances. The assignments of the resonances observed at δ 0.67 and δ 0.25 cannot be made on the basis of coupling constants★, but the assignments are readily made by double resonance spin saturation transfer techniques. If the spin states for nuclei at a given site are saturated and the nuclei migrate to a different site faster than the relaxation time a decrease in the intensity of the resonance at the second site will be observed. At 40° saturation of the resonance at δ 7.12 (assigned to the 2-proton in free imidazole) produces a decrease in the intensity of the resonance at δ 0.67; thus indicating that there is a relatively rapid exchange between free and bound imidazole at this temperature and that the resonance at δ 0.67 should be assigned to the 2-proton of bound imidazole, contrary to the suggestion of Tsutsui *et al.*[6]. Sweeping a saturating RF field through the spectrum while observing the intensity of the resonance at δ 6.71 ((4,5)*f*) produces a decrease in intensity when the RF field corresponds to δ 0.25 and δ 3.91 (see Fig.1). Thus the resonance at δ 0.25 can confidently be assigned to the 4-proton of the bound imidazole considering its proximity to the porphyrin ring; whereas the resonance due to the 5-proton of the bound species must be at δ 3.91 and superimposed upon the quartet of one of the ethyl groups in the porphyrin. Upon raising the temperature to 64° the exchange rate between sites becomes sufficiently fast that broadening of the imidazole resonance becomes evident (see Fig.1). In a solution which contains a ratio of 2/1 bound to free imidazole the resonance assigned to the 4- and 5-protons of the free imidazole ((4,5)*f*) is substantially broader than that of the bound imidazole (4*b*). From the line widths a first order rate constant for nuclei leaving site 4*b* of 10 sec^{-1} is found; whereas a pseudo-first-order constant of 21 sec^{-1} is found for leaving site (4,5)*f*★★. Since the rate constant for leaving 4*b* is one-half of that for leaving (4,5)*f*, no appreciable exchange occurs directly between 4*b* and 5*b*. This point was further verified by the following saturation transfer experiment. Saturation of (4,5)*f* produced

$$\begin{array}{ll} 4b \rightleftharpoons 5b & k(bb) < 2\ sec^{-1} \\ \uparrow\downarrow \quad \uparrow\downarrow & k(bf) = 10\ sec^{-1} \\ 4f \rightleftharpoons 5f & k(ff) > 1000\ sec^{-1} \end{array}$$

a decrease in intensity of 4*b* to 43% of its original value. Simultaneous saturation of 4*b* and 5*b* produced no additional saturation in 4*f* (see Table 1). Since direct 5*b*→4*b* interchange would provide a source of non-zero magnetization transfer in the absence of saturation at 5*b*, one would expect a further decrease in the intensity at 4*b* if appreciable direct interchange of 4*b* and 5*b* were occurring. Thus again no detectable direct interchange of 4*b* and 5*b* is detected.

★Examination of ^{13}C satellites indicates that the coupling between the 4- and 5-protons is the same magnitude as the coupling with the 2-proton ($|J_{45}| = |J_{25}| = |J_{24}| = 1.2$ Hz).

★★At 64° the full widths at half-height of (4,5)*f* and 4*b* were 9.3 and 7.0 Hz respectively. The natural line widths (at 30°) were 2.5 and 2.7 Hz for (4,5)*f* and 4*b* respectively, the difference being attributable to spin coupling.

TABLE 1

OBSERVED SATURATION AT 40°
Reported as 100 x $[M_z(\infty)M_z(0)]$. Other values had average deviations of ±1. The solvent for these measurements was deuteriochloroform containing 5% methylene chloride to provide a lock resonance.

Chemical shift (δ)	7.12	6.71	3.91	0.67	0.25
Assignment	2*f*	4,5*f*	5*b*	2*b*	4*b*
	*[a]	–	–	45	100
	–	*	–	100	43
	–	–	*	100	74
	–	*	*	100	43

[a] The asterisks (*) indicate resonances which were saturated.

The same rate constant ratios hold at a variety of temperatures and additional saturation is not seen in 4*b* upon simultaneous irradiation of (4,5)*f* and 5*b* over irradiation of (4,5)*f* alone at lower temperatures. The rate constant at 64° for leaving the 4*b* site is independent of porphyrin complex concentration over the range studied (0.05*M*–0.35*M*) and imidazole concentration (imidazole/porphyrin–Ru(CO), 1/1 to 30/1)★. These data are consistent with a first order dissociative mechanism with $\Delta F^{\ddagger}_{64}$ = 18.3 kcal/mole which allows equilibration of the bound imidazole with free imidazole in solution and indicates that the predominate pathway for imidazole tautomerism in this complex is via intermolecular exchange rather than an intramolecular shuttling mechanism.

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★Rate studies via the saturation transfer methods are particularly difficult owing to the relatively low solubility of the complex and the short T_1 of the protons in the complex (~0.4 sec). Further studies are in progress.