Dynamic ^{1}H and ^{15}N Nuclear Magnetic Resonance of Free-base ^{15}N-Porphyrins

By Charles S. Irving and Aviva Lapidot*
(Department of Isotope Research, Weizmann Institute of Science, Rehovot, Israel)

Summary ^{15}N n.m.r. chemical shifts, the ^1J(NH) coupling constant, and the change of the band shapes of ^{1}H and ^{15}N resonances with temperature have provided information on the location, degree of hydrogen bonding, and mechanism of tautomerism of the hydrogens in the core of free base $^{15}N_4$-protoporphyrin-IX dimethyl ester and $^{15}N_4$-coproporphyrin-III tetramethyl ester.

The interpretation of vibrational,[1] electronic absorption,[2] and fluorescence spectra[3] of free-base porphyrins, as well as M.O.[4] and force-field calculations require an accurate description of the location and degree of hydrogen bonding of the hydrogens in the core of free-base porphyrins. Although *X*-ray photoelectron spectra[5] and *X*-ray crystallographic structures[6] of porphyrin free bases have revealed that hydrogens are attached to opposite nitrogens, they have not provided estimates of the degree of hydrogen bonding in the core.

The mechanism of NH tautomerism in free-base porphyrins has been discussed for many years[7-9] and remains to be fully characterized. Attempts have been made to detect simultaneous two-proton jumps, in addition to consecutive proton jumps by measuring the deuterium kinetic isotope effect using dynamic ^{1}H and ^{13}C n.m.r. spectroscopy.[7-9] Conflicting results have been reported. We here report that dynamic ^{1}H and ^{15}N n.m.r. spectral measurements on ^{15}N-labelled free base porphyrins provide useful insights into these problems.

[$^{15}N_4$]Protoporphyrin-IX and [$^{15}N_4$]coproporphyrin-III (91·8% total ^{15}N; $^{14}N_4$, 1·5; $^{14}N_3{}^{15}N$, 0·9; $^{14}N_2{}^{15}N_2$, 2·5; $^{14}N^{15}N_3$, 18·8; $^{15}N_4$, 76·3%) were obtained from a mutant of *Rhodopseudomonas spheroides* grown on a medium supplemented with [^{15}N]δ-amino levulinic acid and [^{15}N]glycine, and were converted into their respective methyl esters, (I) and (II).[10]

The proton-coupled ^{15}N n.m.r. spectra of (I) and (II) (Figure 1) at 30 °C display a broad resonance at *ca.* 160 p.p.m. (downfield from 4M $^{15}NH_4Cl$ in 2M HCl). Lowering

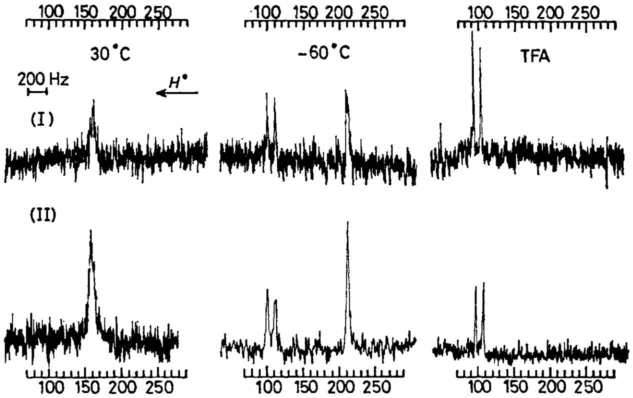

FIGURE 1. ^{15}N n.m.r. (9·12 MHz) spectra of 1 cc samples of (I) and (II) (10—30 mg cc^{-1}) in $CDCl_3$ or TFA (trifluoroacetic acid); pulse length 90°; recycle time, 5 s; accumulations, 5000; spectral width, 6000 Hz; spectral resolution, 0·332 p.p.m./address; field stabilization on the ^{19}F signal of C_6F_6 (room temperature) or $CFCl_3$ (low temperature) contained in a 5 mm concentric tube; chemical shifts are in p.p.m. downfield from 4M $^{15}NH_4Cl$ in 2M HCl.

the temperature to −60 °C in order to reduce the rate of hydrogen tautomerism allowed a ^{15}N n.m.r. spectrum of (I) to be obtained that displayed a doublet ($^{1}J_{\mathrm{NH}}$ 103 ± 4 Hz) at 107 and a singlet at 212 p.p.m. The spectrum of (II) displayed a similar doublet ($^{1}J_{\mathrm{NH}}$ 100 ± 3 Hz) at 105·5 and a singlet at 212 p.p.m. The upfield doublet has a chemical shift similar to that for pyrrole (121 p.p.m., when weakly hydrogen bonded, and 135 p.p.m., when strongly hydrogen bonded)[11] and a $^{1}J_{\mathrm{NH}}$ characteristic of an sp^2 hybridized nitrogen which corresponds to the $^{1}J_{\mathrm{NH}}$ of pyrrole.[12] The downfield singlet has a ^{15}N chemical shift that resembles strongly hydrogen-bonded pyridine (220 p.p.m.) rather than non-hydrogen bonded pyridine (292·8 p.p.m.).[13] It is interesting to note that the spectra of the dications of (I) and (II) display a doublet at 101 p.p.m. with $^{1}J_{\mathrm{NH}}$ 90 ± 3 and 85 ± 3 Hz, respectively. The ^{15}N chemical shift indicates a pyrrolic nature of all four nitrogens and the smaller $^{1}J_{\mathrm{NH}}$ values are characteristic of nitrogen with a hybridization intermediate between sp^2 and sp^3, for the porphyrin dications.

Bridging of a porphyrin inner hydrogen between two nitrogens would result in a ^{15}N spectrum consisting of a single triplet with a $^{1}J_{\mathrm{NH}}$ value reduced by half, which is not consistent with the observed ^{15}N spectra. Pheophytin consists of a non-hydrogen-bonded pair of pyrrole and pyrrolenine groups and a pair of mutually hydrogen-bonded pyrrole and pyrrolenine groups. The ^{15}N chemical shifts of the hydrogen-bonded group (110·9 and 218·5 p.p.m.)[14] correspond exactly to the ^{15}N chemical shifts of free-base porphyrins, while the non-hydrogen-bonded group (102·5 and 272·8 p.p.m.)[14] do not. We therefore conclude that bonding of the inner hydrogens of the porphyrin core is best described by a direct covalent bond to a single nitrogen, with a strong hydrogen bond to an adjacent nitrogen.

The ^{1}H Fourier transform n.m.r. spectrum of (I) was measured over the temperature range 213—283 K (Figure 2). As the temperature is increased, the doublet ($^{1}J_{\mathrm{NH}}$ 103 Hz) of the $^{15}N_4$-compound changes into a quintet, which represents the time-averaged interaction of an inner proton with all four ^{15}N nuclei. Superimposed on this spectrum are the singlet and doublets of the $^{15}N_3{}^{14}N$-compound (*ca.* 20%) which, on increasing the temperature, change into a quartet, representing the time-averaged interaction of an inner proton with only three ^{15}N nuclei. No evidence for intermolecular proton exchange was obtained in the absence of protic compounds. The $^{1}J_{\mathrm{NH}}$ scalar coupling constant remained unchanged over these temperature ranges, as indicated by the constant separation between the highest upfield and lowest downfield multiplets. Intramolecular proton exchange rates were calculated by computer simulation of the total band-shapes of the observed spectra (Figure 2) using the Kubo–Sacks matrix method described by Johnson and Moreland.[15]

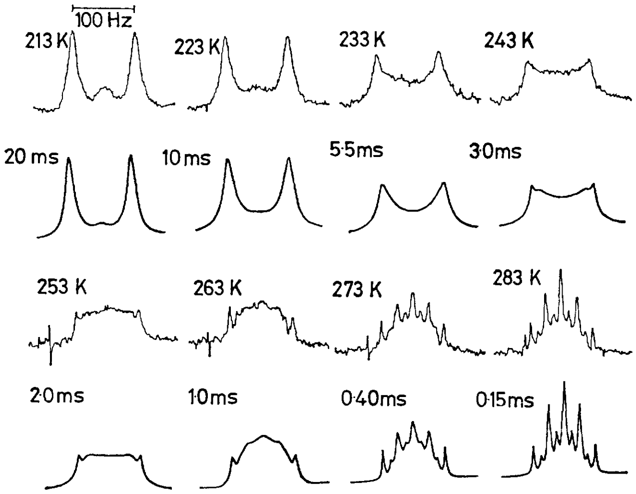

FIGURE 2. The ^{1}H n.m.r. (90 MHz) spectra of the NH protons of ^{15}N-protoporphyrin-IX-dimethyl ester (*ca.* 0·02M) in $CDCl_3$ and the computer-simulated spectra. Spectral conditions: pulse angle, 10°; accumulations, 1024; resolution, 0·75 Hz/address; spectral width, 1500 Hz; field stabilization on the ^{2}H signal of $CDCl_3$; temperature calibrated with a thermocouple immersed in the sample.

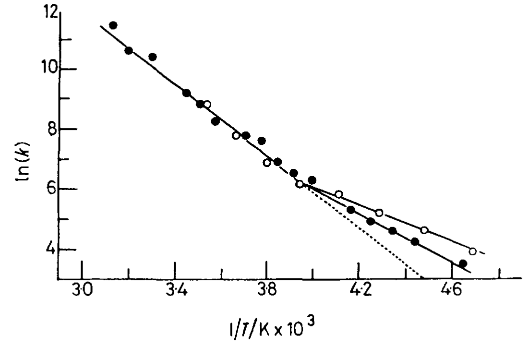

FIGURE 3. Arrhenius plot for tautomerism of (I) measured by ^{1}H n.m.r. (○) and of (II) measured by ^{15}N n.m.r. (●) spectroscopy. The high- and low-temperature least-squares lines correspond to the activation parameters given in the Table.

The coalescence of ^{15}N resonances of the pyrrole and pyrrolenine nitrogens of (II) into a singlet was measured over the temperature range 215—320 K. The intramolecular proton exchange rates, calculated by computer total band-shape analysis, also displayed non-linear Arrhenius (Figure 3) and Eyring plots. At higher temperatures, the rate of tautomerism of (II) was practically identical to that of (I), even though the rates were measured by n.m.r. techniques on different nuclei, monitoring different processes (proton jump and pyrrole–pyrrolenine transition), in two different compounds (I) and (II). The ^{1}H n.m.r. spectra of (II) were very similar to those of (I), but had broader line-widths. It is interesting to note that the similarity in line-widths and intensities of pyrrole and pyrrolenine ^{15}N resonances at 213 K indicates that the rate of proton tautomerism is still fast with respect to dipolar relaxation processes, as is expected from the activation data given in the Table.

Non-linearity in Arrhenius plots can result from proton tunnelling, two independent reaction pathways, or a change in porphyrin structure or solvation with temperature.[16] Evidence for proton-tunnelling has been obtained for NH ··· N proton transfer in the tautomerism of aggregated pyrazole[17,18] and would not be unexpected in porphyrins owing to the symmetrical, narrow, and well defined geometries of their potential barriers. The activation parameters [derived from Arrhenius and Eyring plots of the

Table. Activation parameters for tautomerism of (I) and (II).

Measurement	Temperature range/K	ln A	$\Delta E^\ddagger$ /kcal mol^{-1}	$\Delta S^\ddagger$ /cal K^{-1} mol^{-1}	$\Delta H^\ddagger$ /kcal mol^{-1}	$\Delta G^\ddagger_{265}$ /kcal mol^{-1}
^{15}N n.m.r. of (II)	250—320	29·7±3·7[a]	−11·7±2·0[a]	− 1·5±4·4	−11·2±0·9	−10·8
	215—240	20·7±3·2	− 7·4±1·5	−18·8±4·5	− 6·9±1·0	− 1·8
^{1}H n.m.r. of (I)	263—282	33·7±12·7	−14·0±5·9	− 6·6±17·5	−13·5±4·6	−11·7
	213—253	18·7±3·7	− 6·3±1·7	−22·9±5·6	− 5·8±1·2	0·3

[a] 95% confidence errors of the activation parameters have been estimated from the standard deviation, calculated by the least-squares method, of the slopes and intercepts of the Arrhenius and Eyring plots.

proton-exchange rates of (I) and (II)] for the case of two independent tautomeric pathways are given in the Table. It is interesting to note that the mean activation parameters correspond to those obtained by less precise ^{1}H and ^{13}C n.m.r. measurements.[7–9]

The parameters obtained at both low and high temperatures did not resemble those of sigmatropic hydrogen shifts, as had been previously proposed.[8] However, the large negative entropy and lower activation energy of the low-temperature reaction closely resemble those reported recently for nitrogen to nitrogen proton transfers, involving the separation of charge and the freezing of solvent in the transition state.[19] The more favourable $\Delta S^\ddagger$ but higher $\Delta H^\ddagger$ observed at higher temperatures might represent tautomerism proceeding by two consecutive proton jumps, during which a porphyrin isomer with protons on adjacent nitrogens constitutes a short-lived reaction intermediate.

The state of aggregation of the porphyrin, which might change with temperature, does not affect the proton-exchange rate, since aggregated (0·1M) and non-aggregated (0·02M) (II) had similar rates of tautomerism. However, the detection of change in the solvation of structure of porphyrins must await high resolution ^{1}H and ^{13}C n.m.r. measurements.

We thank Mrs. Hannah Bayer for technical assistance, and the National Institutes of Health for financial support.

(Received, 2nd November 1976; Com. 1217.)

[1] C. Vestling and J. Downing, *J. Amer. Chem. Soc.*, 1936, **58**, 1081; S. Mason, *J. Chem. Soc.*, 1958, 976; G. Badger, R. Harris, R. Jones, and J. Sesse, *ibid.*, 1962, 4329.

[2] G. P. Gurinovich, A. N. Sevchenko, and K. N. Solov'ev, *Optics and Spectroscopy*, 1961, **10**, 396.

[3] M. Gouterman, *J. Mol. Spectroscopy*, 1961, **6**, 138.

[4] M. Sundborn, *Acta Chem. Scand.*, 1968, **22**, 1317.

[5] M. V. Zeller and R. G. Hayes, *J. Amer. Chem. Soc.*, 1971, **95**, 3855; Y. Niea, K. Kobayashi, and T. Tsuchiya, *J. Chem. Phys.*, 1974, **60**, 799.

[6] B. M. L. Chen and A. Tulinsky, *J. Amer. Chem. Soc.*, 1972, **94**, 4144.

[7] C. B. Storm, Y. Teklu, and E. A. Sokoloski, *Ann. New York Acad. Sci.*, 1973, **206**, 631; N. A. Matwiyoff and B. F. Burnham, *ibid.*, 1973, **206**, 365; R. J. Abraham, G. E. Hawkes, and K. M. Smith, *J.C.S. Perkin II*, 1974, 627; C. B. Storm and Y. Teklu, *J. Amer. Chem Soc.*, 1972, **94**, 1745.

[8] R. J. Abraham, G. E. Hawkes, and K. M. Smith, *Tetrahedron Letters*, 1974, **16**, 1483.

[9] R. J. Abraham, G. E. Hawkes, M. F. Hudson, and K. M. Smith, *J.C.S. Perkin II*, 1974, 204.

[10] A. Lapidot and C. S. Irving, in 'Proceedings of the 2nd Internat. Conf. on Stable Isotopes,' ed. P. Klein, USERDA-751027, Argonne, Illinois, 1975.

[11] H. Saito and K. Nukada, *J. Amer. Chem. Soc.*, 1971, **93**, 1072.

[12] W. Bremser, J. I. Kroschwitz, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1969, **91**, 6189.

[13] R. L. Lichter and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 5218.

[14] S. G. Boxer, G. L. Closs, and J. J. Katz, *J. Amer. Chem. Soc.*, 1974, **96**, 7058.

[15] C. S. Johnson, Jr. and G. G. Moreland, *J. Chem. Educ.*, 1973, **50**, 477.

[16] E. F. Caldin, *Chem. Rev.*, 1969, **69**, 135.

[17] A. N. Nesmeyanov, E. B. Zavelovich, V. N. Babin, N. S. Kochetkova, and E. I. Fedin, *Tetrahedron*, 1975, **31**, 1451.

[18] A. N. Nesmeyanov, V. N. Babin, E. B. Zavelovich, and N. S. Kochetkova, *Chem. Phys. Letters*, 1976, **37**, 184.

[19] F. M. Menger, T. D. Singh, and F. L. Bayer, *J. Amer. Chem. Soc.*, 1976, **98**, 5011.