We report here on ^1H NMR experiments on low-spin ferric metMbCN, which demonstrate not only the presence of numerous large NOEs but permit the assignment for the first time of the Ile-99 (-FG5) side chain. metMbCN is a suitable model for MbCO and has the advantage of large magnetic anisotropy whose resulting dipolar field influences both coordinated and noncoordinated residues in the heme pocket.[22,23] The highly resolved upfield portion of the 360-MHz ^1H NMR spectrum of sperm whale metMbCN is illustrated in B of Figure 1. The NOE difference spectrum resulting from saturating peak a is shown in C. Clearly, proton peak f[24] and methyl resonances b and c, as well as some unresolved peak(s) near 0 ppm, exhibit substantial NOEs, indicative of a covalently localized network involving at least two methyls and two single protons.[24] Both Val-E11 and Ile-FG5, as well as Leu-F4, possess such functional groups; the significant line broadening of a,f and b,c eliminates other residues more distant from the iron. The positions of the possible residues are indicated on the projection onto the heme plane in Figure 1A. Saturating individual assigned heme resonances yields zero difference spectra for peaks a, b, c, and f, except for 5-CH_3, which yields a small NOE for peak b (D in Figure 2). Only Ile-99 (-FG5) lies in the proximity of 5-CH_3 but is more directly over pyrrole II with 3-CH_3 and 4-vinyl groups, whose resonances, unfortunately, are unresolved in the crowded diamagnetic region.

Clear identification, however, can be effected by taking advantage of the substitution of deuterohemin (R = H) for hemin (R = vinyl), which yields essentially the same ^1H NMR spectrum whose methyl assignments have established the same heme orientation as the native protein.[5,25] While 3-CH_3 is still obscured in the diamagnetic envelope, the 4-H signal now appears at -15 ppm (A in Figure 2).[25] Saturation of deuterohemin-metMbCN peaks a (B in Figure 2) and 5-CH_3 (D in Figure 2) show the same NOE connectivity as in native metMbCN. However, upon saturation of the deuterohemin-metMbCN peak 4-H (C in Figure 2), significant NOE's are observed for both methyl peaks b and c. Thus the resonances a, b, c, and f in native metMbCN can be definitely assigned to Ile-99 (-FG5).

Our results demonstrate that substantial NOEs are observable even among hyperfine-shifted resonance in paramagnetic hemoproteins and open up the possibility of assigning numerous other important residues in such systems. The unambiguous assignment of Ile-99 (-FG5) in metMbCN now permits investigation of the iron-induced dipolar relaxation, the magnetic coordinate system responsible for the Ile-99 dipolar shifts,[21,22] and the time dependence of the NOEs from which solution spatial orientations and internal mobility of the side chain may be determined.[17,26] Extension of these methods to other side chains may provide insight into the side-chain dynamics considered crucial for the function of oxygen-binding hemoprotein.[27] Variable steric interaction between 4-vinyl and the analogous Val-FG5 have been proposed for the molecular mechanism of cooperativity in hemoglobin.[13] The similarity of the ^1H NMR spectrum of metHbCN[28] to that of metMbCN[4] suggests the present method could be extended to Hb once the relevant heme methyl assignments have been made. Such studies are in progress in this laboratory.

Acknowledgment. This research was supported by grants from the National Science Foundation, CHE-81-08766, and the National Institutes of Health, HL-16087.

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Surface Titration of Silica-Alumina Monitored by Nitrogen-15 NMR with Cross Polarization and Magic-Angle Spinning[1]

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Received July 11, 1983

The characterization of acid sites on solid surfaces is a central problem in catalysis. The total concentration of all acid sites on silica-alumina can be determined by various nonspectroscopic techniques (e.g., standard titration), which are flawed in that they do not distinguish between Brønsted acid sites and Lewis acid sites.[2] Infrared spectroscopy of adsorbed bases such as pyridine is able to distinguish between adsorption at Brønsted sites and adsorption at Lewis sites, but the molar absorbtivities must be estimated.[2-5] In this communication we report an ^{15}N CP/MAS technique that yields independent concentration values for both Brønsted and Lewis acid sites. In addition, this technique is potentially capable of revealing information on the dynamics of molecules adsorbed at the various surface sites. ^{15}N NMR has recently emerged as a promising technique in surface studies.[6-9]

Samples were prepared from silica-alumina[10] that had been activated for 18 h at 160 °C. Following activation, each sample was cooled to room temperature and exposed to a known quantity of 99%-^{15}N-enriched pyridine vapor to give a loading level of 0.19 mmol pyridine/g silica-alumina. On the basis of a reasonable model,[6] this loading is equivalent to a surface coverage of 0.082 monolayer. Each sample was then exposed to a variable but known quantity of *n*-butylamine vapor. Quantitative adsorption was confirmed in all cases by manometer readings. All samples were handled in an inert atmosphere. ^{15}N CP/MAS spectra were obtained at 20.3 MHz on a modified Nicolet NT-200 spectrometer, using a home-built probe. Samples were spun at room temperature at ~2.2 kHz, using dry nitrogen (the boil-off gas from a liquid nitrogen Dewar).

Several samples with *n*-butylamine loading levels ranging from 0 to 11 equiv (relative to pyridine) were examined. Each equivalent of *n*-butylamine is equal to 0.19 mmol/g silica-alumina. The detailed character of the ^{15}N spectra obtained depends markedly on sample history and handling, and reproducibility is difficult. Furthermore, the presence of water in a sample dramatically alters the kinetics of pyridine exchange between surface sites and the relative proportions of sites occupied by pyridine. The ^{15}N CP/MAS spectra of a representative series of samples are shown in Figure 1, which also lists the concentrations of species that we believe to be present in these samples on the basis of our interpretations of the spectra. These interpretations are based on the assumption that *n*-butylamine displaces pyridine from the strongest surface acid sites, with pyridine then occupying the strongest sites not occupied by *n*-butylamine.

The ^{15}N chemical shift of pyridine is a sensitive indicator of chemical environment,[6,7] Natural-abundance *n*-butylamine was

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(10) 75% SiO_2, 25% Al_2O_3, surface area 485 m^2/g by BET.

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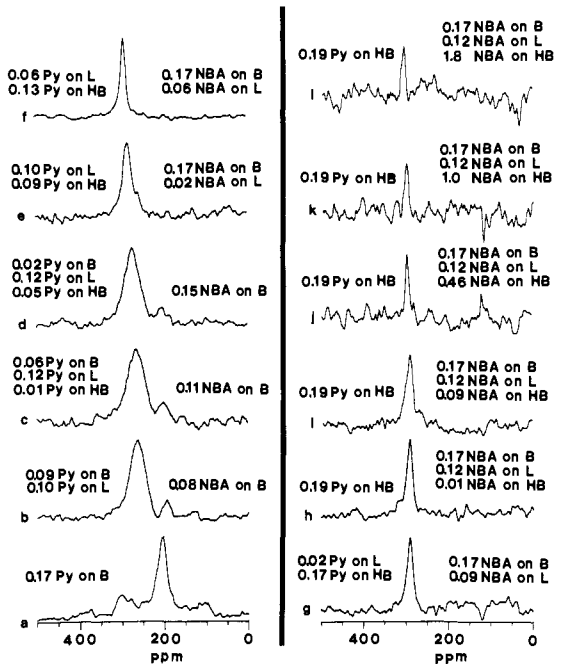

Figure 1. 20.3-MHz ^{15}N CP/MAS NMR spectra of pyridine-^{15}N (Py) adsorbed on silica–alumina in the presence of varying amounts of *n*-butylamine (NBA); 0.19 mmol Py/g silica–alumina (except for 1a for which 0.17 mmol Py/g silica–alumina); mmoles of Py and NBA adsorbed on Brønsted (B), Lewis (L), and hydrogen-bonding (HB) sites indicated numerically. Typical parameters: 2-ms contact time, 1-s repetition time, and 50 000 scans. Chemical shifts in ppm relative to external liquid ammonia (larger numbers, lower shielding). Mole ratio of *n*-butylamine to pyridine: (a) 0; (b) 0.4; (c) 0.6; (d) 0.8; (e) 1.0; (f) 1.2; (g) 1.4; (h) 1.6; (i) 2.0; (j) 4.0; (k) 7.0; (l) 11.

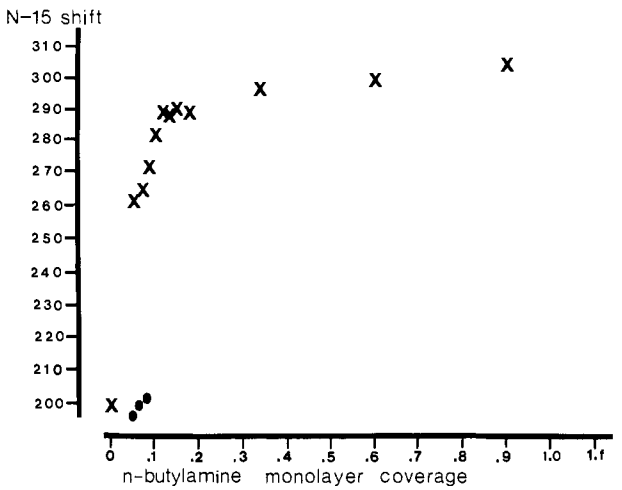

Figure 2. Plot of pyridine ^{15}N chemical shift as a function of total *n*-butylamine surface coverage. (*n*-Butylamine is assumed to occupy the same surface area as pyridine.) For spectra with two clearly-resolved peaks, X represents the major (most intense) peak and ● represents the minor peak.

used in this study, so the ^{15}N spectra contain no signals from this compound. Figure 1a is the spectrum obtained on a sample with no added *n*-butylamine. The ^{15}N chemical shift (198 ppm relative to external liquid ammonia) is characteristic of protonated pyridine,[6,7] indicating that essentially all adsorbed pyridine molecules in this sample are associated with Brønsted acid sites. This observation places a lower limit of roughly 0.17 mmol/g silica–alumina on the concentration of Brønsted acid sites. The two low-intensity signals ~2 kHz to either side of the intense signal are at least largely spinning sidebands. The low relative intensity of these sidebands suggests that molecular motion reduces the ^{15}N powder pattern width substantially (from 782 ppm[11] to less than 400 ppm).

The addition of 0.4–0.8 equiv of *n*-butylamine shifts an increasing fraction of the ^{15}N-resonance intensity of pyridine to 261–271 ppm (Figures 1b–d). When about 1.0 equiv of *n*-butylamine has been added, the pyridine peak associated with Brønsted acid sites is absent (Figure 1e), so an upper limit of roughly 0.19 mmol/g silica–alumina can be established on the concentration of Brønsted sites (strong enough to protonate pyridine). The lower shielding peaks in the pyridine spectra obtained with *n*-butylamine-to-pyridine mole ratios of 0.4–0.8 (Figures 1b–d) are much broader than the main peak in Figure 1a. This broad peak at about 265 ± 5 ppm is characteristic of pyridine coordinated to a Lewis acid site.[6,7]

Spectra of samples with more than 1 equiv of *n*-butylamine show one relatively sharp signal at 289–303 ppm. A chemical shift of 295 ppm is characteristic of hydrogen-bonded pyridine.[6,7] The use of 11 equiv of *n*-butylamine results in a shift of the pyridine resonance to 303 ppm (Figure 1l), a value intermediate between hydrogen-bonded pyridine and that characteristic of neat (liquid) pyridine. The total concentration of adsorbed amine (pyridine + *n*-butylamine) is 2.28 mmol/g silica–alumina for the sample with a *n*-butylamine-to-pyridine mole ratio of 11. Assuming that *n*-butylamine occupies approximately the same surface area as pyridine allows us to calculate a total amine surface coverage for this sample of 0.98 monolayer. With an *n*-butylamine pressure of 30 torr it was found to be impossible to deposit more than 12 equiv of *n*-butylamine on the surface. The latter two observations suggest that this technique can be used to measure the surface area of silica–alumina.

The strong similarities between the results shown in Figure 1 and those of classical titrations prompted us to cast these data in the form of a titration curve, shown in Figure 2. In this "titration" pyridine serves as the indicator and *n*-butylamine as the titrant.

The line widths and relative intensities of the spectra in Figure 1 show wide variations, which can, we believe, be reconciled in terms of molecular motion. The hydrogen-bonded species are expected to be very mobile and have reduced cross-polarization efficiencies. Lewis acid–base complexation requires orbital overlap, a stringent restriction on molecular mobility. We have observed that pyridine adsorbed at Lewis acid sites yields the strongest CP signals, a result consistent with restricted mobility. Further ^{15}N NMR studies of these kinds of systems are in progress.

Acknowledgment. We are grateful to the National Science Foundation for partial support of this work under Grant CHE-8306518 and to the Colorado State University Regional NMR Center, funded by National Science Foundation Grant CHE-8208821.

Registry No. Pyridine, 110-86-1; *n*-butylamine, 109-73-9; silica, 7631-86-9; alumina, 1344-28-1.

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Ethylene Biosynthesis. 2. Stereochemistry of Ripening, Stress, and Model Reactions

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Received July 5, 1983

We recently described models for two potential reactive intermediates in the biosynthesis of the plant growth hormone ethylene.[1] Because of the lability of the natural biosynthetic

(1) See paper 1 in this series: Pirrung, M. C.; McGeehan, G. M. *J. Org. Chem.*, in press.

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