control in the construction of the functionalized cyclopentane framework. The only remaining stereochemical problem is to explore an effective method for allowing the highly selective conversion of the enone side chain into the allylic alcohols that possess the correct, 15*S* configuration. So far tremendous attempts have been made to solve this problem, and a 15*S*/15*R* ratio as high as 92:8 was recorded in the reaction of the enone having a *p*-phenylphenylcarbamoyl protective group and a bulky trialkylborohydride reagent (**8** → **12**)[12] or reduction of the hydroxy enone with diisobutylaluminum 2,6-di-*tert*-butyl-4-methylphenoxide (**9** → **13**).[13] We found that the stereoselectivity displayed by (*S*)-**1** is far superior to that of any of the existing systems currently available. Thus, when the tetrahydropyranyloxy enone **10** was treated with 3 equiv of (*S*)-**1** in THF at −100 °C for 2 h and then at −78 °C for 1 h, there was obtained the 15*S* alcohol **14** of 99.5% stereoisomeric purity[14] in 95% yield. No 1,4-reduction product, the 13,14-saturated ketone, was formed under such conditions. In a like manner, the stereoselective reduction proceeded equally well with the acetoxy enone **10**[7] to afford **15** (99.4% stereochemically pure[14]) in 96% yield. The reaction of the unprotected hydroxy enone **9** gave rise to the 15*S* alcohol **13** *exclusively*, though the isolated yield was modest, 40% (97% yield based on the consumed starting enone).[7,14] Finally, the monocyclic substrate **16** under the standard reduction conditions[7] gave the $PGF_{2\alpha}$ derivative **17** as a single stereoisomer in 76% isolated yield.[14]

The sense and extent of the stereoselection is dependent on the absolute configuration of the binaphthyl moiety in **1.** In the reduction of the THF derivative **10,** for instance, (*S*)-**1** exhibited very high 15*S* stereoselection (15*S*/15*R*, 99.5:0.5), whereas the reduction with the enantiomeric reagent, (*R*)-**1,** showed only moderate, 15*R* selection (15*S*/15*R*, 32:68).[7,14] These observations, coupled with the results obtained with the prochiral substrates **3** and **4,** imply that both the excellent enantioface-recognizing ability of the hydride reagent **1** and diastereomeric influence of the functionalized cyclopentane moieties of the substrates are synergistically operating in exhibiting the exceptionally high stereocontrol.

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(7) The reaction was carried out in THF using 3 equiv of **1** at −100 °C for 2 h and then at −78 °C for 1 h.
(8) Pure (*S*)-(+)-**6** obtained by optical resolution[6] exhibited specific rotation $[\alpha]^{24}_D$ +13.1° (*c* 1.39, CH_3OH). The configuration assignment was made by comparison of the CD curve with that of (*S*)-(+)-**5.**
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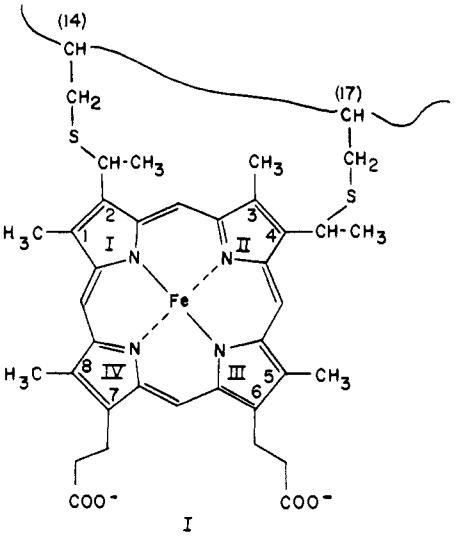
Detection of Localized Conformational Flexibility in Horse Heart Cytochrome *c* by Proton Nuclear Magnetic Resonance

Sir:

Proton nuclear magnetic resonance (^1H NMR) studies of hemoproteins have been found to be extremely useful in characterizing the protein structural and dynamic properties which may influence the heme prosthetic group and hence its function. This is particularly true of paramagnetic hemoproteins where the hyperfine shifted resonances can serve as sensitive probes of protein flexibility in the region surrounding the heme. In the case of ferricytochrome *c*, however, despite extensive NMR studies,[1,2] no direct evidence of protein conformational flexibility in the heme environment has been detected in the physiologically relevant pH range. Detecting and monitoring such flexibility could aid in current investigations devoted to elucidating the changes in conformation that may occur when the protein interacts with its associated oxidase, reductase, and/or peroxidase.

We report here on some preliminary studies on the high-field NMR spectra of horse heart ferricytochrome *c* (cyto c^{III}), which allow characterization of localized protein conformational flexibility in the heme region. The pertinent prosthetic group is depicted in I. The hyperfine shifted region of the 200-



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and 360-MHz spectra of cyto c^{III} are shown in Figure 1.[3] The heme methyl assignments have been proposed based on combined NOE and saturation transfer experiments.[4] The most striking feature of the comparison is the unique variation of line width, δ, of the single Lorentzian signal previously proposed to arise from 3-CH_3. A plot of line width vs. H_0^2 for this methyl is linear for data taken at 100, 200, and 360 MHz. At all fields the line width is independent of protein concentration

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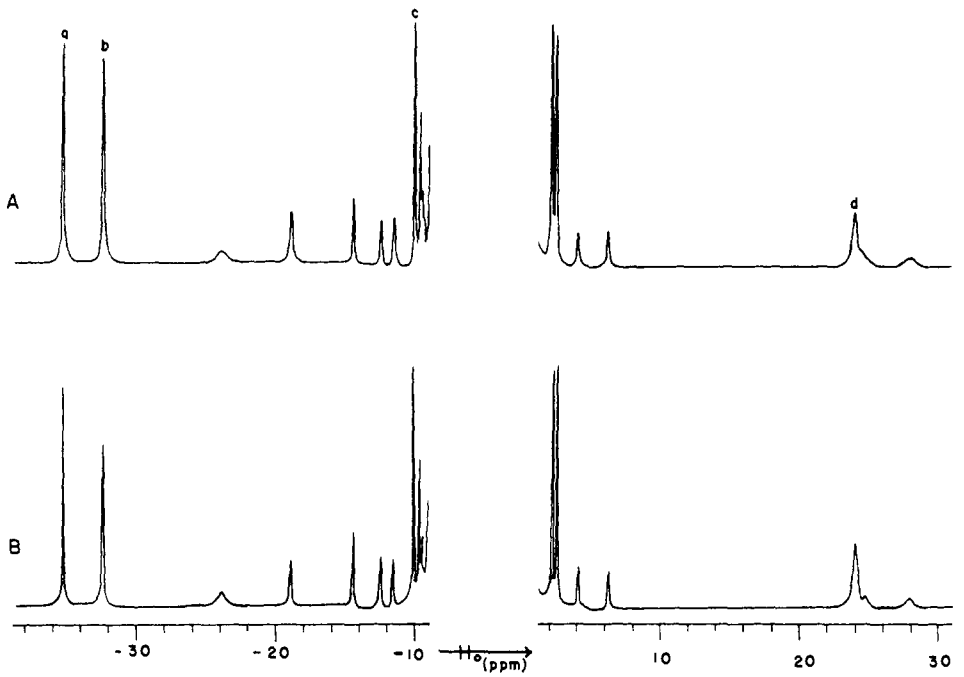

Figure 1. Hyperfine shifted proton resonance regions of cyto c^{III}, pH 5.7 and 25 °C at (A) 200 MHz; (B) 360 MHz. a, b, and c have been assigned to the 8-CH_3, 3-CH_3, and 5-CH_3, respectively; d is the -CH_3 of the coordinated methionine.[4] Line widths are $a = 28$, $b = 33$, $c = 23$, and $d = 100$ Hz at 200 MHz and $a = 33$, $b = 49$, $c = 28$, and $d = 110$ Hz at 360 MHz.

and ionic strength. Since T_1's (180°-t-90°) of the proposed 3- and 8-CH_3 exhibit the expected small increase, while T_2 of the 3-CH_3 decreases dramatically with increasing H_0, line broadening due to dynamic averaging over nonequivalent sites must be occurring in the fast exchange limit. Two likely candidates are hindered methyl rotation and exchange between nonequivalent protein conformations.

Lowering the temperature to 5 °C at pH 5.7 causes further preferential line-width increase of the methyl resonance over all other resolved peaks (A in Figure 2), indicating a slowing of the averaging process. When the pH is raised at 5 °C, the Lorentzian splits into two resolved peaks above pH 6.6 (B in Figure 2). Hence the dynamic process is pH dependent. Although the "split" peak intensities at 5 °C, pH 8.2, are consistent with a rotationally "frozen" methyl group, this possibility is eliminated by observing that the relative intensities change in the pH range 5.0-8.0 (B in Figure 2).

By the addition of methanol-$^2H_4$ to 20% by volume, which has been shown[5] not to significantly perturb the protein conformation, and whose addition does not alter this preferential methyl line broadening, the low-temperature range of the solution can be extended so that the "split" components are resolved over the complete pH range 5-9 at −7 °C. In this pH range, the intensity ratios of the high-field to low-field components vary from 1:1 at low pH to 2:1 at high pH, exhibiting a typical one-proton titration curve with a pK of 6.8. Since the ratio of intensities is essentially independent of temperature at pH 8.2, we estimate that, at pH 6.0 and 25 °C, the equally populated components are interconverting at 10^3 s^{-1}. Moreover, since none of the other 15 resolved hyperfine shifted resonances exhibit significant field-dependent line broadening, the protein conformational change responsible for the two methyl environments must be highly localized, probably involving primarily the amino acid side chain making contact with pyrrole-11, assuming the assignments are correct. The stability of the two conformations is dependent on a titratable residue with a pK of that expected for a histidine.

A similar field-dependent line width at this resonance position is detected in a number of other mammalian cyto c^{III}s but found absent in tuna and *saccharomyces cerevisiae* cyto c^{III}. These findings may be somewhat surprising in light of the

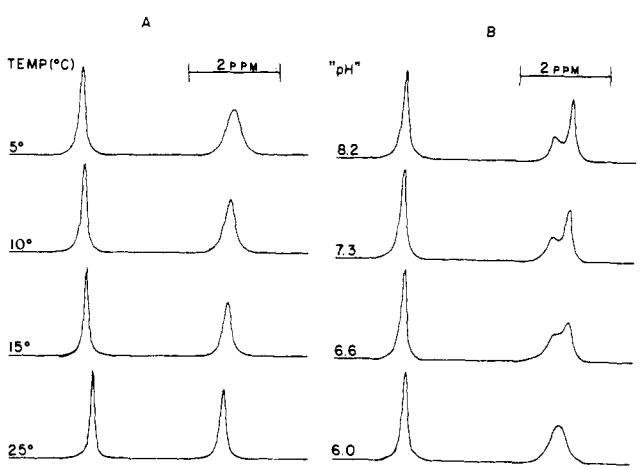

Figure 2. Column A: temperature dependence of the extreme downfield region of the 360-MHz proton spectrum of cyto c^{III} at pH 5.7. Vertical alignment does not indicate a fixed chemical-shift scale; peaks move progressively upfield with increasing temperature. Column B: "pH" dependence of the same region as A at 5 °C. Solvent is ^2H_2O and "pH" is the uncorrected pH.

fact that the heme crevice region is thought to be essentially identical in all of these cytochromes.[6]

Besides the rigid cys-14 through cys-17 peptide sequence which allows for the heme covalent linkage, only phenylalanine-82 (phen-82) makes contact with pyrrole-11.[7] This implies two orientations for phen-82 with respect to 3-CH_3 and requires, at a minimum, a rotation about the C_α-C_β bond.[8] However, how the titration of a histidine (residues 26 and 33 in the horse protein) could account for the pH effect is not exactly obvious owing to the substantial distance between these residues and pyrrole-11.[7]

On the other hand, titration of his-26 may alter the environment of the bottom of the heme crevice around pyrrole-111 (i.e., 5-CH_3) by changing the position of the peptide chain to which it is hydrogen bonded.[9] This peptide chain also contains the only residue alteration that is relatively near the heme, that is, residue 46 which is a phenylalanine in all proteins which exhibit this behavior and a tyrosine in those that do not. These

results may suggest the possibility that the heme methyl groups are misassigned and the field-dependent methyl resonance is the 5-CH_3 not the 3-CH_3. Clearly, more work on a number of cyto c[111] with specific modifications around this region of the heme is in order.

The previously proposed[6] mechanism of electron transport via the heme edge suggests a possible biological relevance for this effect. Alternatively, the different conformations may play a role in the binding of the associated oxidase or peroxidase. Further characterization of this novel conformational flexibility and its kinetic and thermodynamic properties is in progress.

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Simultaneous Emissions Including Intraligand Emission and Charge-Transfer Emission from $[Cu(PPh_3)_2(phen)]^+$

Sir:

Recently, there has been growing interest in metal complexes which exhibit multiple emissions which can be resolved spectrally and temporally. Several systems involving multiple intraligand excited states have been investigated[1] and more recently so have a number of d^6 complexes involving charge transfer (CT) excited states.[2-4] In the case of bis(1,10-phenanthroline)dichloroiridium(III), $Ir(phen)_2Cl_2^+$, emissions assigned to d-d and d-π^* states have been reported,[2] although the results regarding this system have been controversial.[2b,c] Another report has concerned the *fac*-$XRe(CO)_3$(3-benzoylpyridine)$_2$ systems where X is a halogen.[4] The latter systems appear to exhibit emission from d-π^* levels as well as from n-π^* levels of the bichromophoric ligand 3-benzoylpyridine. In the following, we report time-resolved studies of multiple emissions from the d^{10} system $[Cu(PPh_3)_2(phen)]^+$, where PPh_3 represents triphenylphosphine. Upon exciting this system at low temperatures, we have been able to resolve separate emissions with markedly different lifetimes and spectral properties.

The complex was prepared from $Cu(PPh_3)_2NO_3$ as the nitrate salt by the method of Jardine.[5] Recrystallization from ethanol-water containing excess $NaBF_4$ afforded yellow crystals of the tetrafluoroborate salt. Microanalysis showed the complex to be a pure material. (Theoretical percentages: C, 67.40; H, 4.48; N, 3.28. Experimental percentages: C, 67.52; H, 4.32; N, 3.40.) A separate synthesis was carried out by reacting $[Cu(PPh_3)_4]BF_4$ with the phen ligand, and both preparations gave rise to the same emission spectra.

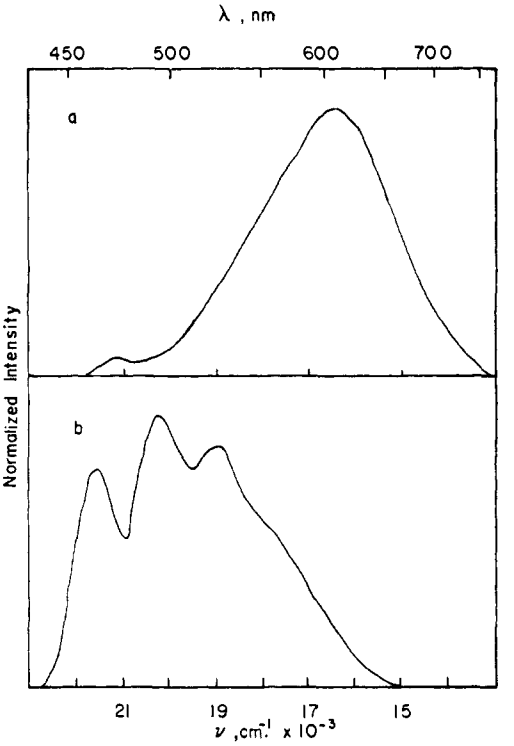

Figure 1. (a) The corrected, total emission spectrum of $[Cu(PPh_3)_2(phen)]^+$ at 77 K, exciting at 370 nm. (b) The corrected spectrum of the long-lived component of the emission detected through the phosphoroscope.

The emission spectra and lifetimes were measured using a 4:1 ethanol-methanol solvent. In the fluorimeter experiments the sample was immersed in liquid nitrogen, while in the laser experiments the sample was cooled to ~90 K using an Air Products cryostat. In either case the sample was cooled below the estimated glass transition temperature of 98 K.[6] In all experiments reported we used freshly prepared samples that had been deoxygenated by repeated freeze-pump-thaw cycles. The methanol (distilled in glass) was used directly as purchased from Burdick and Jackson. Troublesome solvent background emission from impurities in the ethanol was minimized by a careful fractional distillation at reduced pressure. The total emission spectrum was run on a SPEX Fluorolog spectrofluorimeter. The spectrum of the millisecond component, vide infra, of the emission was resolved on an Aminco-Bowman spectrofluorimeter using a phosphoroscope accessory. The instrument used to record the spectra of the shorter lived components will be described elsewhere.[7] Its source is a Phase-R N21K N_2 laser which gives 700-W (peak power), 5-ns (FWHM) pulses at a repetition rate of 30 Hz. The time capabilities of the laser apparatus were calibrated in a standard way using a Stern-Volmer quenching scheme.[8] Sample luminescence was focused through a Corning 0-52 filter (laser scatter attenuation ×1000) into a Jarrel Ash Model 82-405, ¼-m monochromator providing a 4-nm band pass. Emission detection was achieved by an RCA 1P28B photomultiplier specially wired for fast response.[9] Amplification and time resolution was achieved with a Tektronix Model 5S14N sampling oscilloscope for the nanosecond component and a PAR MODEL 162 boxcar integrator for the microsecond component. The lifetime of the phen ligand was measured in

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