Contribution from the Department of Chemistry,
University of Iowa, Iowa City, Iowa 52242

# Rapid Base-Catalyzed Deuterium Exchange at the Ring-Adjacent Methyl and Methylene Positions of Octaalkyl and Natural-Derivative Porphyrins and Metalloporphyrins

Gregory M. Godziela, Susan K. Kramer, and Harold M. Goff*

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An extremely rapid, simple, and inexpensive method is reported for preparation of ring methyl and methylene deuteriated porphyrins and metalloporphyrins. Base-catalyzed ring methyl deuteriation is accomplished at room temperature in an inert atmosphere by the addition of the porphyrin derivative and base to dimethyl-$d_6$ sulfoxide ($Me_2SO$). The ring methyl groups of the iron(III) complexes of etioporphyrin, protoporphyrin IX, deuteroporphyrin IX, and mesoporphyrin IX were at least 74% and in most cases >95% deuteriated in 24 h, with minimal (<5%) porphyrin degradation. The dimethyl ester complexes of the natural-derivative porphyrin complexes also underwent facile deuterium exchange, but the porphyrins were quickly deesterified in the basic solution. Either tetrabutylammonium hydroxide (1 M in methanol) or potassium *tert*-butoxide served as the base. When tetrabutylammonium hydroxide ((TBA)OH) was used as the base, the amount of methanol present in solution was found to be important. Some methanol is presumably required to solvate the hydroxide ion. Both the iron(III) and manganese(III) etioporphyrin I chloride complexes were deuteriated at the ring-adjacent positions by this method. More rigorous conditions were needed to deuteriate the ring methylene positions of octaalkylporphyrins. The ring methylene positions of free-base octaethylporphyrin ($H_2OEP$) were exchanged in refluxing $Me_2SO$-$d_6$ under a nitrogen atmosphere. After a 24-h reflux, 50% of the $H_2OEP$ was recovered and was found to be at least 95% deuteriated at the ring methylene positions. No significant methine or $\beta$-methyl deuteriation (<5%) had occurred.

## Introduction

Deuterium incorporation at specific positions allows for ready assignment of vibrational bands and NMR signals in porphyrins and metalloporphyrins. Label incorporation can be accomplished either by total synthesis or by an exchange of one or more sites on the assembled porphyrin. Total synthesis of deuteriated tetraarylporphyrins is practical in view of their formation by simple one-step condensation of pyrrole and aryl aldehydes.[1] The multistep synthesis of octaalkyl and natural porphyrins dictates the importance of a simple exchange pathway on the preformed porphyrin or metalloporphyrin.

Deuterium labeling of synthetic octaalkylporphyrins has been largely restricted to the study of exchange reactions at the meso positions.[2-4] A single report has been offered for deuterium exchange at ring-adjacent positions of these porphyrins.[5] Deuterium incorporation at both the ring methyl and ring methylene positions was accomplished along with meso exchange in acid solution. We report here a much more facile base-catalyzed deuterium-exchange method for ring methyl and ring methylene labeling of octaalkylporphyrins and the metalated derivatives.

Labeling of natural porphyrin derivatives at the meso positions by deuteriated acids[6,7] or Lewis acids in the presence of a deuterium source is also possible,[8] but no electrophilic deuteriation at any of the ring methyl positions has been reported. Deuterium incorporation at the ring methyl positions of protoporphyrin IX dimethyl ester (see Figure 1 for structures) has been accomplished by total synthesis of the molecule.[9] Evans et al.[10] have shown that the methyl groups adjacent to the vinyl groups of protoporphyrin IX dimethyl ester could be selectively deuteriated, but the authors were unable to label any of the ring methyl positions of mesoporphyrin IX dimethyl ester. We report here a simple, rapid, and inexpensive method for deuteriation of all four ring methyl groups of hemin compounds and their corresponding dimethyl ester derivatives.

## Materials and Methods

Tetrabutylammonium hydroxide ((TBA)OH), as a 1 M solution in methanol, was purchased from Aldrich Chemical Co. Etioporphyrin I ($H_2$ETIO) and octaethylporphyrin ($H_2OEP$) were synthesized by literature methods.[11] Iron or manganese was inserted by the dimethylforamide-reflux method[12] with anhydrous ferrous chloride or manganous acetate, respectively. Zinc was incorporated into $H_2$ETIO by the chloroform-reflux method with zinc acetate.[13] Iron protoporphyrin (hemin) was purchased from Aldrich. Deuterohemin[13] and mesohemin[14] and the respective dimethyl ester derivatives[15] were prepared by literature methods.

Proton and deuterium NMR spectra were recorded at 25 °C on a Bruker WM-360 FT spectrometer operating at 360 and 55 MHz, respectively. Signal positions were referenced to $Si(CH_3)_4$, and the solvent signal was used as a secondary reference. The solvent deuterium signals for $CHCl_3$ ($CDCl_3$), $CH_2Cl_2$ ($CHDCl_2$), and $Me_2SO$ were taken as 7.24, 5.32, and 2.49 ppm, respectively.

The extent of deuteriation was measured by integration of the proton NMR spectra. Porphyrin reference peaks were chosen after deuterium NMR spectra of the exchanged material showed that no significant deuterium incorporation had occurred at these positions. These peaks included the $\beta$-$CH_3$ signal of (ETIO)FeCl, the $\beta$-$CH_3$ signal of $H_2OEP$, and the 6,7-$\alpha$-$CH_2$- signals of the natural iron porphyrin derivatives.

Deuterium-exchange reactions of (ETIO)FeCl and the natural porphyrins were conducted under an inert atmosphere in order to avoid isotopic dilution by atmospheric moisture and air oxidation of the ferrous porphyrin species (the iron(III) porphyrin in $Me_2SO$ solution is immediately reduced upon addition of the base). Deuteriation of the ring methyl groups of (ETIO)FeCl was accomplished by dissolution of the solid material in $Me_2SO$-$d_6$ (typical concentrations were 6 mM) followed by the addition of 2–5 equiv of (TBA)OH (as a 1 M solution in methanol) with a micropipet. At various times aliquots of the reaction were quenched by the addition of $CHCl_3$ and 1 M aqueous HCl. Following removal of the acid layer, the chloroform solution was washed with distilled water three times. The chloroform was evaporated under a nitrogen stream, and any remaining water was removed in a vacuum desiccator at room temperature. The iron(III) porphyrin was dissolved

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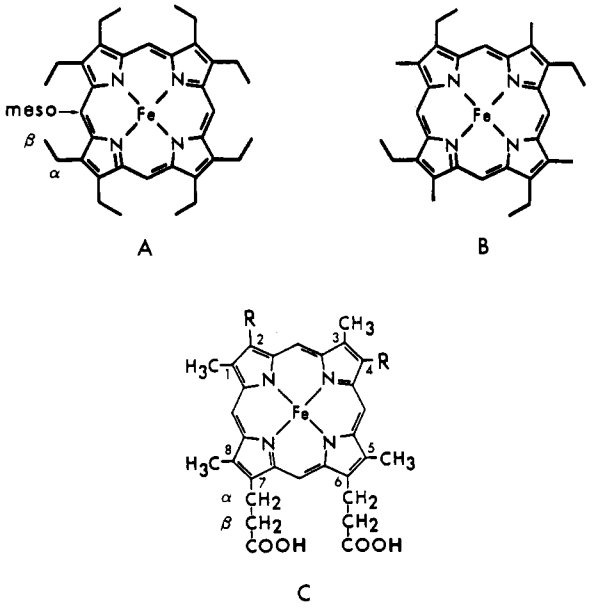


**Figure 1.** Structures of iron porphyrins (axial ligands and charges not included): (A) iron octaethylporphyrin; (B) iron etioporphyrin I; (C) natural porphyrin derivatives (R = $-CH{=}CH_2$, protohemin; R = H, deuterohemin; R = $-CH_2CH_3$, mesohemin).

in $CHCl_3$ and purified by passage through an alumina column.

Ring methylene deuteriation of $H_2OEP$ was accomplished by the addition of 5 equiv of (TBA)OH to 50 mg of porphyrin in 10 g of $Me_2SO$-$d_6$. After being purged with $N_2$ for 30 min, the solution was brought to reflux for the desired length of time. Following the reflux period, the solution was allowed to cool, and the free-base porphyrin was recovered by the procedure used for (ETIO)FeCl described above.

Protohemin, deuterohemin, and mesohemin as the chloride complexes and mesohemin dimethyl ester as the sulfate complex were subjected to deuterium-exchange conditions as 6 mM solutions in $Me_2SO$-$d_6$ containing from 3 to 7 equiv of (TBA)OH. The reactions were quenched at the appropriate times through precipitation of the iron porphyrin by the addition of 1 M aqueous HCl. The iron porphyrin was separated by centrifugation (10 min at 7000 rpm), and the solid was rinsed twice with distilled water. The wet solid was dried at room temperature in a vacuum desiccator. Solutions of the iron porphyrins to be examined by proton NMR spectroscopy were prepared by dissolution of the dry solid in 0.3 mL of $Me_2SO$-$d_6$, with subsequent addition of 10 μL of a 2.0 M solution of KCN in $D_2O$ (at least a 6-fold excess of $CN^-$). Samples for deuterium NMR spectroscopy were prepared by dissolution of the dry solid in 0.3 mL of $Me_2SO$, with subsequent addition of 10 μL of a 2.0 M solution in KCN in $H_2O$.

## Results and Discussion

**Synthetic Octaalkylporphyrin Derivatives.** Addition of 3 equiv of (TBA)OH to a solution of (ETIO)FeCl in $Me_2SO$-$d_6$ causes immediate one-electron reduction of the iron(III) center.[16] A proton NMR spectrum of this solution, recorded approximately 1 h after mixing, shows ring methyl and ring methylene signals in the 12–17 ppm range, consistent with known spectra for other high-spin iron(II) porphyrins.[17,18] However, the intensity of the ring methyl signal is considerably smaller than expected (as compared to the ring methylene signals). A deuterium NMR spectrum of this species in $CHCl_3$ reveals signals for the ring methyl and ring methylene deuterons, indicating that a facile deuterium exchange has occurred with the solvent. Base-catalyzed deuteriation of (ETIO)FeCl in $Me_2SO$-$d_6$ also occurs when excess solid potassium *tert*-butoxide is used as the base.

The relatively faster rate for ring methyl as compared to ring methylene exchange is readily explained by differences in proton acidity. A study dealing with the acidity of benzylic hydrogens shows that those of toluene are 9 times more acidic than those of ethylbenzene.[19] The faster ring methyl deuteriation can be directly attributed to the greater acidity of these protons.

**Table I.** Room-Temperature Deuterium Exchange of (ETIO)FeCl as a Function of Base Concentration[a]

| equiv of base | reacn time, h | %[b] α-$CH_3$ deuteriation | %[b] α-$CH_2$– deuteriation |
|---|---|---|---|
| 2 | 5 | 76 | 36 |
| 3 | 5 | 79 | 27 |
| 4 | 5 | 77 | 22 |
| 5 | 5 | 58 | 13 |
| 2[c] | 24 | 64 | 35 |
| 3[c] | 24 | 69 | 40 |
| 4[c] | 24 | 59 | 38 |

[a] Exchange reactions were run in $Me_2SO$-$d_6$ solutions. The solutions were 6–7 mM in porphyrin, and tetrabutylammonium hydroxide (1 M in methanol) was used as the base. [b] Deuteriation percentages were obtained by integration of the proton NMR spectra (6–7 mM in $CD_2Cl_2$ at room temperature), and Uncertainties are ±5%. [c] Methanol was removed under Ar prior to reaction commencement.

The deuterium exchange of the ring-adjacent protons of (ETIO)MnCl, (ETIO)Zn, and $H_2$ETIO was also evaluated under the same reaction conditions (3 equiv of (TBA)OH in $Me_2SO$-$d_6$). At room temperature the manganese compound showed partial deuterium exchange after 1 h, whereas the zinc compound and the free-base porphyrin showed no (<5%) ring methyl deuterium exchange during this period. Increasing the temperature to 75 °C and the reaction time to 2 days allowed for partial deuterium exchange of the free-base porphyrin. Hence, the apparent exchange rates are metal ion dependent, but no absolute requirement for a redox-active metal ion is found.

Factors that might affect the deuterium-exchange rates and specificity of exchange were systematically investigated. The room-temperature deuterium-exchange profile for ring-adjacent positions of (ETIO)FeCl as a function of base concentraion is shown in Table I. In reactions where methanol was present, 2–4 equiv of base had no effect on the degree of deuteriation at the ring methyl position within 5 h. Addition of 5 equiv of base caused a decrease in the fraction of ring methyl deuteriation. Under the same conditions, as the base concentration was increased, the percent deuteriation at the ring methylene position was also dramatically decreased. In another set of experiments, the (TBA)OH was transfered to a vial and the methanol was removed with an argon stream. $Me_2SO$-$d_6$ was then added to the dry base, and this basic solution was added to the porphyrin. In this instance the rate of deuteriation at the ring methyl positions was slightly decreased, but exchange at the ring methylene positions remained essentially constant for 2–4 equiv of base. The effect of added $D_2O$ on the deuteriation reaction was also investigated. Addition of up to 0.5% $D_2O$ (on a volume basis) did not quench the deuterium-exchange reaction.

Dimethyl sulfoxide cannot form hydrogen bonds with an anionic species such as the hydroxide ion. Thus, the activity of hydroxide ion is greater in $Me_2SO$ than in a solution in which it is partially solvated by hydrogen bonding. The presence of a strong base in $Me_2SO$ induces formation of small amounts of methylsulfinyl carbanion (the conjugate base of $Me_2SO$).[20] In solutions of potassium methylsulfinyl the kinetic base strength reaches a maximum at a 2:1 ratio of alcohol to base and drops off as more methanol is added.[21] Hence, slower exchange rates for metalloporphyrin side chains with either large excesses of (TBA)OH–methanol or a lack of methanol may be explained by methanol solvation of $OH^-$. Base-catalyzed exchange appears to be most efficient when there is at least some solvation of the hydroxide ion.

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**Table II.** Room-Temperature Deuterium Incorporation at the Ring Methyl Positions of Natural Porphyrin Derivatives[a]

| porphyrin | equiv of base added[b] | methyl position[c] | % deuteriation 6 h | 12 h | 24 h |
|---|---|---|---|---|---|
| protohemin | 3 | 8 | 29 | 44 | 43 |
| | | 5 | 36 | 51 | 59 |
| | | 3 | *d* | *d* | *d* |
| | | 1 | 93 | *d* | *d* |
| protohemin | 5 | 8 | 61 | 68 | 80 |
| | | 5 | 63 | 69 | 77 |
| | | 3 | *d* | *d* | *d* |
| | | 1 | 94 | *d* | *d* |
| deuterohemin | 5 | 8 | 80 | 94 | *d* |
| | | 5 | 52 | 92 | *d* |
| | | 3 | 95 | *d* | *d* |
| | | 1 | *d* | *d* | *d* |
| mesohemin | 5 | 8, 5 | 84 | 92 | *d* |
| | | 3 | 93 | *d* | *d* |
| | | 1 | 94 | *d* | *d* |
| mesohemin dimethyl ester | 7 | 8, 5 | 37 | 46 | 55 |
| | | 3 | 71 | 79 | 83 |
| | | 1 | 71 | 77 | 82 |

[a] Deuteriation percentages were obtained by integration of the proton NMR spectra of the dicyano derivative ($Me_2SO$-$d_6$ solvent, room temperature), and uncertainties are ±5%. [b] Tetrabutylammonium hydroxide (1 M in methanol) was used as the base. [c] Assignments were made by comparison with spectra of the same species in $CD_3OD$. [d] The signal was too small to integrate accurately (>95% deuteriation).

The slow, room-temperature deuterium-exchange rates at the ring methylene positions of (ETIO)FeCl indicated that more rigorous conditions were needed to obtain nearly quantitative deuterium exchange at the ring methylene positions. This is demonstrated for the metal-free $H_2OEP$ compound. Ten grams of $Me_2SO$-$d_6$ was added to 50 mg of $H_2OEP$ and 5 equiv of (TBA)OH (in methanol), and the solution was brought to reflux under nitrogen. After a reflux period the free-base porphyrin was removed from $Me_2SO$, and the product was purified on an alumina column. After a 5-h reflux, approximately 40% of the porphyrin had been degraded and the purified $H_2OEP$ was 37% deuteriated at the ring methylene positions. After a 24-h reflux, approximately 50% of the porphyrin had been degraded and the remaining purified $H_2OEP$ was >95% deuteriated at the ring methylene positions. A deuterium NMR spectrum of the product revealed that no β-methyl or meso deuteriation had occurred.

**Natural Porphyrin Derivatives.** Results of deuterium-exchange reactions for the natural porphyrin derivatives are described in Table II. Specific methyl group assignments have been reported for methanol[22] and $Me_2SO$[23] solutions of the hemin cyanide complexes. It is apparent that the use of 5 equiv of (TBA)OH at ambient temperature is often sufficient to exchange >95% of the ring methyl protons of the hemins in 24 h. The material recovered after 24 h showed that no major porphyrin degradation had occurred (<5%) for any of the porphyrins. As expected, hemin dimethyl esters were deesterified in the basic $Me_2SO$ solution. The progressive proton NMR spectra for the mesohemin deuterium-exchange reaction are shown in Figure 2. For purposes of NMR analysis the isolated iron porphyrin was converted to the dicyano complex in order to generate sharp, well-defined ring methyl and methylene signals. Each methyl group gives three signals as partial deuteriation is achieved. This has been reported previously by Evans et al.[10] and is attributed to differential chemical shifts for $-CH_3$, $-CH_2D$, and $-CHD_2$ due to isotope effects.[24]

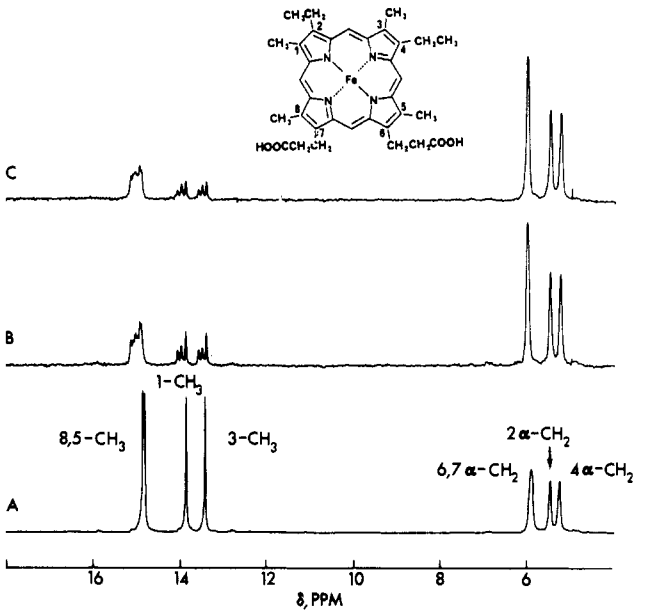


**Figure 2.** Downfield region of proton NMR spectra of (mesohemin)-$(CN)_2^-$ isolated from the exchange reaction with 7 equiv of (TBA)OH in $Me_2SO$-$d_6$ at ambient temperature. Spectra were recorded at 25 °C in $Me_2SO$-$d_6$, and all peaks are referenced to $Si(CH_3)_4$. Exchange times: (A) 0 h; (B) 6 h; (C) 12 h.

Deuterium NMR spectra of each of the resulting hemins showed signals for the ring methyl positions and no significant signals (<5% exchange) due to the 6,7-α-$CH_2$- groups. Also, the deuterium spectrum of (deuterohemin)$(CN)_2^-$ showed no indication of deuteriation at the ring 2- and 4-protons, in which case signals in the upfield region would be anticipated.

Methyl group exchange rates were compared for two concentrations of (TBA)OH. As the amount of base added to a $Me_2SO$-$d_6$ solution of protohemin was decreased from 5 to 3 equiv, the exchange rates of the 8- and 5-methyl groups decreased, while those of the 1- and 3-methyl groups remained invariant (and essentially completely exchanged in 24 h). Two equivalents of base must be consumed by the iron porphyrin carboxyl protons (as well as by the deesterification reaction). Hence, the addition of 3 molar equiv of hydroxide ion would leave only 1 equiv for the base-catalyzed exchange process. Although exchange of protohemin methyl protons does take place under such conditions, addition of 5 equiv of base speeds the exchange. As seen in Table II, use of 7 equiv of (TBA)OH in methanol appears to slow the exchange rate for the mesohemin dimethyl ester complex (the ester linkages presumably are displaced rapidly and hence do not perturb the net exchange seen over a 24-h period).

## Conclusion

Optimal (TBA)OH concentrations appear to differ for the synthetic tetraalkylporphyrins and the natural-derivative porphyrins in view of the fact that hemins consume 2 equiv of base for deprotonation of the carboxyl groups. Three equivalents of 1 M (TBA)OH in methanol is recommended for the synthetic tetraalkylporphyrin derivatives, and 5 equiv appears to be optimal for the natural iron porphyrin derivatives.

The ambient-temperature exchange conditions or relatively short heating periods required for the base–$Me_2SO$-$d_6$ exchange technique are much more attractive than the 8-day *o*-dichlorobenzene/deuteriotoluenesulfonate reflux method. Moreover, recovery of product from $Me_2SO$ is rather trivial, as compared to that from the vacuum distillation required for the *o*-dichlorobenzene solvent. Direct application to the metalated porphyrin rather than to the free-base porphyrin (as is the case for the acid-exchange method) is also most desirable.

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