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Supplementary Material Available: Physical and spectral data of the adducts (**3a**, **3b**, and **3c**) and photoelectron spectrum of **2** (2 pages). Ordering information is given on any current masthead page.

Demethylation of *N*,*N*-Dimethylaniline and *p*-Cyano-*N*,*N*-dimethylaniline and Their *N*-Oxides by Cytochromes $P450_{LM2}$ and $P450_{CAM}$

David C. Heimbrook, Ralph I. Murray, Karen D. Egeberg, and Stephen G. Sligar*

Department of Biochemistry
University of Illinois
Urbana, Illinois 61801

Michael W. Nee and Thomas C. Bruice*

Department of Chemistry
University of California at Santa Barbara
Santa Barbara, California 93106

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Cytochrome P450 catalyzes the efficient oxidation of secondary and tertiary amines, providing an important pathway of xenobiotic metabolism. The chemical mechanism of oxidative amine dealkylation has been biomimetically studied by using (*meso*-tetraphenylporphinato)iron(III) chloride ((TPP)$Fe^{III}Cl$) and the exogenous oxidants *N*,*N*-dimethylaniline *N*-oxide (DMANO)[1] and *p*-cyano-*N*,*N*-dimethylaniline *N*-oxide (*p*-CNDMANO).[2] To help clarify the enzymatic mechanism of cytochrome P450 dependent amine dealkylation reactions, we have tested amine *N*-oxides as oxygen donors to purified rabbit liver cytochrome $P450_{LM2}$[3] and bacterial cytochrome $P450_{CAM}$.[4] Intra- and intermolecular isotope effects on amine dealkylation were used to compare the mechanism of these exogenous oxidant supported reactions with that of the pyridine nucleotide/O_2 dependent ones.

Several conceptual pathways for enzymatic dealkylation of amines should be considered; direct hydroxylation of the methyl carbon, with or without formation of an intermediate *N*-oxide, or electron-transfer oxidation of the nitrogen is the most probable of the proposed mechanisms.[5] To distinguish between these various reaction pathways we have synthesized *N*,*N*-dimethylaniline (DMA) and *p*-cyano-*N*,*N*-dimethylaniline (*p*-CNDMA) with specific deuterium contents: *N*-methyl-*N*-(trideuteriomethyl)aniline (DMA-D_3); *p*-cyano-*N*-methyl-*N*(trideuteriomethyl)aniline (*p*-CNDMA-d_3); and their corresponding *N*,*N*-bis(trideuteriomethyl) compounds (DMA-d_6 and *p*-CNDMA-d_6). The deuterated dimethylaniline *N*-oxides DMANO-d_3, *p*-CNDMANO-d_3, DMANO-d_6, and *p*-CNDMANO-d_6 were synthesized from the corresponding dimethylanilines by oxidation with *m*-chloroperoxybenzoic acid.[7]

Table I. Isotope Effects on Oxidative Demethylation by Cytochrome P450

	exogenous oxidant		NAD(P)H + O_2	
	p-CNDMANO	DMANO	*p*-CNDMA	DMA
$P450_{LM2}$				
intermolecular (d_0 vs. d_6)	1.1	1.0	1.0	1.0
intramolecular (d_3)	2.0	3.0	3.9	2.6
$P450_{CAM}$				
intermolecular (d_0 vs. d_6)	1.7	1.4	*a*	*a*
intramolecular (d_3)	2.5	4.3	*a*	*a*

[a] Due to the extreme substrate specificity of this enzyme, $P450_{CAM}$ will not catalyze the demethylation of dimethylaniline. Statistical errors on all isotope effects average ±8%.

Purified rabbit liver cytochrome $P450_{LM2}$ was found to efficiently utilize the exogenous oxidant DMANO, generating *N*-methylaniline (NMA) and formaldehyde in a second-order reaction with a velocity of 0.0231 turnovers min^{-1} mM^{-1}. Negligible amounts of heme oxidation were observed in contrast to results obtained with peroxides, peroxy acids, and iodosylbenzene. No saturation of the *N*-demethylation reaction was observed over the concentration range 0.2–50 mM. At very high concentrations of *N*-oxide, a significant autocatalytic effect was observed with the reaction velocity increasing with time.[8] Cytochrome $P450_{CAM}$ was also observed to efficiently catalyze the demethylation of DMANO and *p*-CNDMANO. Presumably due to its substrate specificity, cytochrome $P450_{CAM}$ has not been shown to oxidatively dealkylate tertiary amines. On mixing DMANO with $P450_{CAM}$, linear production of NMA and formaldehyde was observed over a wide range of concentrations, from 0.2 to 50 mM. No aniline or dimethylaniline was produced in the reactions with either $P450_{LM2}$ or $P450_{CAM}$ suggesting that the N-demethylation reaction occurs very rapidly after the generation of the active oxygen intermediate, in contrast to results obtained in similar experiments with (TPP)$Fe^{III}Cl$.[1,2] This hypothesis is supported by the observation that the normal substrate of $P450_{CAM}$, camphor, is not hydroxylated when the *N*-oxides are used as exogeneous oxidants. The addition of the electron-withdrawing *p*-cyano group would be expected to increase the oxygen-transfer potential of the aniline *N*-oxide and also to deactivate the nitrogen to electron-abstraction reactions.[2] Substitution of *p*-CNDMANO for DMANO, however, did not significantly alter the kinetics of the demethylation reaction or allow the hydroxylation of camphor, suggesting that the resulting *p*-CNDMA did not have time to leave the cage following the transfer of the oxygen of *p*-CNDMANO to iron.

To further probe the mechanisms of oxygen atom transfer by this oxidant, intra- and intermolecular isotope effects were quantified using DMANO-d_3 [$C_6H_5N(CH_3)(CD_3)O$] and a mixture of DMANO-d_6/DMANO-d_0 (1:1). In addition, the NADPH/O_2 dependent demethylations of DMA-d_3 and of a mixture of DMA-d_0/DMA-d_6 (1:1) were determined for cytochrome $P450_{LM2}$ (Table I). In these experiments an intramo-

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(7) Craig, J. C.; Purushothaman, K. K. *J. Org. Chem.* **1970**, *35*, 1721–1722.
(8) The increase in reaction velocity was observed by monitoring production of DMA via HPLC, GC, and UV spectrophotometry and by monitoring production of formaldehyde via the Nash reaction.[11] Detailed studies were performed to determine the source and mechanism of this autocatalytic effect. These studies included measuring the effects of *N*-methylaniline, lipid, oxygen, and buffer composition on the catalysis. Anaerobisis or changes in buffer composition had no effect on reaction velocity or autocatalysis. Reactions performed in buffer containg the product of the reaction, *N*-methylaniline, showed a small increase in the initial velocity of the reaction, but this increase was not significant enough to explain the observed autocatalysis. Lipid concentration effects were measured and showed the expected dependence with maximal reaction velocities obtained at 30 μg DLPC per mL. Higher or lower concentrations did not eliminate autocatalysis. The origin of this effect at very high *N*-oxide concentrations is unclear, and all reported velocities are derived from the initial slope of product formation, which was linear for over 4 h.

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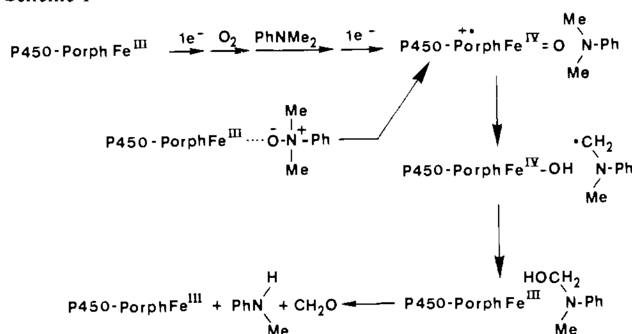
Scheme I



lecular isotope effect is defined as the preferential removal of the protio- vs. the deuteriomethyl group when both are present in the same molecule of aniline. Intermolecular isotope effects occur as the result of preferential N-demethylation of the d_0 vs. the d_6 substrate when both substrates are present in the reaction mixture, i.e., an isotope competition experiment. Demethylation of both DMANO and p-CNDMANO proceeded with a significant intramolecular isotope effect with both cytochrome $P450_{CAM}$ and $P450_{LM2}$, suggesting that carbon–hydrogen bond breaking is contributing to the rate-limiting step of demethylation. The porphyrin model system showed comparable intramolecular isotope effects.[2,9] It is also evident that $P450_{CAM}$ displays a larger preference for hydrogen abstraction from CH_3 vs. CD_3 than does $P450_{LM2}$. Small but significant intermolecular isotope effects were observed with $P450_{CAM}$, while this is effectively masked in the $P450_{LM2}$ reactions.

Cytochrome $P450_{LM2}$ will also catalyze the oxidative demethylation of dimethylaniline (K_m = 126 μM, V_m = 16.3 nmol/min/nmol P450) and p-cyanodimethylaniline (K_m = 50 μM, V_m = 7.7 nmol/min/nmol P450) in the presence of NADPH, molecular oxygen, and saturating amounts of NADPH–cytochrome P450 reductase. Both the intra- and intermolecular isotope effects observed with these reactions are similar to those seen with the corresponding *N*-oxides, suggesting that the active hydrogen-abstracting species generated in both cases are similar. These results are also consistent with those reported for the demethylation of *N*,*N*-dimethylphentermine by purified rat liver P450.[10] If the observed intramolecular isotope effects for the demethylation of DMA and p-CNDMA by cytochrome P450, through the pyridine nucleotide/O_2 dependent reaction, vs. the *N*-oxide-supported case had been substantially different it would imply that the *N*-oxide could not be an intermediate prior to hydrogen abstraction in the reaction path. The fact that these two isotopes effects are of the same magnitude does not preclude the formation of an *N*-oxide intermediate in the normal NADPH/O_2 reaction, but neither does it require such an intermediate. It does suggest that the species active in carbon–hydrogen bond scission is similar in both cases. Thus, these studies point to reaction of exogenously supplied *N*-oxide via oxygen atom transfer to the heme iron, followed by abstraction of a methyl hydrogen and subsequent oxygen radical capture to generate a carbinolamine intermediate, which decomposes to the observed products. The lack of significant intermolecular isotope effects in demethylation of dimethylanilines by the reconstituted cytochrom P-450 systems and in demethylation of dimethylaniline *N*-oxides by the iron(III) cytochrome P450's must be due to a commitment to reaction that occurs prior to C–H bond breaking. These commitment steps may involve (Scheme I) the formation of the reactive cytochrome P450 species following the second 1e$^-$ transfer from reductase and oxygen transfer from *N*-oxide to P450-PorphFe^{III}. The significant intramolecular isotope effects show that bond breaking is at least partially rate determining in the demethylation step. The inability of either *N*-oxide to support normal substrate oxygenation is probably related to the proximity and orientation effects common to all enzymes, which would produce an enormous enhancement of the rate of N-demethylation relative to the rate of dissociation of the heme/oxygen–dimethylaniline complexes.

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Two-Dimensional Exchange NMR in Static Powders: Interchain ^{13}C Spin Exchange in Crystalline Polyethylene

Hommo T. Edzes* and Jan P. C. Bernards

Department of Molecular Spectroscopy
Faculty of Science, University of Nijmegen
Toernooiveld, 6525 ED Nijmegen, The Netherlands
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Two-dimensional (2D) NMR spectroscopy has established itself as a valuable method for the study of exchange processes. In solution,[1] in powdered solids under magic angle spinning conditions,[2] and in single or plastic crystals[3] exchange is measured between well-resolved peaks. We here want to demonstrate the application of 2D exchange NMR to a powdered solid with chemical shielding anisotropy (2DECSA). The experiment exploits the dependence of the nuclear resonance frequency on the molecular orientation. The time dependence of spin exchange or molecular jumps and the change in orientation of the chemical shielding tensor can be studied on a time scale of the order of 10^{-2}–10^2 s.

A detailed description of 2D exchange NMR can be found elsewhere.[1,2,4] The pulse sequence used is shown in Figure 1. The measured 2D NMR spectrum $F(\omega_1,\omega_2;\tau)$ represents the correlation function between the NMR resonance frequencies ω_1 before and ω_2 after an exchange time τ. Any exchange process that couples different resonance frequencies ω_1 and ω_2 will manifest itself by off-diagonal signal intensity in the 2D spectrum.

In the case of anisotropic chemical shielding the nuclear resonance frequency depends on the molecular orientation and is given by

$$\omega = \omega_0(1 - \lambda_{11}^2\sigma_{11}^2 - \lambda_{22}^2\sigma_{22}^2 - \lambda_{33}^2\sigma_{33}^2) \quad (1)$$

where ω_0 is the chemical shift reference frequency, the σ_{ii} are the principal elements of the chemical shielding tensor, and the λ_{ii} are the direction cosines of the angles between the magnetic field and the tensor principal axes. In a powder all orientations contribute to the spectrum, which shows a characteristic line shape (Figure 2a) with discontinuities at frequencies ω_{ii} that correspond to the σ_{ii} values. An exchange process involving a change in molecular orientation alters the resonance frequency. The resulting 2D powder spectrum[4] typically contains a large signal along the diagonal from molecules that are in the original orientation, whereas exchange is reflected by off-diagonal ridges and humps. The off-diagonal pattern is specific for the change in orientation of the shielding tensor and allows for a detailed characterization of the exchange process.

2DECSA NMR spectra for the ^{13}C methylene resonance of polyethylene are shown in Figure 2. The spectra reflect mainly

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