Dealkylation of *N*-Nitrosodibenzylamine by Metalloporphyrin/Oxidant Model Systems for Cytochrome P450

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***N*-Nitrosodialkylamines are alkylating carcinogens which are metabolically activated to α-hydroxy nitrosamines through oxidative dealkylation by cytochrome P450. In this study, the dealkylation step in the activation of *N*-nitrosodibenzylamine (NDBz) was investigated with metalloporphyrin/oxidant model systems under non-aqueous conditions, as biomimetic chemical models of cytochrome P450. In the model systems, NDBz was dealkylated and benzaldehyde was released. The catalytic activity required a porphyrin ring with a central metal that can interact with an oxidant. The oxidative activity of the model varied with the oxidant used in the order of *tert*-butyl hydroperoxide > cumene hydroperoxide > iodosobenzene, and also with the central metal of porphyrin used in the order of tetraphenylporphyrinatoiron(III) chloride > its manganese derivative. It was confirmed that *N*-nitrosodialkylamine undergoes oxidation to α-hydroxy nitrosamine in chemical model systems which are free of protein. These biomimetic models should be useful in elucidating the mechanisms of the metabolism of *N*-nitrosodialkylamines.**

Key words *N*-nitrosodialkylamine; cytochrome P450 model; metalloporphyrin; dealkylation; oxidation; metabolism

N-Nitrosodialkylamines are alkylating carcinogens which are metabolically activated through oxidative dealkylation by cytochrome P450.[1] The major pathway for the metabolic activation by cytochrome P450 is considered to be α-hydroxylation (Chart 1).[2] α-Hydroxy nitrosamines spontaneously break down to alkanediazohydroxides by dealkylation, releasing aldehydes, and the alkylating species formed, alkyldiazonium ions, are the ultimate electrophilic species which alkylate DNA.

Some α-hydroxy nitrosamines were chemically isolated[3] by deoxygenation of the corresponding α-hydroperoxy nitrosamines.[4] They alkylated water, deoxyribonucleosides or amino acids to form the respective alkylated products.[5] There was a good correlation between the mutagenic activities of α-hydroxy nitrosamines in the absence of a metabolic activation system and *N*-nitrosodialkylamines in the presence of the activation system.[5] These facts support the view that α-hydroxylation is the key pathway in the metabolic activation of *N*-nitrosodialkylamines.

Numerous reports have indicated that the cytochrome P450 2E1 or 2B1 family plays a major role in the activation of *N*-nitrosodialkylamines.[6] However, the molecular mechanism prior to α-hydroxylation by cytochrome P450 is not well understood. A pathway through an α-nitrosamino radical has been proposed for the formation of α-hydroxy nitrosamines.[7]

In investigating the metabolism of xenobiotics, drugs or chemical carcinogens, enzymes are troublesome to deal with, because there are restrictions in the available reaction conditions (temperature, solvent or pH), and enzymes easily lose their activity during the operations. Consequently, biomimetic chemical models for enzymes are helpful in studies of drug metabolism.[8] The chemical model systems are free of protein, and are advantageous for isolating oxidized products or reactive intermediates. Since the first report by Groves *et al.*, who employed a metalloporphyrin model system as a mimic of cytochrome P450-dependent monooxygenases,[9] a number of model systems have been established and applied to study mechanisms of drug metabolism.[8] Various oxidative reactions similar to those of cytochrome P450 have been reported for many compounds with the chemical models. Although there have been many studies on *N*-dealkylation of tertiary amines with models,[10] little work has been reported on *N*-dealkylation of *N*-nitrosodialkylamines.

We have already confirmed that some *N*-nitrosodialkylamines are oxidized with metalloporphyrins and oxidants to form aldehydes under aqueous or non-aqueous conditions.[11] Furthermore, in the presence of tetrakis(pentafluorophenyl)porphyrinatoiron(III) chloride and *tert*-butyl hydroperoxide (*tert*-BuOOH), *N*-nitrosodialkylamines (alkyl = methyl, ethyl, propyl and butyl) showed mutagenicity towards *Salmonella typhimurium* YG7108.[12]

In this study, dealkylation of *N*-nitrosodibenzylamine (NDBz) was observed with metalloporphyrin/oxidant model systems under non-aqueous conditions. The forma-

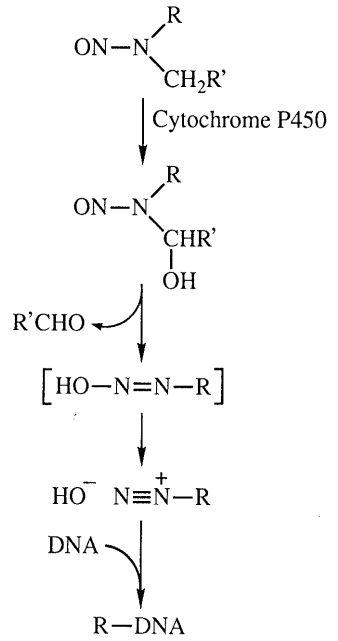

Chart 1. Metabolic Activation of *N*-Nitrosodialkylamine

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tion of benzaldehyde through oxidative dealkylation of *N*-nitrosodialkylamines *via* α-hydroxylation was used as a marker to evaluate the efficiency of the model systems. The metalloporphyrins used were tetraphenylporphyrinatoiron(III) chloride (FeTPPCl) and its manganese derivative (MnTPPCl), while the oxidants were *tert*-BuOOH, cumene hydroperoxide (cumene-OOH) and iodosobenzene (PhIO).

Experimental

Materials FeTPPCl and MnTPPCl were synthesized by metalation of tetraphenylporphyrin (TPP) with $FeCl_2 \cdot 4H_2O$ or $MnCl_2 \cdot 4H_2O$ according to the method of Kobayashi *et al.*[13] *tert*-BuOOH as a solution of 2,2,4-trimethylpentane and cumene-OOH were purchased from Aldrich. PhIO was prepared according to the published procedures[14] and its purity was determined by iodometry.[15] NDBz was synthesized by nitrosation of dibenzylamine and purified by recrystallization from ethyl acetate–hexane.[16]

Oxidation Procedure NDBz (500 μmol) and metalloporphyrin (0.5 μmol) were dissolved in 1 ml of benzene, and an oxidant (50 μmol) was added to the solution to initiate the reaction. The reaction mixture was incubated at 25 °C under air. The formation of benzaldehyde was measured as a marker of the oxidative dealkylation *via* α-hydroxylation.

Quantitative Analysis of Benzaldehyde Benzaldehyde formed from the dealkylation of NDBz was analyzed by reversed-phase HPLC after conversion to the corresponding 2,4-dinitrophenylhydrazones.[17] HPLC determinations were performed with a Hitachi Model 655A-11 liquid chromatograph and a 655A UV-visible spectrophotometric detector. Separations were carried out on a LiChrosorb RP-18 column (10 μm, 4.6 (250 mm i.d.) with a mixture of methanol–acetonitrile–water (20%/45%/35% by volume) at 1 ml/min. Absorbance was monitored at 340 nm.

Results and Discussion

In the oxidation of NDBz with FeTPPCl and *tert*-BuOOH, the formation of benzaldehyde was rapid, being completed in the first 10 min (Fig. 1). In the absence of either FeTPPCl or *tert*-BuOOH, no benzaldehyde was detected, demonstrating that the dealkylation of NDBz required both FeTPPCl and *tert*-BuOOH. No reaction occurred in the system of TPP without a central metal or that of tris(acetylacetonato)iron(III), which is an iron complex without a porphyrin ring. Thus, an active catalyst for the dealkylation requires a porphyrin ring with a central metal that can interact with an oxidant.

Figure 2 shows the effect of the oxidant on the dealkylation of NDBz. The amounts of benzaldehyde formed were plotted against the concentration of oxidant. The formation of the aldehyde was the highest with *tert*-BuOOH, followed by cumene-OOH, and then PhIO. At higher concentrations of oxidant no further increase in dealkylation was observed.

The effect of the metal in a metalloporphyrin was investigated using iron- and manganese-porphyrin. The amounts of benzaldehyde formed in the dealkylation of NDBz were plotted against the concentration of metalloporphyrin (Fig. 3) up to 20 min (FeTPPCl) or 3 h (MnTPPCl). In the model with manganese porphyrin, the reaction was very slow compared to that with iron porphyrin. The iron porphyrin showed higher efficiency and quickly reached its maximum activity. The reason for the effect of metal on the reactivity is not known at present, though differences in electronic properties or in the high valent metal-oxo species generated from the metalloporphyrin–oxidant complex[8] may be involved. Lindsay

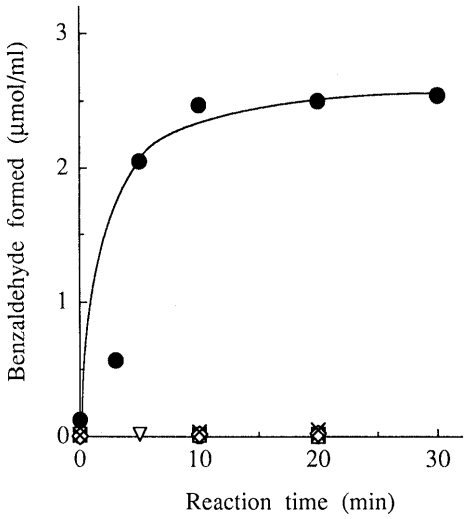

Fig. 1. The time Course of the Formation of Benzaldehyde in the Dealkylation of NDBz by FeTPPCl and *tert*-BuOOH

NDBz (500 μmol), FeTPPCl (0.5 μmol) and *tert*-BuOOH (50 μmol) were incubated in benzene (1 ml) at 25 °C. The complete system contained NDBz, FeTPPCl and *tert*-BuOOH (●). Control systems were as follows: NDBz alone (○), −NDBz (▽), −FeTPPCl (□) and −*tert*-BuOOH (△). A system with tris(acetylacetonato)iron(III) (◇) or TPP (×) instead of FeTPPCl was also examined.

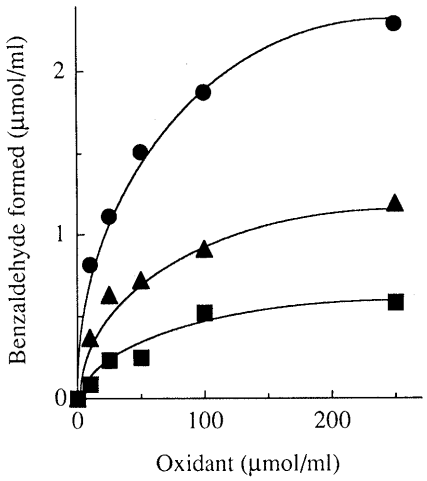

Fig. 2. The Effect of Oxidant on the Dealkylation of NDBz by FeTPPCl

NDBz (500 μmol), FeTPPCl (0.5 μmol) and an oxidant (0—250 μmol) were incubated in benzene (1 ml) at 25 °C for 20 min. (●), *tert*-BuOOH; (▲), cumene-OOH; (■), PhIO.

Smith *et al.* reported oxidative dealkylation of NDBz using oxidants and metalloporphyrins, FeTPPCl or MnTPPCl.[18] They found little difference in the catalytic activity between FeTPPCl and MnTPPCl, in contrast to our present data. The concentrations of substrate and metalloporphyrin used in their reactions were higher than those used in this study, and this may account for the difference.

Since the benzaldehyde formed was possibly oxidized as a substrate in the model system, the stability of benzaldehyde was investigated in each oxidation system (Figs. 4 and 5). The decomposition of benzaldehyde was confirmed in all the systems, and the rate of the reaction was determined by the metalloporphyrin used rather than the oxidant. In the model with *tert*-BuOOH as an oxidant, the decomposition of benzaldehyde was very slow with

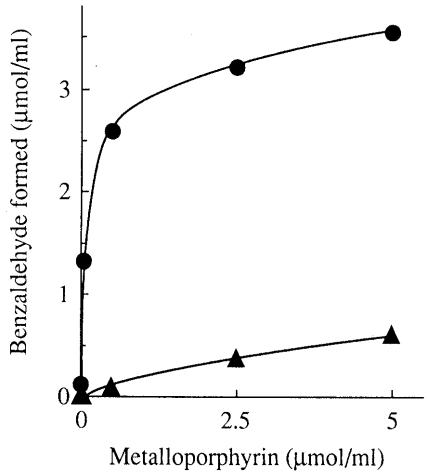

Fig. 3. The Effect of Metal on the Dealkylation of NDBz by Metalloporphyrin and *tert*-BuOOH

NDBz (500 μmol), a metalloporphyrin (0—5 μmol) and *tert*-BuOOH (50 μmol) were incubated in benzene (1 ml) at 25 °C for 20 min with FeTPPCl (●) or for 3 h with MnTPPCl (▲).

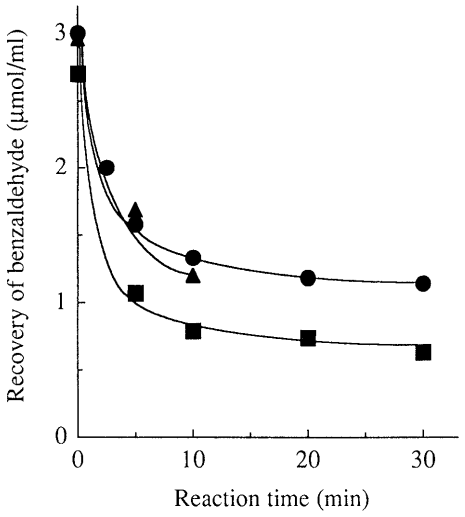

Fig. 4. The Effect of Oxidant on the Decomposition of Benzaldehyde in Metalloporphyrin/Oxidant Model Systems

Benzaldehyde (3 μmol), FeTPPCl (0.5 μmol) and an oxidant (50 μmol) were incubated in benzene (1 ml) at 25 °C. (●), *tert*-BuOOH; (■), cumene-OOH; (▲), PhIO.

MnTPPCl compared to that with FeTPPCl (Fig. 5), as was the case for the formation of benzaldehyde. This suggested that the same active species catalyzed both the formation and the decomposition of benzaldehyde.

In this study, chemical model oxidation systems based on a metalloporphyrin and an oxidant were used to investigate the mechanism of metabolic activation of *N*-nitrosodialkylamines. In the model systems, NDBz was dealkylated by metalloporphyrin and oxidant as in the enzymatic system, to release benzaldehyde, and the aldehyde formed was also oxidized by the models. The rate of aldehyde formation varied with the oxidant used in the order of *tert*-BuOOH > cumene-OOH > PhIO, and also with the central metal of the porphyrin (FeTPPCl > MnTPPCl). While the rate of aldehyde decomposition also varied with the central metal of porphyrin used (FeTPPCl > MnTPPCl), no significant difference in the rate of decomposition was observed with the oxidant used.

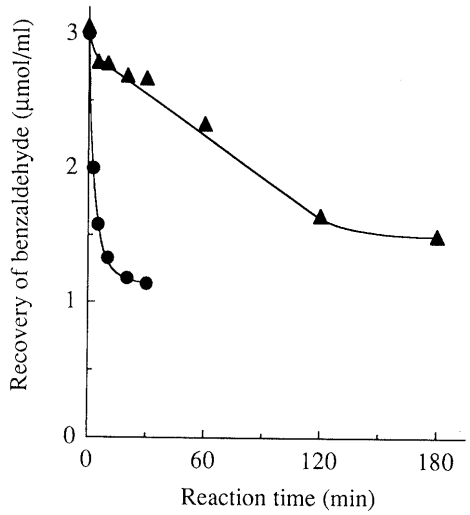

Fig. 5. The Effect of Metalloporphyrin on the Decomposition of Benzaldehyde in Metalloporphyrin/Oxidant Model Systems

Benzaldehyde (3 μmol), a metalloporphyrin (0.5 μmol) and *tert*-BuOOH (50 μmol) were incubated in benzene (1 ml) at 25 °C. (●), FeTPPCl; (▲), MnTPPCl.

Taking account of the rate of decomposition of the aldehyde once formed, FeTPPCl/*tert*-BuOOH model system showed higher activity of benzaldehyde formation. The dealkylation of *N*-nitrosodialkylamines catalyzed by metalloporphyrin/oxidant did not proceed smoothly. It is possible that the oxidant may be entirely consumed in the reaction, and oxygen transfer to the substrate may not be efficient. In addition, the metalloporphyrin may undergo oxidative self-destruction, or the alkylating species derived from *N*-nitrosodialkylamines may react with the metalloporphyrin to form *N*-alkylporphyrin, with loss of the catalytic activity. However, these possibilities remain to be examined. Chauhan and Satapathy reported the dealkylation of *N*-nitrosodialkylamines in chemical model systems and indicated that the sterically hindered and electron withdrawing manganese-porphyrin acted as an effective catalyst in the reaction.[19] In the present study, there was little effect of substituents on the phenyl ring in the *meso*-position of iron-porphyrin on the oxidation of *N*-nitrosodialkylamines (data not shown).

It is confirmed that *N*-nitrosodialkylamine undergo dealkylation, possibly through oxidation to α-hydroxy nitrosamine, in chemical model systems which are free of protein. These biomimetic models will be useful in elucidating the mechanisms of the metabolic pathway of *N*-nitrosodialkylamines.

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