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The Metabolic *N*-Oxidation of Carcinogenic Arylamines in Relation to Nitrogen Charge Density and Oxidation Potential

by Fred F. Kadlubar,* Peter P. Fu,* Hyewook Jung,† Ali U. Shaikh,† and Frederick A. Beland*

The *N*-oxidation of carcinogenic arylamines to form *N*-hydroxy arylamines has long been regarded as a necessary metabolic step for conversion to proximate carcinogenic derivatives. In contrast, arylamine *ring*-oxidation has been generally considered to be an important detoxification mechanism. Both enzymatic reactions are carried out in the liver and usually involve the cytochrome P-450 monooxygenases. Studies on the metabolic oxidation of certain arylamines have indicated that the relative charge density on nitrogen versus *ring*-carbon atoms for a nitrenium/carbenium ion-enzyme intermediate correlates with the relative proportion of *N*-versus *ring*-hydroxylated products that are formed. A further examination of this approach now shows that positive charge density on the nitrogen, as estimated by Hückel molecular orbital calculations, is consistent with the formation of *N*-hydroxy arylamines from aniline, 4-aminoazobenzene, 2-naphthylamine, 4-aminobiphenyl, 2-aminofluorene, and 6-aminochrysene, but not from 1-naphthylamine, 1-aminopyrene, 6-aminobenzo[a]pyrene, or 7-aminobenz[a]anthracene. Since greater positive charge on the arylamine nitrogen implies a greater charge localization during the transition state of the enzyme-substrate complex, we envisioned that higher oxidation potentials for arylamines, which might be expected to correlate inversely with the ease of total oxidation, would instead be predictive of the relative extent of *N*-oxidation. When the half-wave oxidation potentials ($E_{1/2}$'s) of the above-mentioned arylamines, along with those of 1-amino-6-nitropyrene and 1-amino-8-nitropyrene (which also undergo *N*-oxidation but are not amenable to simple Hückel calculations), were experimentally determined by linear sweep voltammetry, an $E_{1/2}$ of greater than +0.60 V was found to be generally predictive of the ability of these arylamines to undergo metabolic *N*-oxidation. Thus, these methods may be applicable to other arylamines whose extent of *N*-oxidation and carcinogenic potential are unknown.

Introduction

The metabolic activation of carcinogenic arylamines generally involves enzymatic *N*-oxidation to form highly mutagenic *N*-hydroxy arylamines (*1,2*). These electrophilic *N*-hydroxy metabolites can react directly with cellular DNA or be converted to even more reactive *O*-esters by several different enzymatic conjugation mechanisms (*3*). Arylamine *ring*-oxidation, on the other hand, generally results in the formation of phenolic derivatives, which undergo conjugation reactions to form stable excretory products. Although *N*-oxidation and *ring*-oxidation are carried out by several monooxygenases, liver cytochrome P-450IA2 possesses the highest catalytic activity (*4,5*). In our previous studies on primary arylamine metabolism, the cytochrome P-450-dependent-*N*-oxidation of the carcinogens 2-aminofluorene and 2-naphthylamine, but not of the noncarcinogen 1-naphthylamine, was observed; while *ring*-oxidation of all three arylamines was detected (*6*). In order to rationalize the specificity of cytochrome P-450 for arylamine *N*-and *ring*-oxidation in relation to the mechanisms of enzyme action, simple Hückel molecular orbital calculations were employed to estimate the charge density at the nitrogen and/or carbon atoms being hydroxylated. From these calculations, we showed that product distribution was consistent with a two-electron transfer mechanism in which there was formation of a nitrenium/carbenium ion intermediate prior to oxygen rebound and release of hydroxylated metabolites. Moreover, the relative positive charge at nitrogen versus carbon atoms was predictive of the extent of *N*-oxidation and of the formation of potentially carcinogenic *N*-hydroxy arylamines (*4,6*).

The discovery of several polycyclic nitroaromatic hydrocarbons as potent environmental mutagens and carcinogens (*7*) and their enzymatic reduction to polycyclic arylamines has led us to examine the metabolic acti-

*Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.
†Department of Chemistry, University of Arkansas at Little Rock, Little Rock, AR 72204.
Address reprint requests to F. Kadlubar, National Center for Toxicological Research, Jefferson, AR 72079.

vation of several of these arylamines to DNA-bound products (*8*). In this latter study, evidence was presented for the microsomal *N*-oxidation of 1-amino-6-nitropyrene and 1-amino-8-nitropyrene, the *N*- and *ring*-oxidation of 6-aminochrysene, and the *ring*-oxidation of 1-aminopyrene, 6-aminobenzo[a]pyrene, and 7-aminobenz[a]anthracene. In the present report, we have further examined the relation between charge distribution in the nitrenium/carbenium ion intermediate and the ability of 12 different arylamines to undergo *N*- versus *ring*-oxidation. Under thermodynamic control of the transition state, greater positive charge localization for an enzyme-substrate cationic intermediate would be expected to be inversely related to the ease of substrate oxidation. Thus, we have also determined the oxidation potentials of all 12 arylamines as a predictive measure of the enzymatic formation of their potentially carcinogenic *N*-hydroxy metabolites.

Materials and Methods

Aniline, 4-aminoazobenzene, 4-aminobiphenyl, and 2-aminofluorene were obtained from the Aldrich Chemical Co., Milwaukee, WI; and 1-naphthylamine and 2-naphthylamine were purchased from the Sigma Chemical Co., St. Louis, MO. The polycyclic arylamines were prepared from their corresponding nitroaromatic hydrocarbons (obtained from Chemsyn Science Laboratories, Lenexa, KS) by reduction with hydrazine hydrate and palladized charcoal by the method of Bavin (*9*). Their identity was determined by electron impact mass spectrometry (Finnigan 4000), and their purity was judged to be > 95% by high pressure liquid chromatography (*5*).

Molecular orbital calculations were performed by the simple Hückel approximation (*10*) using a Digital VAX780 computer. For the diagonal elements, $\alpha + n\beta$, the parameters for n were: C(H) = 0 and N(H) = 0.5. For the nondiagonal elements, $m\beta$, the parameters for m were: C—C = 1.0 and C—N = 1.0.

Oxidation potentials for each arylamine were determined in 0.1 M tetraethylammonium perchlorate in anhydrous dimethyl sulfoxide by linear sweep voltammetry using a Bioanalytical Systems Model 100A electrochemical analyzer. The voltage scan rate was 10 mV/sec; the reference electrode was Pt/Ag/AgCl; and half-wave potentials ($E_{1/2}$) were taken as the first well-defined oxidation peak.

Results and Discussion

Molecular Orbital Calculations of Arylamine Charge Density at Nitrogen and Carbon Atoms That Undergo Metabolic Oxidation

Since the metabolic oxidation of 4-aminobiphenyl, 2-aminofluorene, and 1- and 2-naphthylamine to *N*-hydroxy and/or *ring*-hydroxy products correlated well with the positive charge density of the atoms undergoing a two-electron oxidation (*5,6*), we sought to apply this method to eight other arylamines including those derived from several polycyclic nitroaromatic hydrocarbons. As shown in Table 1 for all 12 arylamines, a net positive charge on the nitrogen appears to be necessary in order to form the *N*-hydroxy arylamine metabolite. 1-Amino-6-nitropyrene and 1-amino-8-nitropyrene, which were found to undergo *N*-oxidation predominantly, are not suitable for simple Hückel approximations; however, the strong negative inductive effect of the nitro group would be expected to appreciably increase the positive charge at the nitrogen, as compared to that calculated for the nitrogen of 1-aminopyrene. These results are again consistent with the collapse of substrate cation~Fe^{III}-OH anion caged complex as the final enzyme intermediate prior to product formation (*4,6*). Moreover, the relative positive charge on the carbon atoms is also consistent with the extent of *ring*-oxidation observed for each of these compounds.

For the transition state of the enzyme-substrate complex, the ability to achieve an increased positive charge on the arylamine nitrogen indicates that there is a decrease in the extent of delocalization of π electrons in the aromatic system as well as a greater charge localization in the enzyme-nitrenium/carbenium ion intermediate. Since oxidation potentials are inversely related to the extent of π electron delocalization, we sought to determine experimentally the $E_{1/2}$ of each of the arylamines under consideration.

Oxidation Potentials of Arylamines That Undergo Metabolic Oxidation

Using linear sweep voltammetry under conditions (*11*) that would allow the electrochemically oxidized intermediates ($-e^-$) to oxidize further ($-2e^-$) to hydroxylated products, the $E_{1/2}$'s for all 12 arylamines were determined. As shown in Table 2, the higher oxidation

Table 1. Calculation of charge density at nitrogen and carbon atoms of arylamines using the Hückel molecular orbital approximation.[a]

Aromatic amine	Charge density	
	Nitrogen	Carbons[b]
Aniline	+ 0.307	+ 0.181(2), + 0.218(4)
2-Naphthylamine	+ 0.187	+ 0.332(1), + 0.103(6)
4-Aminobiphenyl	+ 0.180	+ 0.146(3), + 0.063(4')
2-Aminofluorene	+ 0.180	+ 0.057(5), + 0.063(7)
4-Aminoazobenzene	+ 0.160	+ 0.140(3)
1-Naphthylamine	+ 0.081	+ 0.233(2)
1-Amino-6-nitropyrene	—	—
1-Amino-8-nitropyrene	—	—
6-Aminochrysene	+ 0.004	+ 0.049(1), + 0.163(12)
1-Aminopyrene	− 0.069	+ 0.148(6), + 0.146(8)
7-Aminobenz[a]anthracene	− 0.088	+ 0.134(3), + 0.290(12)
6-Aminobenzo[a]pyrene	− 0.159	+ 0.151(1), + 0.146(3)

[a] The calculations were performed on the arylamine cations; parameters for the elements are described in "Materials and Methods."

[b] The position of the *ring*-carbon atom is designated by the number in parentheses.

Table 2. Oxidation potentials[a] of arylamines and their extent of *N*- and *ring*-oxidation.[b]

Aromatic amine	$E_{1/2}$	*N*-oxidation	*Ring*-oxidation
Aniline	+ 1.31	+	+ +
4-Aminoazobenzene	+ 0.97	+ +	+
2-Naphthylamine	+ 0.86	+ +	+ +
4-Aminobiphenyl	+ 0.80	+ +	+
1-Naphthylamine	+ 0.77	−	+ +
2-Aminofluorene	+ 0.74	+ +	+
1-Amino-6-nitropyrene	+ 0.77	+ +	−
1-Amino-8-nitropyrene	+ 0.74	+ +	−
6-Aminochrysene	+ 0.62	+	+
1-Aminopyrene	+ 0.51	−	+ +
6-Aminobenzo[a]pyrene	+ 0.44	−	+ +
7-Aminobenz[a]anthracene	+ 0.30	−	+ +

[a] The $E_{1/2}$'s are expressed in volts versus Ag/AgCl.

[b] See Gorrod and Manson (*2*), Kadlubar and Hammons (*4*), Butler et al. (*5*), Hammons et al. (*6*), and Kadlubar et al. (*8*).

potential was indeed consistent with greater positive charge density at the arylamine nitrogen and was generally predictive of *N*-oxidation, including 1-amino-6-nitropyrene and 1-amino-8-nitropyrene. Furthermore, it would appear that an $E_{1/2}$ of $> +0.60$ V may be necessary for the enzymatic formation of potentially carcinogenic *N*-hydroxy arylamines. The only apparent anomaly to this correlation is 1-naphthylamine, which is not *N*-hydroxylated yet shows a positive nitrogen charge density and a relatively high oxidation potential. However, these thermodynamic considerations do not take into account the known regioselectivity of the cytochrome P-450 monooxygenases. With 2-naphthylamine, for example, of the eight different isozymes examined, five were active in catalyzing *ring*-oxidation, but only cytochrome P-450IA2 catalyzed its *N*-oxidation (*6*). Thus, while determination of $E_{1/2}$'s may have predictive value for assessing the metabolic activation potential of other arylamines, the possibility of false-positive results must be recognized.

Perspective

Structure-activity studies on arylamines should be of value in assessing the human health risks posed by this class of compounds, especially the specific structural features that correlate with their tumorigenic potency. In this paper, we have demonstrated that both the half-wave potentials ($E_{1/2}$'s) and the relative positive charge distribution at nitrogen versus carbon atoms are important electronic parameters for prediction of the relative carcinogenic activity of arylamines. Nevertheless, there are two reservations that should be taken into consideration:

a) It is known that aryl *ring*-oxidation can result either from direct hydroxylation or from epoxidation followed by nonenzymatic rearrangement to the corresponding phenol(s) or enzymatic hydration to a dihydrodiol (*12*). However, we determined only the relative positive charge at the nitrogen versus carbon atoms for prediction of the relative extent of *N*-oxidation. This implies that these correlations hold independent of the involvement of an epoxidation mechanism. Thus, this discrepancy exists and a more sophisticated theoretical treatment will be needed to test fully this hypothesis.

b) Most of the arylamines considered thus far are those derived primarily from noncarcinogenic aromatic hydrocarbons, such as naphthalene, biphenyl, fluorene, and pyrene. Accordingly, the only metabolic activation pathway available may be *N*-oxidation to form *N*-hydroxy arylamines. However, if the arylamine is derived from a carcinogenic polycyclic aromatic hydrocarbon, such as benzo[a]pyrene, it is highly probable that *ring*-oxidation may also serve as an activation pathway. The identification of 6-aminochrysene *trans*-1,2-dihydrodiol, a highly tumorigenic metabolite of 6-aminochrysene (*13*), and of 3-nitrobenzo[a]pyrene *trans*-7,8-dihydrodiol, a potent mutagenic metabolite of the environmental contaminant 3-nitrobenzo[a]pyrene (*14*), is consistent with this possibility. Consequently, epoxidation pathways leading to *ring*-hydroxylation and dihydrodiol formation should be considered in future studies with polycyclic arylamines.

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