Oxidation of Tryptophan and N-Methylindole by N_3 , Br_2 , and $(SCN)_2$ Radicals in Lightand Heavy-Water Solutions: A Pulse Radiolysis Study

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Reactions of N_3^{\bullet} , $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ with tryptophan (TRPH) and N-methylindole (NMI) have been investigated in H_2O and D_2O solutions. The main transients produced were the TRP[•] radical and the TRPH⁺⁺ radical cation from TRPH and the NMI⁺⁺ radical cation from NMI. Their extinction coefficients (ϵ) as well as the rate constants for their formation and decay were determined in the pH range 3–10. The pK of the TRPH⁺⁺ radical cation was confirmed to be 4.2 ± 0.1 . These rate constants were within experimental error, independent of pH, and the differences between the rate constants in H_2O and D_2O were small. On the basis of the results, it was concluded that of the two possible reactions—electron or hydrogen atom transfer—the data are more consistent with electron transfer in all cases. However, secondary acid-base reactions have an important effect on the overall redox equilibria, especially with N_3^{\bullet} . Experimental data on these effects have also been obtained.

Introduction

Reactions involving radicals and radical anions produced in reactions $1-3^1$ may be important in radiation biology and food

$$H_2O \longrightarrow H^{\bullet}, OH^{\bullet}, e_{aq}^{-}, H_2, H_2O_2, H^{+}$$
 (1)

$$OH^{\bullet} + X^{-} \rightarrow (XOH^{\bullet-}) \rightarrow X^{\bullet} + OH^{-},$$

$$X^{-} = CI^{-}, Br^{-}, I^{-}, N_{3}^{-}, SCN^{-} (2)$$

$$X^{\bullet} + X^{-} \rightleftharpoons X_{2}^{\bullet-}$$
(3)

irradiation, since they can react with enzymes and other cellular components.²⁻⁶ In addition, the reactions of $(SCN)_2^{\leftarrow}$, Br_2^{\leftarrow} , and N_3^{\bullet} have been used to investigate selective one-electron oxidation reactions of amino acids and enzymes.^{2-4,7-11} The mechanism of these oxidations has generally been assumed to be a direct electron transfer to the radical or radical anion.^{8,12,13} In the case of the reaction of Br_2^{\leftarrow} with tryptophan (TRPH), experimental evidence for the electron-transfer mechanism in neutral aqueous solutions has been reported.²

$$Br_2^{\bullet-} + TRPH \rightleftharpoons TRPH^{\bullet+} + 2Br^{-}$$
 (4)

At pH's above the pK of the TRPH radical cation (TRPH^{•+}), deprotonation follows until acid-base equilibrium is established:

$$\text{TRPH}^{*+} \xrightarrow{k_3}_{k_{-3}} \text{TRP}^* + \text{H}^+ \qquad (5, -5)$$

Unfortunately, not all cases are as clear-cut. For example, in the reactions of $Cl_2^{\bullet-}$, both electron transfer and hydrogen atom abstraction seem to take place.¹⁴

For the reaction of N_3^{\bullet} with TRPH, the possibility of hydrogen atom transfer, instead of electron transfer, i.e., reaction 7 instead of reaction 6, was suggested:¹⁰

$$N_3^{\bullet} + TRPH \rightleftharpoons N_3^{-} + TRPH^{\bullet+}$$
(6)

$$N_3^{\bullet} + TRPH \rightleftharpoons HN_3 + TRP^{\bullet}$$
(7)

Subsequent to these publications,^{2,10} the reduction potential of the azide radical has been established¹⁵ as 1.33 V, and the re-

duction potentials of TRPH and several other indoles have been determined more accurately ($E^{\circ}(\text{TRPH}^{++}/\text{TRPH}) = 1.24^{16}$ and 1.21 V¹⁷). This information, along with the known pK values of HN₃ (4.74)¹⁸ and the TRPH⁺⁺ radical cation (4.3),⁷ makes it possible to calculate $\Delta G^{\circ}_{6} = -10$ (± 2) kJ mol⁻¹ and to show that ΔG°_{7} is about 2.5 kJ mol⁻¹ more negative. Thus, from a thermodynamic point of view, reaction 7 is slightly favored. It seemed therefore that further study of the oxidation of TRPH by N₃[•] was warranted. The approach included making direct comparisons between TRPH and N-methylindole (NMI), which has a similar reduction potential¹⁶ (1.23 V as compared with 1.24 V for TRPH)

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but can only undergo electron transfer.

The spectrum of the NMI*+ radical cation does not appear to have been published. For the radical cation formed from Nmethyltryptophan there is no evidence for the loss of any ring protons, as shown by the spectrum, up to pH 10. Its spectrum is similar to that of the TRPH⁺⁺ radical cation.^{19,20} The pK of the TRPH^{•+} radical cation⁷ is 4.3, and the proton that is lost on deprotonation is derived from the H atom on the indole nitrogen. This H is also rapidly exchanged for D on solution of TRPH in D_2O^{21} Furthermore, this would also be the atom abstracted, if reaction 7 occurred. Thus, if the rates of oxidation by N_3^* were compared in light- and heavy-water solutions, the rate in heavy water would be lower by a factor of 2-3 because of the isotope effect,^{22,23} if reaction 7 were the dominant reaction. On the other hand, the rates would be similar (except for the viscosity effect of heavy water), if reaction 6 were the dominant reaction.

The main aim of this work was to study the reactions of N_3^* , Br_2^{+} , and $(SCN)_2^{+}$ with TRPH and NMI in H_2O and D_2O at pHs above and below the pK of the TRPH⁺⁺ radical cation. By comparing trends in the rate constants for oxidation by N₃[•] with those for Br2., for which electron transfer is the accepted mechanism, it was expected that the relative importance of reactions 6 and 7 as a function of pH could be decided. At the same time, NMI served as a model in which, even for N_3^* , only electron transfer could occur.

Experimental Section

Initial experiments used the 1.5-MeV Van de Graaff electron accelerator at the University of Calgary.²⁴ Transient spectra and kinetics of species formed in solutions of both TRPH and NMI in H₂O and D₂O were obtained with the 3-MeV Van de Graaff electron accelerator at Mülheim,²⁵⁻²⁷ and the NMI-N₃^{•-} equilibrium

$$N_3^{\bullet} + NMI \rightleftharpoons N_3^{-} + NMI^{\bullet+}$$
(8)

was studied, using the 8-MeV linear electron accelerator in the Radiation Laboratory at the University of Notre Dame.²⁸ Details of these facilities and dosimetry methods have been previously described;²⁴⁻²⁸ density corrections for D_2O solutions were made. The experiments were performed at room temperature (20 ± 2) °C), and the OD/cm values were normalized to a dose of 10 kGy.

Solutions. All solutions were prepared with triply distilled or "Millipore/Milli-Q" water. The heavy water was supplied by AECL Research (isotopic purity, 99.95%) and was distilled over potassium permanganate prior to use. The TRPH was recrystallized from water solutions before use. The NMI (Merck) was distilled under argon and stored in a refrigerator in an air-free container. Because of the potential volatility of this compound, the concentrations of the solutions used in the study of the N_3^*/NMI system equilibrium (reaction 8) were determined from the absorbances (optical density, OD) at 280 nm before and after the pulse radiolysis experiments, and their average was used in the calculations. The largest change observed was 6%. The nitrous oxide was passed through columns containing "Oxisorb" (Messer



Figure 1. Transient absorption spectra obtained 10 μ s after the pulse in H_2O on reaction of N_3^* with TRPH at pH = 3 (A1) and at pH = 8-10 (A2). A3 results from A1 at a few milliseconds after the end of the pulse. Insert I: Absorption spectra of the same transients, but produced in D₂O at pH = 3 (B1) and pH = 8-10 (B2). Insert II: Plot of optical density versus pH to obtain pK. All OD/cm values are at 580 nm and normalized to 10 Gy. OD, and OD, are the values for acidic and basic solutions, respectively. Solutions: 5×10^{-4} M TRPH, 5×10^{-2} M NaN₃, and 2.8 \times 10⁻² M N₂O.

TABLE I: Spectroscopic Characteristics and Decay Kinetics (2k) of Transients Produced by Radical Reactions with TRPH and NMI in Acid (pH = 3) and Neutral/Basic Solutions (pH = 7-10)

	pH of	λ	E	$10^{-8}2k$, N	, M ⁻¹ s ⁻¹ , in	
species	soln	nm	M ⁻¹ cm ⁻¹	H ₂ O	D ₂ O	
TRPH						
cation	3	335	4750 🛳 200	5.1 🛳 0.3	4.8 ± 0.2	
radical,		560	3000 🕿 150			
TRPH•+						
neutral	7-10	325	3670 🛳 200	5.3 ± 0.3	4.9 单 0.2	
radical,		510	2300 ± 150			
TRP•						
NMI						
cation	3-10	335	5800 ± 250	6.7 🗨 0.2	6.5 ± 0.2	
radical,		560	2600 ± 180			
NMI++						

Griesheim) to remove traces of oxygen, prior to use. All other chemicals were reagent grade and were used as received. For control of pH, use of phosphate buffer, HClO₄, and NaOH was made, as needed.

Results and Discussion

A. Tryptophan. 1. Absorption Bands and pK. Transient absorption spectra obtained on pulse radiolysis of N2O-saturated solutions of TRPH (5 \times 10⁻⁴ M) containing sodium azide (5 \times 10⁻² M) have been measured in the pH range from 3 to 10 (Figure 1). In addition to the two major bands observed in the 320- and 520-nm regions^{2-4,7,8,10} very soon after the electron pulse (10 μ s), due to TRPH*+/TRP*, a weak transient absorption due to an unidentified product of decay is observed at ~ 2 ms after the pulse (Figure 1). The absorption spectra of the transient species formed from TRPH in D₂O solutions are similar (Figure 1, I).

From the change in optical densities as a function of pH, the pK for reaction 5 has been calculated to be 4.2 ± 0.1 (Figure 1). This compares well with the previously reported value⁷ of 4.3. Consistent with the earlier studies,^{2-4,7,8,10} the same TRPH*+/TRP* species were also produced on reactions of Br2*- and (SCN)2*with TRPH.

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2. Radical Yields and Extinction Coefficients. The objective of this part of the study was to obtain absorption spectra and extinction coefficients of the TRP[•] and TRPH^{•+} species separately. For the case of TRP[•], this can be done at pHs considerably higher than the pK of TRPH^{*+}. The appropriate yields of N_3^{\bullet} , $Br_2^{\bullet-}$, and $(SCN)_2$ for 5 × 10⁻² M salt solutions are 5.5 molecules per 100 eV each, as they are formed only from 'OH. The H' atoms make no contributions.29

To ensure that radical-radical reactions were not causing inaccuracies in the absorbances per unit dose, the intensities in azide solutions at pH 10.5 \pm 0.3 were determined as a function of dose per pulse and concentration of TRPH, in 10⁻² M solutions of NaN₃. The results showed that the OD values were, in effect, independent of dose up to 7 Gy per pulse for 5×10^{-4} M tryptophan. Also, the dependence on TRPH concentration above 10^{-4} M for doses of 3 Gy per pulse was small. Because the radical plus radical reaction rate constants for $Br_2^{\bullet-}$ and $(SCN)_2^{\bullet-}$ are lower than that³⁰ for N₃[•], it can be assumed that the effects in those cases would have been smaller. The extinction coefficients for TRP[•] given in Table I are the averages of the values obtained from the absorbances per unit dose at TRPH concentrations of 5×10^{-4} M and doses per pulse of 7 Gy or less, with the yield of oxidizing radicals taken as 5.5 molecules per 100 eV for all the three species.

Determination of the spectrum of the TRPH⁺⁺ radical cation requires that the pH be well below the pK of 4.3, and so pH 3 was chosen. In the case of bromide solutions, the Br₂^{•-} yield was taken as 5.5, the same as in alkaline solutions. According to Ye and co-workers,²⁹ the yield of N_3^{\bullet} at pH 3 should be 6.1. Here, the conversion of the hydrated electron into hydrogen atoms and the production of N_3 through the following reactions of the hydrogen atoms become important:13,30-32

$$HN_3 \rightleftharpoons H^+ + N_3^- \tag{9}$$

$$\mathbf{e}_{\mathbf{aq}}^{-} + \mathbf{HN}_{3} \rightarrow \mathbf{HN}_{3}^{-} \tag{10}$$

$$\mathbf{e}_{\mathbf{a}\mathbf{a}}^{-} + \mathbf{H}^{+} \to \mathbf{H}^{\bullet} \tag{11}$$

$$H^{\bullet} + N_{3}^{-} \rightleftharpoons HN_{3}^{\bullet-}$$
(12)

$$HN_3^{\bullet-} + H^+ \rightarrow H_2 + N_3^{\bullet}$$
(13)

$$H^* + HN_3 \rightarrow H_2 + N_3^* \tag{14}$$

$$OH^{\bullet} + HN_3 \rightarrow N_3^{\bullet} + H_2O \tag{15}$$

Because of the complexity of this scheme, the yield of N₃^{*} at pH 3 in H_2O was determined directly by titration with $Fe(CN)_6^4$ in the reaction

$$N_{3}^{\bullet} + Fe(CN)_{6}^{4-} \rightarrow N_{3}^{-} + Fe(CN)_{6}^{3-}$$
 (16)

with concentrations of $Fe(CN)_6^4$ chosen so that the overall rates of reaction toward N_3^* were equivalent to those seen for TRPH. These studies confirmed that the $G(N_3^{\circ})$ at pH 3 was 6.1 ± 0.2. This latter value was used as the yield of the TRPH⁺⁺ radical cation in azide solutions at this pH. It is pertinent to point out that calculations based on the OH* reaction rates indicate that approximately one OH[•] radical per 100 eV would probably have reacted directly with TRPH at pH 3 in 5×10^{-2} M azide solutions. However, this should not affect the spectra and calculated extinction coefficients, since at this pH the OH[•] adduct rapidly decays to the TRPH ** radical cation.

Radical yields in D_2O are generally assumed to be 5% higher than in H_2O^{33} In view of this small difference and the fact that

TABLE II: Rate Constants^a (k) for the Reactions of N₃^{*}, Br₂^{**}, and $(SCN)_2$ with TRPH and NMI in H₂O and D₂O

pH region		$k/10^9 \text{ M}^{-1} \text{ s}^{-1}$				
	radical	TRPH		NMI		
		H ₂ O	D ₂ O	H ₂ O	D ₂ O	
3	Br2*-	1.4	1.5			
	N ₃ *	4.2	3.9	6.6	5.9	
	Br ₂ •-	1.3	1.2	3.5	2.5	
	-	0.8		2.4°		
		0.8 ^d				
7-10	N ₃ •	4.3	4.0	7.1	6.5	
		4.1 ^b		13°		
	(SCN)2*-	0.30 0.27 ^{d,e}	0.29	1.9	1.5	
	он.	131		12		
				10°		

^aRate constants (k) in H₂O are subject to a standard deviation of 10% and in D₂O of 15%. ^bReference 34. ^cReference 11. ^dReference 9, pH 7. 'Reference 3, pH 7. 'Reference 35a,b.

similar absorbance intensities were observed in the two solvents, the extinction coefficients were taken to be the same in D_2O .

3. Kinetics. The kinetics of oxidation were examined by following the growth of the OD of the TRP[•] species at high pH (8-10) and of the TRPH*+ species at pH 3.0 and 3.8. This was done at different TRPH concentrations $((1-10) \times 10^{-5} \text{ M})$, and the overall second-order rate constant of the oxidation process was determined in the standard way from plots of the pseudo-first-order growth rates versus TRPH concentration. Salt concentrations were varied between 1×10^{-3} and 5×10^{-3} M. The second-order rate constants (Table II) were independent of pH, and those in H₂O are generally in good agreement with the previously reported data.3,9,11,34,35

At this point it should be recognized that our observations require acid-base equilibria to be established on the same time scale as the oxidation process, whether it be reaction 6 or 7. Thus at high pH, with all three radical species, if reaction 5 did not occur on the same time scale as (4) (or (6), if it is the main process for the reaction of N_3 , there will be a time lag between the development of the TRP[•] spectrum and that of TRPH^{•+}. Likewise, if reaction 7 is the main process with N_3^* , at low pH, the back-reaction -5 must proceed on the same time scale; otherwise there will be a lag in the development of TRPH⁺⁺ from TRP⁺. Since neither phenomenon was observed in the presence of $2 \times$ 10^{-2} M phosphate buffer, it must be concluded that at both low and high pH the rates of the secondary acid-base reactions were similar to the rates of the oxidation process.

The rate constants for the reactions of N_3^{\bullet} , Br_2^{\bullet} , and $(SCN)_2^{\bullet}$ in D₂O are generally the same as those in water, within experimental error. Any differences, if real, are similar to the ratio of the viscosities³⁶ of H₂O and D₂O, which is 0.8904 cP/1.0952 cP or 0.813. Since the reactions involving N₃[•] occur near the diffusion-controlled limit, this fact argues against a hydrogen atom transfer mechanism and in favor of the electron-transfer mechanism.

At both low and high pH, the "transients" decayed on a millisecond time scale. The 2k value of 5.1×10^8 M⁻¹ s⁻¹ at low pH (Table I) for the bimolecular reaction of the TRPH⁺⁺ radical cation

$$TRPH^{+} + TRPH^{+} \rightarrow products$$
(17)

is only slightly less than that for the uncharged TRP[•] radical, reaction 18, observed at higher pH. However, the full effect of

$$TRP^{\bullet} + TRP^{\bullet} \rightarrow products$$
 (18)

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Figure 2. Absorption spectra of transients produced in H₂O by the reaction of N₃[•] with NMI at pH = 3 (A1) and pH = 8-10 (A2), both measured at 5 μ s after pulse. Spectrum A3 represents long-lived transients (~0.5 ms after the end of the pulse). Insert: Transient absorption spectrum obtained 5 μ s after the pulse in D₂O (pH = 3) by the reaction of N₃[•] with NMI. All OD/cm values are normalized to 10 Gy. Solutions: 5 × 10⁻⁴ M NMI, 5 × 10⁻² M NaN₃, and 2.8 × 10⁻² M N₂O.



Figure 3. Absorption spectra of transients produced in H₂O by the reaction of NMI with Br₂⁻⁻ at pH 3-10 (A1) or $(SCN)_2^{--}$ at pH = 8-10 (A2) measured at 10 μ s after the pulse. All OD/cm values are normalized to 10 Gy. Solutions: 5×10^{-4} M NMI, 5×10^{-2} M KBr or KSCN, and 2.8×10^{-2} M N₂O.

the charge is partially masked by the ionic strength of the solutions $(\sim 10^{-1} \text{ M})$. The 2k values in D₂O are within experimental error the same as those in H₂O, and any effect of viscosity is small. The earlier 2k value⁶ of $5.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ for pH 6.1 in H₂O is in good agreement with the present result.

B. N-Methylindole. 1. Spectra and Extinction Coefficients. The absorption spectra of transients obtained in the reaction of N_3^{\bullet} with NMI in H_2O and D_2O solutions are shown in Figure 2. Those produced in the reaction with $Br_2^{\bullet-}$ at pH 3-10 and with $(SCN)_2^{\bullet-}$ at pH 8-10 are presented in Figure 3. The spectrum and intensity of the absorption per reacting radical seen with $Br_2^{\bullet-}$ were independent of pH and similar to those observed with $(SCN)_2^{\bullet-}$ at high pH. A comparison of the curves in Figures 2 and 3 shows that a similar spectrum and intensity were produced by N_3^{\bullet} at pH 3. The extinction coefficients at the two maxima based on the average of all of these intensities are listed in Table I.



Figure 4. (A) Plot of the reciprocal of OD, the absorbance intensity of NMI⁺⁺ at 335 nm, versus θ in accord with expression 19: circles, NaN₃ concentration 0.025 M; triangles, NaN₃ concentration varied from 0.005 to 0.05 M. (B) Plot of k_{obs} , the pseudo-first-order rate constant for oxidation of NMI by N₃⁺ at pH 9, in accord with expression 20. Squares, triangles, and circles correspond to (N₃⁻) = 0.50, 0.025, and 0.0125 M, respectively.

In contrast to the low pH range, the intensity of the NMI^{*+} absorbance produced by N_3^{*} at high pH (9) was about half of that seen with Br_2^{*-} for similar conditions (Figure 2). Also, the magnitude of the OD at 335 nm per mole of N_3^{*} radicals initially formed depended on the ratio of the concentrations of the azide ion and NMI, in accord with the relation

$$\frac{1}{\text{OD}} = \frac{1}{\text{OD}_{\infty}} + \frac{\theta}{K_8^0 \text{OD}_{\infty}}$$
(19)

Here K_8^0 is the equilibrium constant of reaction 8 at zero ionic strength, $\theta = (N_3^-)(\gamma_{\pm})^2/(NMI)$, and γ_{\pm} is the activity coefficient for a univalent electrolyte calculated by using the Debye-Hückel limiting law.³⁷ OD is the absorbance per N₃^{*} radical formed, and OD_w is the value of OD when reaction 8 proceeds to completion, i.e., the extinction coefficient of NMI^{*+}. Figure 4A shows a plot of OD⁻¹ versus θ for some of our data. The reciprocal of the intercept gives OD_w = 5714, in good agreement with $\epsilon(335)$ = 5800 \pm 250 in Table I for NMI^{*+}. The ratio of the intercept to the slope yields $K_8^0 = 86$. Further evidence that reaction 8 reaches an equilibrium at high pH is given below. The reason why stoichiometric oxidation yields are seen at pH 3 with NMI and at both high and low pHs with TRPH will be explained later.

2. Kinetics. The kinetics of oxidation of NMI by N_3^{\bullet} , $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ were investigated in the same way as those for TRPH. The second-order rate constants obtained are summarized in Table II.

In the case of N_3^{\bullet} at the higher pH, the plots of the observed pseudo-first-order rate constants (k_{obs}) for growth of NMI⁺⁺, versus NMI concentration, exhibited significant intercepts which increased with $N_3^{\bullet-}$ concentration. This is consistent with a pseudo-first-order relaxation rate in which both the forward and the back reactions play a role.³⁸ Therefore, the data were plotted

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in accordance with the expression

$$\frac{k_{\rm obs}}{(\gamma_{\pm})^2(N_3^-)} = k_8 \frac{(\rm NMI)}{(\gamma_{\pm})^2(N_3^-)} + k_{-8}$$
(20)

as shown in Figure 4B. A least-mean-squares fit yielded $k_8 =$ $(7.1 \pm 0.4) \times 10^9$ M⁻¹ s⁻¹ from the slope and $k_{-8} = (7.8 \pm 0.9)$ $\times 10^7$ M⁻¹ s⁻¹ from the intercept. The ratio of these leads to K_8^{0} = 91, in excellent agreement with 86 calculated above from the absorbance intensities after equilibrium was reached. The kinetic traces indicated that this occurred well before significant radical-radical recombination took place.

The data in Table II show that the rate constants for oxidation of NMI by Br_2^{-} and N_3^{-} are up to a factor of 2 higher than for the oxidation of TRPH. The reason for this is not entirely clear, though the data of Merényi and co-workers^{11,16} do suggest that $E^{\circ}(NMI^{+}/NMI)$ is slightly lower for NMI than for TRPH. For the present purposes, however, the main point is that the trends between H_2O and D_2O solutions are the same for NMI as for **TRPH.** This observation strongly reinforces the conclusion that reactions 6 and 8 are the rate-controlling processes for these indoles.

3. Equilibrium Constants and Yields of Oxidized Radicals. The average value of K_8^0 for NMI from our data for absorbance and kinetics is 88, which leads to $E^{\circ}(N_3^{\circ}/N_3^{-}) - E^{\circ}(NMI^{\circ+}/N_3^{-})$ NMI) = 0.114 V. Using $E^{\circ}(N_3^{\circ}/N_3^{\circ}) = 1.33$ V from Stanbury's recent review¹⁵ and ref 39, one finds $E^{\circ}(NMI^{+}/NMI)$ for NMI = 1.22 V. This agrees well with 1.23 ± 0.01 V determined earlier by Merényi, Lind, and Shen¹⁶ with ClO₂[•] as a reference. A later study by the same group,⁴⁰ using N₃⁻ as a reference, gave the E° value as 1.20 ± 0.02 V. This study also reports $k_8 \sim 10^{10}$ and $k_{-8} \sim 5 \times 10^7$ M⁻¹ s⁻¹. These various studies have served to "connect" the $ClO_2^{\bullet}/ClO_2^{-}$ potential and the N_3^{\bullet}/N_3^{-} potential and demonstrate excellent agreement. Such comparative checks of key potentials like N_3^*/N_3^- and ClO_2^*/ClO_2^- are essential in confirming the validity of the overall one-electron "redox" scale.

Attention is now directed to the reasons why the yield of oxidized indole with N3° was quantitative with NMI at low pH and with TRPH at both low and high pH. The activities of TRPH*+ and NMI⁺⁺ at equilibrium are determined by secondary acid-base equilibria as well as by reactions 6 and 8, respectively. Thus, for the case of N_3^* as the oxidant, N_3^- enters into a second equilibrium with H⁺, which becomes increasingly important as the pH falls below the pK_{HN} , of 4.7. Likewise for TRPH, the TRPH⁺⁺ radical cation is subject to deprotonation above the pK of 4.3. Writing the total concentration of $HN_3 + N_3^-$ as (Az) the total concentration of (TRP[•] + TRPH^{•+}) or the concentration of NMI^{•+} as ('Ind_{ox}), and the concentration of TRPH or NMI as Ind, the following relationship can be derived:

$$K_{\text{overall}} = \frac{(\text{Az})(^{*}\text{Ind}_{\text{ox}})}{(^{*}\text{N}_{3})(\text{Ind})} = \exp\left[\frac{F\Delta E^{\circ}}{RT} - \ln\left\{\frac{K_{\text{HN}_{3}}\gamma_{\text{N}_{3}^{-}}}{K_{\text{HN}_{3}} + a_{\text{H}^{+}}\gamma_{\text{N}_{3}^{-}}}\right\} - \ln\left\{\frac{a_{\text{H}^{+}}\gamma_{\text{TRPH}^{+}}}{a_{\text{H}^{+}} + K_{\text{TRPH}^{+}}\gamma_{\text{TRPH}^{+}}}\right\}\right] (21)$$

Here, F is the Faraday constant and, for simplicity, the activity coefficients of uncharged species and of TRPH, Ind, and TRP* have been taken as unity.

As pointed out above for NMI at high pH, where no secondary acid-base equilibria occur

$$K_{\text{overall}} = K_8^0 = \exp[F\Delta E^\circ / RT]$$
(22)

and has a value of 88. In acid solution, as $a_{H^+}\gamma_{N_0^-}$ begins to exceed K_{HN_0} , the second term causes $K_{overall}$ to exceed K_8^0 and it becomes \sim 3000 at pH 3. Thus, quantitative yields of the radical cations are observed. Note that the last term in the square bracket of eq 21 does not apply for NMI.

Turning to the case of TRPH, it can be shown that a similar effect occurs in acid solution. In addition, the third term in the square bracket of eq 21 now contributes increasingly as the pH rises above 4.3, the pK of TRPH^{•+} radical cation, until at pH 8 $K_{overall}$ is about 3×10^5 . Thus, interestingly, the azide radical oxidation of TRPH is an electron-transfer reaction, which is proton driven at low pH and base driven at high pH. The minimum in K_{overall} (~180) occurs at pH 4.5. In this case, of course, exp- $(F\Delta E^{\circ}/RT)$ is equal to K_6° .

Conclusions

Comparison of the ratios of the rates of reactions of N_3^{\bullet} , Br_2^{\bullet} , and $(SCN)_{2}$, in H₂O and D₂O solutions shows that the changes from H_2O to D_2O are basically of the same magnitude for TRPH and NMI over the pH range studied. Since only electron transfer takes place in the latter case and H/D transfer should lead to significant (i.e., factor of \sim 2) differences for TRPH in the two solvents, it is reasonable to conclude that electron transfer also dominates for this molecule—both above and below the pK of the radical cation. For the case of Br_2^{-} , this conclusion is in agreement with the earlier work of Butler and co-workers.² It is also in agreement with the interpretation of the reaction between (SCN)₂^{•-} and TRPH given earlier by Posener and co-workers.⁷

An additional point of interest is the significantly slower rate of reaction of the X2^{•-} dimeric anion radical species (Br2^{•-} and $(SCN)_2^{-}$). Indeed, in these cases, it may be that adduct formation by X[•] transfer to the indoles (Ind) is a prerequisite to successful electron transfer, viz.

$$X_2^{\bullet-} + \operatorname{Ind} \xrightarrow{(23a)} X^- + (X - \operatorname{Ind})^{\bullet} \xrightarrow{(23b)} 2X^- + \operatorname{Ind}^{\bullet+}$$
 (23)

There are good grounds for proposing this in the case of Br2*-, where the transferred electron must enter a relatively high energy antibonding orbital, unless dissociation of Br^* from the (Br_2^{*-}) radical ion has occurred.⁴¹ In contrast to the X_2^{*-} species in reaction 23a, N₃[•] would not need to undergo a dissociation on reaction with indoles. This should enhance the rate. In fact, for this species the reaction may be an outer-sphere process with no adduct involved.

Finally, the spectra of the radical cations of TRPH and NMI can be compared by reference to Figures 1-3. The value of OD for NMI solution at 335 nm is seen to be somewhat higher than for the TRPH solution, and there is no shoulder at 370 nm. Apart from this, the spectra of the two radical cations (TRPH** and NMI⁺⁺) are quite similar. Reliable extinction coefficients for the NMI*+ cation do not appear to have been reported previously. The present values for the TRPH^{•+} radical cation and TRP[•] neutral radical are somewhat higher than those in ref 9. This may reflect a loss of oxidizing radicals to radical-radical reactions in the earlier study. As noted above, precautions were taken to avoid that problem in this work.

Acknowledgment. N.G. and S.S. thank the Natural Sciences and Engineering Research Council of Canada for visiting fellowships. The authors are also indebted to the Max-Planck Institut in Mülheim and the Radiation Laboratory at the University of Notre Dame for the use of their pulse radiolysis facilities.

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