

AN ELECTRON SPIN RESONANCE STUDY OF RADICALS FROM CHLORAMINE-T—1

SPIN TRAPPING OF RADICALS PRODUCED IN ACID MEDIA

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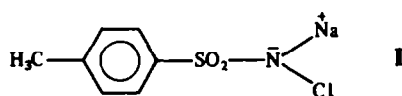
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Abstract—Under acid conditions aqueous solutions of chloramine-T form nitrogen-centred radicals via loss of the chlorine atom. The nitrogen radicals produced have been studied by ESR spectroscopy using the spin-trapping method. Adducts of the spin trap phenyl-t-butyl nitron are oxidized by chloramine-T in acid media to give a paramagnetic product in which the unpaired electron interacts with two inequivalent nitrogen atoms. The spin trap 5,5-dimethyl-2-pyrrolidine-1-oxide is oxidized rapidly to 5,5-dimethyl-2-pyrrolidone-1-oxyl by chloramine-T under acid conditions. The water soluble trap α -4-pyridyl-1-oxide-N-t-butyl nitron forms a stable nitroxide adduct with a nitrogen radical of chloramine-T in acid solution. Identical results were obtained with chloramine-B (sodium salt of N-chlorobenzene sulfonamide), indicating the involvement of the N-chloramine group in radical formation.

INTRODUCTION



Chloramine-T (I) (sodium salt of N-chlorotoluene sulfonamide) is a powerful disinfectant used in hospitals, kitchens, breweries, etc. The development of asthmatic symptoms in a number of factory workers has been shown to be due to occupational exposure to chloramine-T.¹ Sera taken from these patients was found to contain specific IgE antibodies which were shown to be directed against several different proteins treated with chloramine-T.² We have recently shown that chloramine-T forms covalent conjugates with proteins and causes extensive modification of amino acid side chains.³ It was found in these protein binding experiments that the greatest binding ratio between chloramine-T and the proteins occurred under slightly acid conditions, but that under more basic conditions the same binding ratio could only be achieved by irradiation with ultraviolet light. The nature and mechanism of this covalent protein modification is of great importance in the understanding of the allergic response produced by this chemical agent. Published studies suggest⁴⁻⁶ that chloramine-T may react via free radicals in some of its oxidation reactions. However, these suggestions come largely from the results of kinetic data, and there has been no direct evidence of free radical intermediates in reactions of chloramine-T.

The object of this work was therefore to obtain direct evidence for the involvement and characterization of free radicals in reactions of chloramine-T, and to suggest mechanisms by which such radicals could cause the protein modifications and the binding between

chloramine-T and soluble proteins observed in our studies. (Since secondary N-chloroamines are known to produce radicals at acid pH,⁷ and the results of our binding experiments between chloramine-T and proteins indicated maximum binding ratios occurred at acid pH, we have examined the production of free radicals from chloramine-T under acid conditions.)

We report on the radicals produced with irradiation under basic conditions in a subsequent study.⁸

To study any short-lived radicals produced in this system we have employed the spin-trapping method, in which diamagnetic molecules (spin traps) react with free radicals to form stable nitroxide radicals which have spectral parameters characteristic of the adduct.⁹

EXPERIMENTAL

Materials. Chloramine-T (BDH), chloramine-B, *p*-toluene-sulfonamide (Fluka), phenyl-t-butyl-nitron (PBN), 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), and α -4-pyridyl-1-oxide-N-t-butyl-nitron (POBN) (Aldrich) were used as received.

Procedure. ESR spectra were recorded on a Varian E3 spectrometer operating at 9.5 GHz (X-band). Samples were placed in the cavity in quartz or pyrex aq sample cells.

Preparation of samples. The spin traps DMPO, PBN and POBN were made up in aq soln (0.01 M) and diluted as necessary. All solns of spin traps were degassed with N₂ and stored in the dark in a refrigerator.

Aq solns of chloramine-T and the various model compounds were made to a strength of 0.05 M and diluted as necessary.

Before mixing, solns for ESR spectroscopy were degassed with N₂ for a few min to remove dissolved O₂.

Solns were made acid by the addition of 0.1 M HCl; the pH was measured on a pH meter.

RESULTS

When aqueous solutions of chloramine-T containing the spin trap PBN were made acid (pH 5) by the

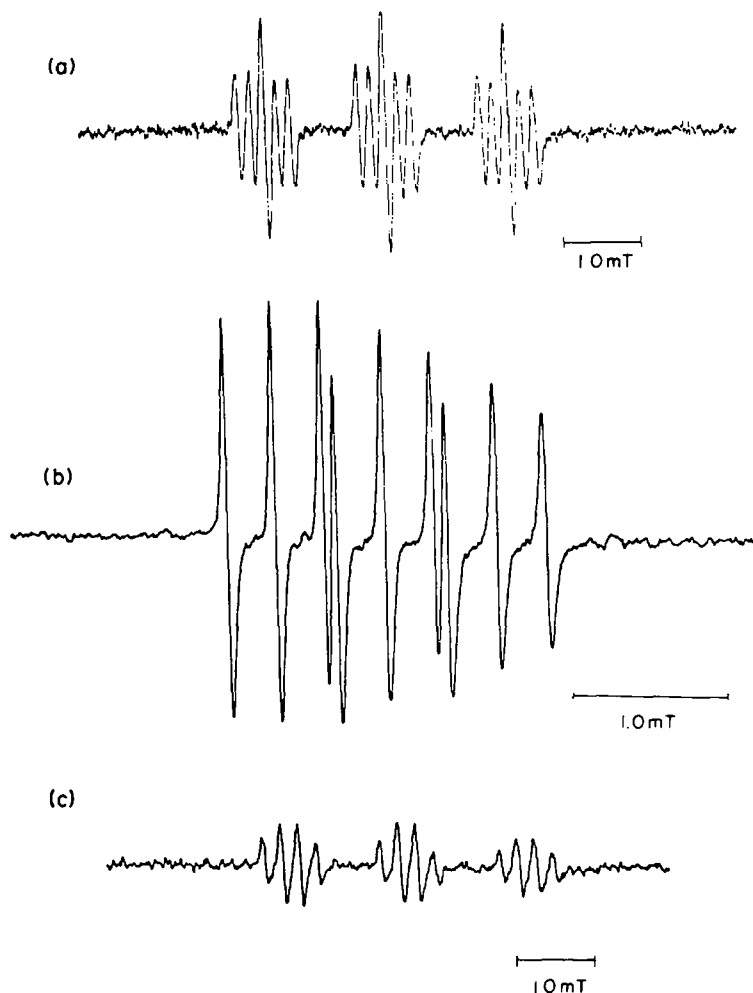


Fig. 1. (a) ESR spectrum obtained from acid solution of chloramine-T in the presence of PBN. (b) ESR spectrum obtained from (a) after allowing the solution to stand for approximately 5 min. (c) ESR spectrum obtained from acid solution of chloramine-T in the presence of POBN.

addition of 0.01 M HCl, the spectrum shown in Fig. 1(a) was obtained. When no spin trap was included no ESR spectrum was obtained. When the solution of chloramine-T and PBN were mixed at alkaline pH no ESR signals were observed. The spectrum shown in Fig. 1(a) suggests interaction between the PBN and an N-centred radical giving the following splitting constants: $a_{N_1} = 1.563$, $a_H = 0.338$, $a_{N_2} = 0.175$ mT. Within a few minutes this spectrum changed to a different spectrum shown in Fig. 1(b). This shows the interaction of the unpaired electron with two inequivalent N atoms ($a_{N_1} = 0.71$, $a_{N_2} = 0.42$ mT). This we believe could indicate a rearrangement of the first trapped species to give a complex in which the N of the spin trap is directly bonded to the N of the chloramine-T.

When chloramine-B was used instead of chloramine-T, identical ESR spectra were obtained. *p*-Toluenesulfonamide failed to produce any ESR signals at acid pH in the presence of PBN.

When the more water soluble spin trap POBN was used instead of PBN, then at acid pH solutions of chloramine-T produce the spectra shown in Fig. 1(c). This can be analysed in terms of the unpaired electron interacting with the nitroxide nitrogen, a β -H and an

α -N atom of equal coupling ($a_{N_1} = 1.500$, $a_{N_2} = 0.225$, $a_H = 0.225$ mT). This adduct remained stable with time and no other species were seen.

When an aqueous solution of chloramine-T containing the spin trap DMPO was made acid (pH 5) the spectrum shown in Fig. 2(a) was obtained. This showed the presence of several species, one of which was a triplet ($a_N = 0.71$ mT) split into further triplets in the ratio 1:2:1 ($a_{H(2H)} = 0.42$ mT). The other species decayed quickly and it was not possible to identify them. When an aqueous acid solution of $KMnO_4$ was added to a solution of DMPO the spectrum shown in Fig. 2(b) was obtained. This shows a species with the same splitting constants as the major species seen in Fig. 2(a), and probably represents an oxidation product of the spin trap.

DISCUSSION

The results of the spin-trapping experiments show that under acid conditions in aqueous solution chloramine-T reacts via a free radical mechanism. The spectrum obtained with PBN (Fig. 1(a)) is consistent with the trapping of an N-centred radical of

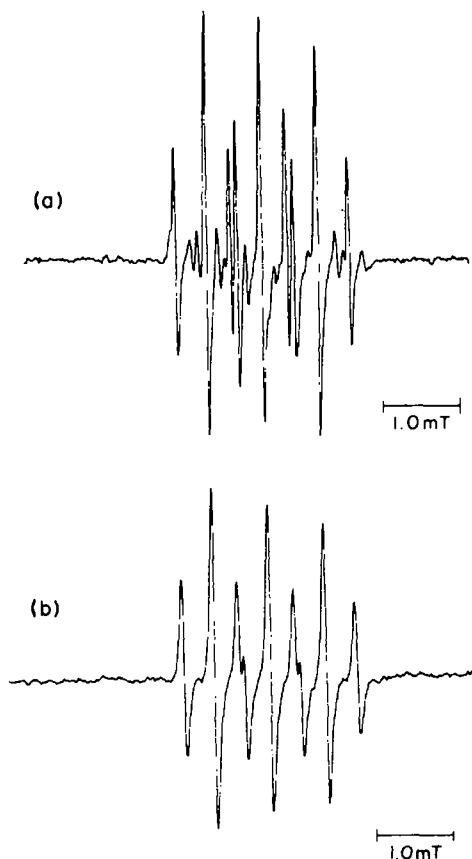
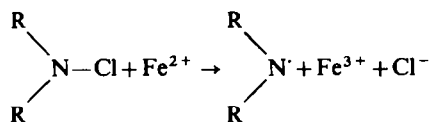


Fig. 2. (a) ESR spectrum obtained from acidified solution of chloramine-T in the presence of DMPO. (b) ESR spectrum obtained from the addition of acidified KMnO_4 to a solution of DMPO.

chloramine-T. This could be formed via loss of the Cl atom by homolytic cleavage of the N—Cl bond in a manner analogous to Hofman-Löffler radical formation in secondary N-chloramines.¹⁰ The chloramine-T N-centred radical could then be added to

PBN in the normal way; this structure accounts for the splitting constants obtained from Fig. 1(a). Further evidence for the involvement in this mechanism comes from the observation that adduct 1 is formed at a much faster rate by the addition of Fe^{2+} to the acidic aqueous solution of chloramine-T/PBN. Catalysis of the homolytic cleavage of the N—Cl bond by Fe^{2+} is well known in radical reactions of the Hofmann-Löffler

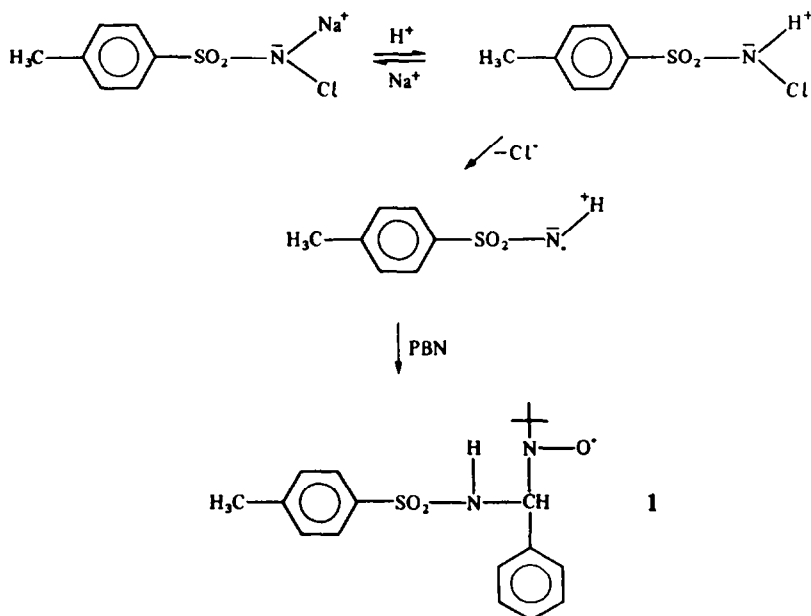


type.¹⁰ The observations that chloramine-B reacts with PBN to give spectra identical to those obtained with chloramine-T, whilst *p*-toluenesulfonamide gives no ESR signal under the same conditions, gives additional support to the involvement of the N—Cl bond in radical formation.

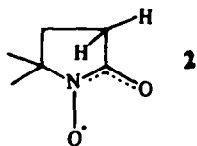
Adduct 1 is seen to rearrange or be replaced by a different adduct (Fig. 1(b)) after a few minutes. This new species is characteristic of a radical in which the unpaired electron interacts with two inequivalent N atoms; the N splittings ($a_{\text{N}} = 0.71$, $a_{\text{N}} = 0.42$ mT), however, are not characteristic of a normal nitroxide adduct of PBN. This species could arise from direct bonding of the chloramine-T N atom to the N atom of the spin trap perhaps via oxidation of an intermediate oxaziridine.¹¹

The results using the more water soluble trap POBN show that an N-centred radical is produced at acid pH, but not at alkaline pH, confirming the acid dependence of the reaction. As with PBN, the rate of formation of the ESR signals was much greater when Fe^{2+} ions were added to the solution. Reaction of spin trap POBN with chloramine-T gave rise to ESR spectra with slightly different splitting constants from those obtained with PBN; the spectra were consistent with the addition of the radical to POBN.

The powerful oxidizing properties of chloramine-T are evident from the formation of an oxidized form of



the spin trap DMPO (the major species in Fig. 2(a)). This radical has been reported by other investigators¹² in redox reactions using DMPO as spin trap, and has been assigned to 5,5-dimethyl-2-pyrrolidone-1-oxyl (2). When DMPO was added to dilute acidic KMnO_4 the same radical was formed (Fig. 2(b)).



The results of the spin trapping experiments give direct evidence that in acid solution chloramine-T reacts to form free radicals. The ESR spectra of the trapped chloramine-T radical show an N-centred radical, which is formed via acid catalysed homolytic cleavage of the N—Cl bond. Further evidence to support the generation of Cl^\cdot is the production of 3-chlorotyrosine in reactions of chloramine-T with proteins³ under mildly acidic conditions. The N-centred chloramine-T free radical plays an important role in the oxidizing properties of chloramine-T, and such properties are emphasized by the oxidation of the spin trap DMPO into the paramagnetic derivative (2). The rearrangement of the PBN-chloramine-T adduct

to produce a paramagnetic compound in which two inequivalent nitrogens are bonded together is also thought to arise from a similar oxidation reaction.

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