

SPIN TRAPPING OF FREE RADICALS FORMED DURING VISIBLE IRRADIATION OF AN ACRIDINE DYE: 3,6-DIAMINOACRIDINE (PROFLAVINE)

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Summary

Reactions of proflavine during visible irradiation in aqueous and alcohol-water solutions have been investigated.

Photolysis of the dye generates free radicals which were spin trapped using nitroso-*t*-butane and identified with the help of electron spin resonance spectroscopy.

Several types of radicals were found: the hydrogen adduct radical, the alcohol adduct radical, the di-*t*-butyl nitroxide radical and a radical characteristic of the semi-reduced form of the dye.

1. Introduction

It is well known that the acridine dyes are efficient sensitizers [1]. Most of the photosensitized reactions involve the triplet state of the dye. The most efficient sensitizers are those with a long lifetime and a triplet state of high quantum yield [2].

It is clear that irradiation of the dye in the long-wavelength absorption band produces intermediate semi-oxidized and semi-reduced photoproducts. To characterize these radical products formed during the production of excited triplet states and their interaction with the solvent, the spin trapping technique, which involves the formation of stable free radicals by attachment of primary labile radicals to the nitroso compound, has been used.

In the present work the visible photolysis at room temperature of aqueous, alcoholic or mixed solutions of proflavine (DAA) in the presence of nitroso-*t*-butane (NtB) has been studied. The nitroso compound can react with the short-lived radicals; long-lived radicals are then formed (reaction (1)) which can be identified by electron spin resonance (ESR) spectroscopy:



By following the decay of the spin-trapped radicals, the complex spectra due to several radical species could be elucidated.

2. Materials and methods

2.1. Chemicals

DAA was purchased from the Aldrich Chemical Company and was recrystallized three times from ethanol-water (50 vol.%). The molar extinction coefficient ϵ was 4×10^4 . Stock solutions (10^{-3} M) in distilled water or in ethanol were kept in the dark. NtB was also obtained from Aldrich and was stored under nitrogen at 5 °C in the dark.

2.2. Sample preparation and photolysis

Solutions of NtB (1 mg ml^{-1}) were prepared by stirring overnight in the dark. The solution was light blue and had an optical density of 0.34 at 662 nm in a 10 cm cell. The two solutions were mixed together and were bubbled in the dark prior to the irradiation with either N_2 or O_2 for at least 20 min. The solutions were photolysed in the cavity of the spectrometer using a high pressure mercury lamp (Osram HBO 500 W) equipped with an interferential filter ($\lambda_{\text{max}} = 440 \text{ nm}$, bandwidth = 20 nm).

2.3. Measurements

A Varian E-3 X band (9.5 GHz) ESR spectrometer was used. The magnetic field modulation frequency was 100 kHz. Care was taken to avoid power saturation and in general 5 mW of microwave power was used. All the ESR measurements were carried out at room temperature.

The radical species were observed during and after direct irradiation. Such observations (light on and light off) proved helpful in analysing the ESR spectra containing more than one radical species.

3. Results and discussion

Photolysis by monochromatic light ($\lambda = 440 \text{ nm}$) of aqueous solutions containing NtB and DAA produced three different radical species. The corresponding sets of lines are labelled 1,2 and S in the spectrum shown in Fig. 1. Each spectrum exhibits primary splitting of the triplet caused by the ^{14}N nucleus of the nitroxide group along with secondary splittings which originate from magnetic nuclei in the part of the nitroxide radical derived from the trapped radical.

No radical has been detected in the illuminated solution of NtB alone, but in the presence of DAA a symmetrical triplet (line S) with a primary splitting



Fig. 1. The ESR spectrum observed during irradiation of nitrogen-saturated aqueous solution of DAA (10^{-3} M) in the presence of NtB (1 mg ml^{-1}).

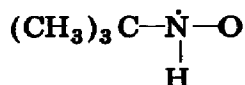
of 17.0 G is observed. This radical is characteristic of the di-*t*-butyl nitroxide radical S:



The value of the measured splitting is in very good agreement with those reported by Kominami *et al.* [3] ($a_{\text{N}} = 16.8 \text{ G}$, $g = 2.0055$), Kawamura *et al.* [4] ($a_{\text{N}} = 16.75 \text{ G}$, $g = 2.0055$) and Sargent *et al.* [5] ($a_{\text{N}} = 17.0 \text{ G}$) during radiolysis of aqueous solutions.

In the presence of oxygen the spectrum of this radical is very intense and overlaps considerably the other components of the total resonance. In another experiment, when a D_2O solution was photolysed, the structure of this spectrum did not change; this is a good support for the assignment made earlier.

The ESR pattern marked 1 consists of six lines arising from the interaction of an unpaired electron with a nitrogen nucleus and a proton. The spectrum is consistent with the spin-trapped radical produced by hydrogen atom addition. This radical is not stable and decays rapidly as the light is turned off. The splitting constants are respectively $a_{\text{N}} = 14.34 \text{ G}$ and $a_{\text{H}} = 13.85 \text{ G}$ corresponding to the structure 1:



In D_2O solutions, the lines 1 disappear and a new pattern of lines (a triplet of triplets) appears in the vicinity of the lines S. Although these lines overlap part of the spectrum, one can measure the splitting of the deuterium spin adduct: $a_{\text{D}} = 2.1 \text{ G}$. This is in good agreement with what is expected for replacement of a proton splitting by a deuterium splitting.

The third pattern of lines marked 2 consists of three lines of width larger than that of the other lines. The nitrogen coupling constant is equal to

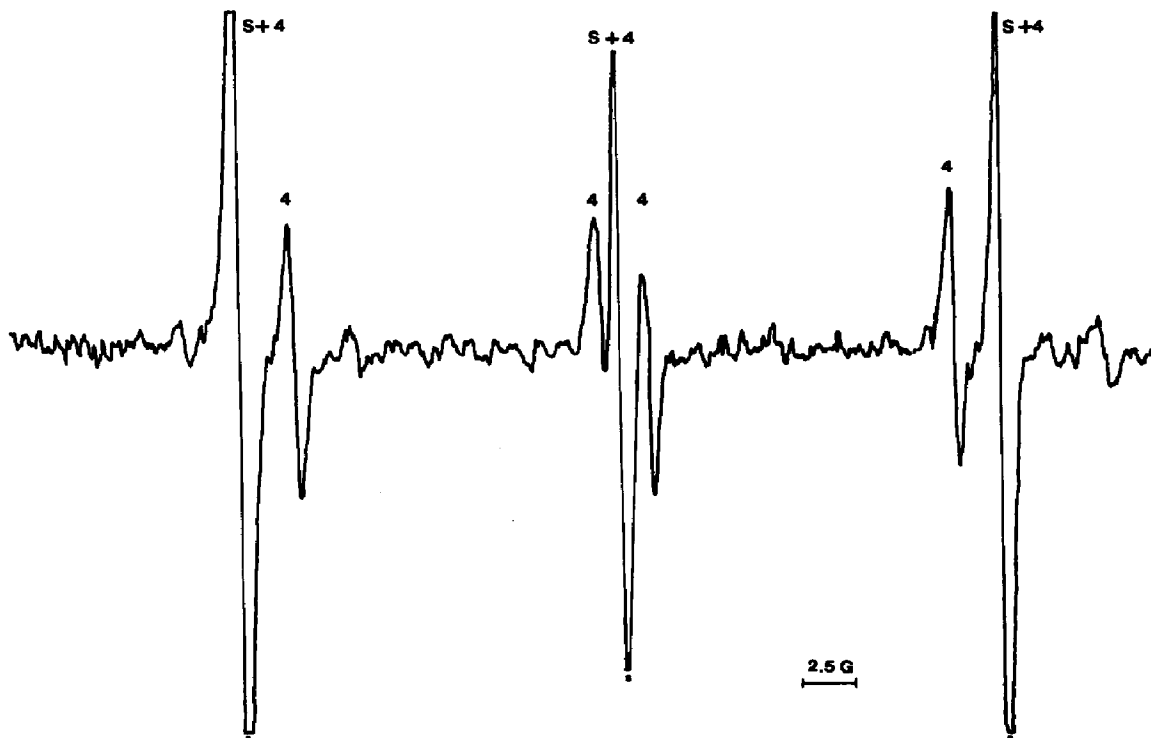
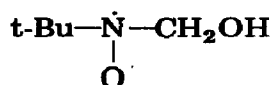


Fig. 2. The ESR spectrum observed after irradiation of a nitrogen-saturated water-ethanol (50 vol.%) solution of DAA (10^{-3} M) in the presence of NtB (1 mg ml^{-1}). The H spin adduction disappeared.

$a_N = 14.4 \text{ G}$; when the irradiations are performed in oxygen-bubbled solutions, this signal cannot be observed. Moreover, when the solutions are bubbled with nitrogen prior to the irradiation, the relative intensities of lines S and 2 are modified, the latter being more intense in the absence of O_2 . These facts indicate that radical 2 is formed through the triplet state probably after a triplet-triplet annihilation. Under these conditions, it is suggested that the observed DAA spin adduct is the trapped semi-reduced form of the dye.

When alcoholic or mixed (alcohol-water) solutions are studied, the lines characteristic of the ethanol or methanol adduct radicals (Figs. 2 and 3) can be observed. These radicals are formed through the abstraction of a hydrogen atom from the alcohol during excitation of the dye. The resulting spin adducts are



from CH_3OH

Radical 3

and

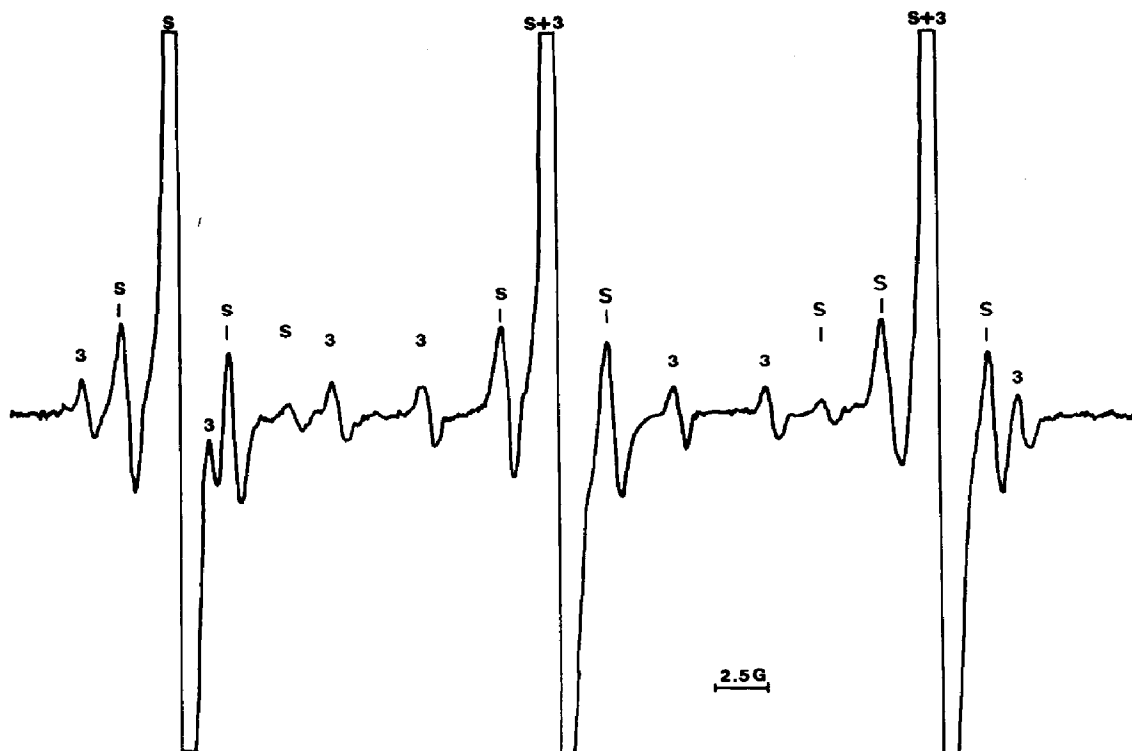
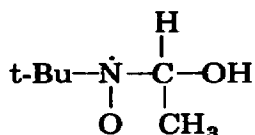


Fig. 3. The ESR spectrum observed after irradiation of a nitrogen-saturated water-methanol (50 vol.%) solution of DAA (10^{-3} M) in the presence of NtB (1 mg ml^{-1}).



from $\text{CH}_3-\text{CH}_2\text{OH}$

Radical 4

The main splittings for methanol are $a_{\text{N}} = 15.2 \text{ G}$ and $a_{\text{H}} = 5.45 \text{ G}$ and for ethanol are $a_{\text{N}} = 15.2 \text{ G}$ and $a_{\text{H}} = 2.06 \text{ G}$. These splittings are in good agreement with those reported by Sargent and Gardy [6] for the radicals obtained by γ radiolysis of methanol and ethanol. Moreover, the signal characteristic of the hydrogen spin adduct is still present (not shown on the figure); this is consistent with the hydrogen donor properties of the alcohol.

4. Conclusion

The interaction between DAA and NtB during monochromatic irradiation leads to the production of free radicals characteristic of each of the compounds of the solution: the spin trap, the dye, the solvent. The di-*t*-butyl radical is formed by electron transfer from the dye to the spin trap. It is favoured by the presence of oxygen in the solution. In contrast, the signal

due to the dye is favoured by the absence of oxygen. This is in good agreement with the formation of the semi-reduced form by triplet-triplet annihilation. The hydrogen spin adduct formation could proceed from two mechanisms: the first is the electron transfer to the spin trap followed by protonation, the second is the direct transfer of a hydrogen atom. In any case, the spin adduct is the same and only the origin of the proton is different: in the first case the proton comes from the solvent, in the second case the proton comes from the excited dye. Which excited state of the dye is involved in the proton transfer is difficult to know: it could be either the triplet state of the dye or the semi-reduced form. The pK values of the triplet state (pK_T) and of the semi-reduced form of the dye both differ from the value pK_G of the ground state. The DAA case has not been elucidated but a recent study [7] has shown that for acridine orange $pK_T = pK_G = 10.25$ whereas the pK of the semi-reduced form was about 5.1.

As a consequence, it is quite possible that at neutral pH the semi-reduced form liberates a proton simultaneously with the electron which could lead to a hydrogen spin adduct. As far as the alcoholic solutions are concerned the free radical formation is due to the extraction of a hydrogen atom of the alcohol by reaction with the semi-reduced form to give the leucodye.

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