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Photophysics of methyl substituted alloxazines in water: efficiency of singlet oxygen generation

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Abstract

The photophysical properties of some methyl substituted alloxazines in water are reported. Excited singlet and triplet have been studied and excited singlet state relaxation in the absence of oxygen shown to be dominated by non-radiative relaxation pathways. In the presence of oxygen, efficient singlet oxygen generation is observed. The observed photophysica are discussed in terms of the hydrogen bonding nature of the solvent.

Keywords: Alloxazines; Singlet state; Photophysics; Singlet oxygen

1. Introduction

Alloxazines represent a class of nitrogen heterocycles related to lumazines and biologically important flavins. The early interest in the photophysical and photochemical properties of alloxazines, including lumichrome (7,8-dimethylalloxazine) was mainly driven by comparison with isoalloxazines, mostly as their photoproduct. The early interest in alloxazines was limited largely to their excited state phototautomerism [1–3]. Alloxazines unsubstituted at the N(1) position can undergo excited state proton transfer from N(1)–H to N(10) to the corresponding isoalloxazinic form. The excited state reaction occurs in the presence of compounds having proton-donor and acceptor groups, able to form correct hydrogen bonds with alloxazine molecules [2–4]. Recently, the interest in the photochemistry and photophysics of alloxazines has become more intense because of other interesting facts. Alloxazines are well known as photochemical decomposition products of flavins. Substituted alloxazines, mainly lumichromes, have been found in many biological materials [5,6]. Alloxazine nucleosides are potentially of interest as fluorescent probes and have been predicted to exhibit hydrogen bonding characteristics similar to those of thymidine [7]. It has been suggested that lumichrome may be used as an optical transistor device with a thin film of lumichrome on conductive SnO_2 glass [8].

Recently, the interest in the photochemistry of alloxazines has focused on the study of the interactions which occur between alloxazines and oxygen in the presence of light. It has been shown that alloxazines are good and efficient photosensitisers of singlet oxygen [9,10]. It has also been proposed that lumichrome may play an important role in the process of photodegradation of a polyamidehydroxyurethane type of polymer in aqueous solution. It has been suggested that a possible mechanism of photodegradation involves singlet oxygen [11]. The singlet oxygen producing capacity of lumichrome in water was examined under UVA [12]. A further point of interest is the possibility of using alloxazines to sensitise the photooxidation of substituted phenols in water [13].

In water, little spectroscopic data has been published for the methyl substituted alloxazines studied here [14]. To our knowledge no data are available on their excited singlet and triplet states in water. In order to study the interaction between alloxazines and oxygen in water it is first necessary to determine the photophysics of the alloxazines in their singlet and triplet states. In the first part of this paper we present spectroscopic and photophysical data for both the excited singlet and triplet states of methyl substituted alloxazines in water. In the next part, we present the results of measurements of the emission at 1270 nm, which is highly specific to the $O_2(^1\Delta_g) \rightarrow O_2(^3\sum_g{}^-)$ transition, under laser

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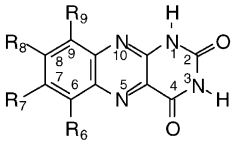

R_6=CH_3,	R_7=H,	R_8=H,	R_9=H,	6-methylalloxazine (6MAll)
R_6=H,	R_7=CH_3,	R_8=H,	R_9=H,	7-methylalloxazine (7MAll)
R_6=H,	R_7=H,	R_8=CH_3,	R_9=H,	8-methylalloxazine (8MAll)
R_6=H,	R_7=H,	R_8=H,	R_9=CH_3,	9-methylalloxazine (9MAll)

Fig. 1. Structures and abbreviations for methyl substituted alloxazines studied in the present work.

excitation at 355 nm of the alloxazines in air-equilibrated D_2O solutions. The results concerning singlet oxygen include the quantum yields of photosensitised production of singlet oxygen $O_2(^1\Delta_g)$, ϕ_Δ, and singlet oxygen lifetimes, τ_Δ. The structure and abbreviations for the alloxazines studied here are presented in Fig. 1.

2. Experimental

Fluorescence lifetimes were measured by exciting alloxazines in aqueous solution at 355 nm using the frequency-doubled output of a mode-locked, argon-ion synchronously pumped, cavity-dumped dye laser for excitation and a fast Hamamatsu microchannel plate detection system. The description of the time correlated single photon counting measuring system used can be found elsewhere [15].

The laser flash photolysis experiments were carried out by exciting samples at 354.7 nm in a quartz cell. The experiments were performed using an LKS50 instrument from Applied Photophysics. The laser was a Q-switched Nd:YAG Spectron Laser System, the output from which was frequency tripled to give, typically, 17–25 mJ/pulses with a width of ca. 8 ns. A description of the system has been presented previously [16].

For the singlet oxygen measurements, air-equilibrated D_2O solutions of the alloxazines were optically matched (±0.01 absorbance units) at the excitation wavelength (355 nm) to a standard reference solution. The solutions were prepared in 1 cm^2 capacity cells with an absorbance at 355 nm equal to 0.20. Emission from singlet oxygen following laser excitation was detected by time resolved spectroscopy as described previously [9,10]. Singlet oxygen was detected by monitoring the 0,0 vibronic band of its phosphorescence the $O_2(^1\Delta_g) \rightarrow O_2(^3\sum_g{}^-)$ centred at 1270 nm, using a Judson germanium photodiode coupled to a Judson PA100 preamplifier. Quantum yields of singlet oxygen, ϕ_Δ, were determined relative to riboflavin in D_2O as a standard, for which a value of $\phi_\Delta = 0.3$ has been reported [17].

UV–visible absorption spectra were recorded on an HP 8453 diode array spectrophotometer. Steady-state fluorescence spectra were recorded on a Spex Fluoromax spectrofluorometer. All measurements were performed at room temperature. Distilled, deionised and unbuffered water was used in all experiments and the pH of all of the aqueous solutions prepared was ca. 6.

3. Results and discussion

The absorption spectra of 8-methylalloxazine (8MAll) and 9-methylalloxazine (9MAll) together with the corresponding corrected emission spectra of alloxazines, are shown in Fig. 2. Alloxazines exhibit absorption spectra with two electronic transitions in the region 300–400 nm. The molar absorption coefficients and positions of the band maxima are listed in Table 1. The UV–visible absorption and emission bands of alloxazines are all assignable to the electric dipole allowed π–π^* transitions. In aprotic solvents the absorption bands undergo small red shifts with increasing polarity of the solvent. In water, on cellulose and in the presence of hexafluoroisopropanol the red shift in the absorption and emission spectra is much greater [10,14,18].

The fluorescence emission spectra of methyl substituted alloxazines exhibit a broad, structureless single band with a maximum at about 480 nm, whose exact position depends on the position of the substituents. The exact wavelengths of the fluorescence maxima are listed in Table 1. The maximum of the alloxazines fluorescence in aqueous solution is shifted to longer wavelengths by about 45 nm, relative to that of the alloxazines in acetonitrile [10,18]. Recently, it has been shown that the fluorescence emission maxima of lumichrome and lumichrome substituted with methyl groups

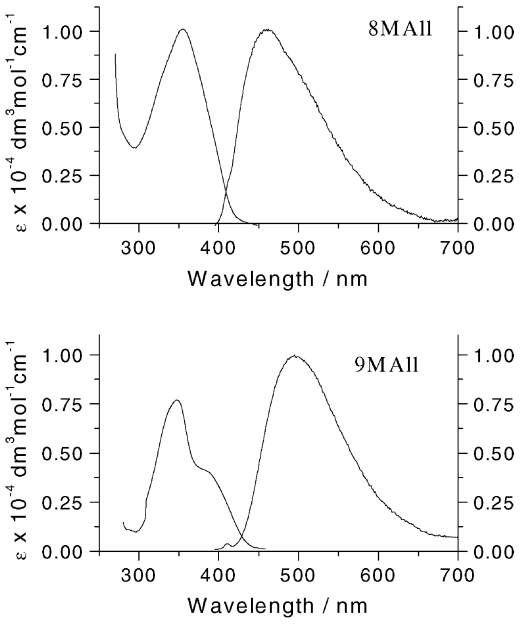

Fig. 2. Ground-state absorption spectra together with normalised fluorescence spectra of 8MAll, and 9MAll, in aqueous solution at pH ≅ 6.

Table 1
Spectroscopic and photophysical data for the singlet states of alloxazines in air-equilibrated water (pH ≅ 6)[a]

Compound	$\lambda_{max}{}^2$ (nm)	$\lambda_{max}{}^1$ (nm)	λ_F (nm)	ϕ_F	τ_F (ns)	$k_r{}^b$ ($\times 10^7\ s^{-1}$)	$\Sigma k_{nr}{}^c$ ($\times 10^9\ s^{-1}$)
6-Methylalloxazine	349 (11500)	385 (s)	512	0.049	8.58	0.6	0.11
7-Methylalloxazine	328	391 (5700)	475	0.046	3.15	1.5	0.30
8-Methylalloxazine	355 (10800)	–	461	0.023	1.79	1.3	0.54
9-Methylalloxazine	347 (7700)	386 (s)	495	0.053	7.33	0.7	0.13

[a] $\lambda_{max}{}^1$ and $\lambda_{max}{}^2$ are the maxima of the two long-wavelength bands in the absorption spectra; the molar absorption coefficients are in parentheses. The fluorescence emission maximum is λ_F, the fluorescence quantum yields, ϕ_F, the lifetime of fluorescence, τ_F. The radiative rate constant is k_r, and the sum of non-radiative rate constants Σk_{nr}.
[b] Calculated as $k_r = \phi_F/\tau_F$.
[c] Calculated as $\Sigma k_{nr} = (1-\phi_F)/\tau_F$.

at the N(1) position in aqueous solution are shifted to longer wavelengths relative to the fluorescence maxima in other non-aqueous solvents previously used [9].

The two bands located in the long wavelength region of the alloxazine spectra reflect single electronic transitions. According to quantum mechanical predictions and excitation anisotropy experiments both bands at 335 and 380 nm, reflect two independent $\pi-\pi^*$ transitions. An additional very weak $n-\pi^*$ transition may be expected in the long wavelength region [19,20]. It has been suggested that in the case of alloxazines in low polarity solvents the closely spaced $n-\pi^*$ and $\pi-\pi^*$ transitions are shifted in opposite directions as a result of increased solvent polarity. Alloxazines are multifunctional molecules with numerous proton-donor and proton-acceptor sites. It is reasonable to expect that for alloxazines in water solutions different hydrogen bonds can be formed between alloxazine and water molecules. The spectra are sensitive to solvent polarity, and in particular the long-wave absorption bands exhibit linear correlations with the polarity of solvents expressed by *Z*-values [14]. However, for solvents such as water and acetic acid deviations from that correlation were observed suggesting the possibility of hydrogen bonding formation. It is very interesting to compare the spectra of alloxazines in water with those in solution in the presence of hexafluoroisopropanol, a hydrogen donor agent, or to those on cellulose [18,21,22]. For example in the emission spectra of alloxazines in water strong long wavelength shifts of the maxima are observed, and similar shifts were noted of the fluorescence maxima of 1,3-dimethyllumichrome and 3-methyllumichrome in the presence of hexafluoroisopropanol in 1,2-dichloroethane and fluorescence spectra of different alloxazines including 1-methyllumichrome on cellulose [21,22]. Szafran et al. [22] suggested that hydrogen bonds between hexafluoroisopropanol and 3-methyllumichrome are formed first at N(10), then at N(5) and at both carbonyl oxygen atoms (at C(2) and C(4)).

Fluorescence quantum yields (ϕ_F), radiative and the sum of the non-radiative rate constants (k_r and Σk_{nr}, respectively) and fluorescence lifetimes, τ_F, for methyl substituted alloxazines in aqueous solution together with some other spectroscopic data are summarised in Table 1. The measurements have shown that ϕ_F is low and similar for all alloxazines in aqueous solution but higher than those obtained for examined alloxazines in other non-aqueous solvents [4,10,18]. According to the values of fluorescence intensity of the three forms, neutral and two monoanions of lumichrome (see Fig. 6 from Ref. [23]), at pH ≅ 6 the only emitting component is the neutral form. By analogy to lumichrome it is expected that at pH 6, methyl substituted alloxazines are also in the neutral form [23,24]. For all methyl substituted alloxazines at pH ≅ 6 in water, the fluorescence decays are well represented by single exponential functions, as shown in Fig. 3. The goodness of fit was evaluated using the reduced chi-square, χ^2, Durbin–Watson, and ordinary-runs tests as well as by inspection of the distribution of the weighted residuals and the autocorrelation functions. The corrected fluorescence excitation spectra of all methyl substituted alloxazines are identical to the corresponding absorption spectra throughout the near UV–Vis range, the emission spectra are independent of the excitation wavelength, and the excitation spectra are independent of emission wavelength. Thus, it is reasonable to assign the emitter to the lowest excited singlet state of the neutral form of corresponding alloxazines. The lifetimes determined for all alloxazines in water are the longest if compared to non-polar 1,2-dichloroethane, polar aprotic acetonitrile, polar protic ethanol and other solvents previously used [18]. It has been reported for alloxazines that increases in the solvent polarity cause a strong red shift of the emission maxima and an increase in the fluorescence quantum yield [14,18]. It is believed that in acetonitrile the possibility of mixing and enhancement of emission from pure $\pi-\pi^*$ transition is observed.

The results also indicate that the fluorescence lifetimes of the alloxazines vary according to the position of the methyl substituent and the shorter lifetimes are observed for 7MAll and 8MAll, while the longest one for 6MAll and 9MAll. The position of methyl substituents affects very markedly the directions of the transition moments of alloxazines [19,20]. Quantum mechanical predictions show that methyl substituent in position C(7) and C(8) enforce the orientation of the first electronic transition as parallel to the long axis of the molecules, whereas those at C(6) and C(9) along short axis. Those observations suggest some role of

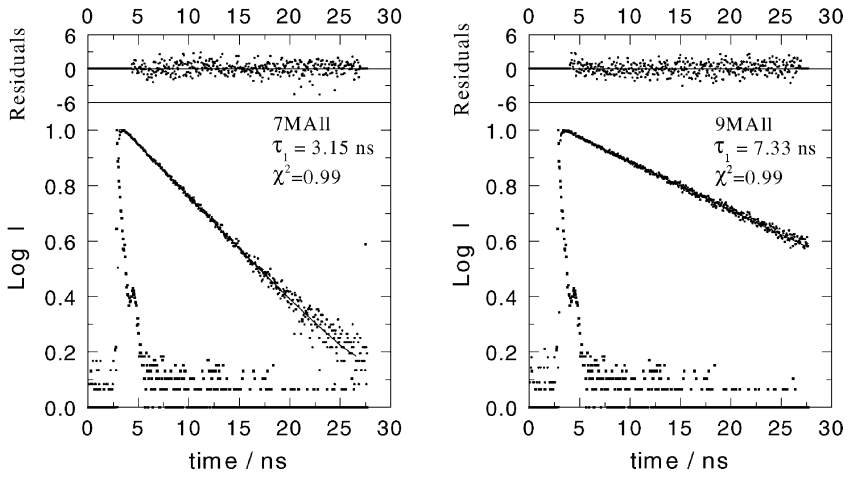

Fig. 3. Normalised fluorescence emission decays of 7MAll, and 9MAll, in air-equilibrated aqueous solutions, pH≅6.

the position of the methyl substituent on the deactivation of the first excited singlet state.

The fluorescence quantum yields and excited state lifetimes may be used to calculate the kinetic decay parameters of the neutral forms of the alloxazines in aqueous solution using the standard relationships

$$k_r = \frac{\phi_F}{\tau_F} \quad (1)$$

$$\sum k_{nr} = \frac{1 - \phi_F}{\tau_F} \quad (2)$$

where k_r is the rate constant for the radiative decay of the emitting S_1 state and Σk_{nr} the sum of the rate constants of all parallel first order and pseudo-first order processes by which S_1 decays non-radiatively. The data are presented in Table 1 and show that the decay of the excited singlet state of alloxazines is dominated by the rates of non-radiative processes. These spectroscopic and photophysical properties of the alloxazines in their excited singlet states are similar to those recently reported by us for lumichrome and lumichrome substituted with methyl group at N(1) position in aqueous solution [9]. The results presented in Table 1 confirm the influence of the methyl substituent position on the photophysics of alloxazine molecules.

Nanosecond flash photolysis of methyl substituted alloxazines in aqueous solution reveal transient species that decay on a microsecond timescale. All alloxazines appear to exhibit very similar photochemistry in aqueous solution in terms of the nature of the transient species observed following nanosecond flash excitation. Transient difference spectra for 7-methylalloxazine (7MAll) and 9-methyllumichrome as representatives of the studied alloxazines, are presented in Fig. 4. Similar spectra, not shown, have been recorded for 6-methylalloxazine (6MAll) and 8-methyllumichrome. These spectra are similar to those previously reported for the transient absorption of lumichromes and alloxazine in aqueous solution [9,25–28]. The transient absorptions have maxima at about 280, 360, 440 and 500 nm, with the addition of "negative absorbance" features at about 330 and 390 nm which are due to ground-state depletion. The decay lifetimes were measured as a function of the ground-state alloxazines concentration in the limit of low laser intensities. For the concentration range of alloxazines from 6×10^{-5} to 1×10^{-5} mol dm^{-3} no changes in the triplet lifetime have

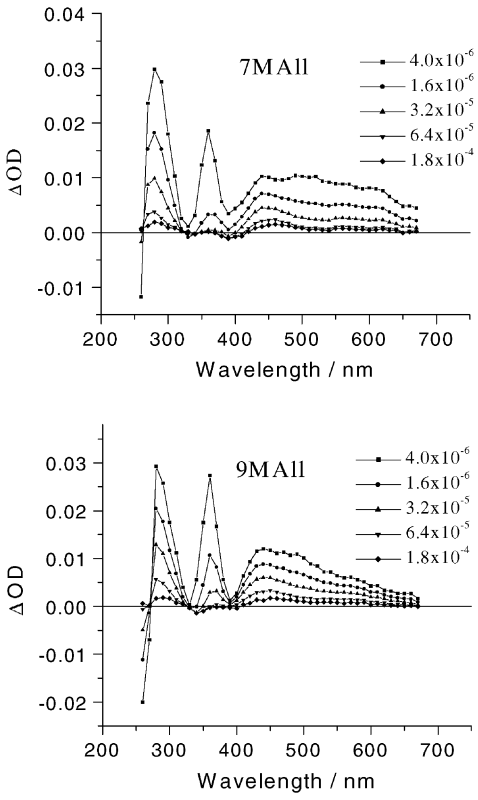

Fig. 4. Transient absorption spectra of 7MAll, and 9MAll, in deoxygenated aqueous solutions at room temperature. The numbers refer to the time after laser excitation at 355 nm, cell pathlength 1 cm, pH≅6.

Table 2
Photophysical data for the lowest triplet states of alloxazines in water at pH ≅ 6[a]

Compound	τ_T (μs)	$k_t^{O_2}$ ($\times 10^9$ dm^3 mol^{-1} s^{-1})	ϕ_Δ	τ_Δ (μs)
6-Methylalloxazine	14	1.4	0.45	51
7-Methylalloxazine	13	1.5	0.42	61
8-Methylalloxazine	15	1.5	0.37	56
9-Methylalloxazine	14	1.5	0.39	58

[a] The triplet state lifetime is τ_T; the rate constants for quenching of the triplet states by oxygen are $k_t^{O_2}$, and the quantum yields of photosensitised production of singlet oxygen are ϕ_Δ. The singlet oxygen lifetimes in air-equilibrated D_2O solutions are τ_Δ.

been observed. Transient difference spectra of alloxazines show the presence of at least two species with the lifetimes of the order of micro- and milliseconds. Non-monoexponential kinetic decays are observed. The short-lived species has a lifetime sensitive to oxygen, and lifetimes recovered from the decay of the depletion and the decay of short-lived species were identical, which suggest that short-lived species is the lowest triplet of the alloxazine.

The photophysical data obtained from flash photolysis experiments for the alloxazines studied in aqueous solution at pH ≅ 6 are summarised in Table 2. The previously reported lifetimes of the triplet states are 17.0 μs for lumichrome in aqueous solution at pH ≅ 6 [9], 11.0 and 10.0 μs for lumichrome and alloxazine in acetonitrile [10], and 13 μs for alloxazine in ethanol [26]. Note that the previous assignment of the transient absorption spectrum of lumichrome in water at pH = 2.0 to the neutral triplet was an error [25]. The transient absorption spectrum of lumichrome in water at pH = 2.0 can be assigned as absorption due to the protonated triplet state [28]. From the spectra and kinetics, it is seen that several different species are present. The photophysics and photochemistry of alloxazine and lumichrome in aqueous solutions, in both air-equilibrated and oxygen free environments, have been discussed in detail by Dekker et al. [26]. These authors suggest that the photochemical decomposition of alloxazines include several different free radicals as intermediates. The final products in deoxygenated water include dihydroalloxazine, hydroxyalloxazine, semiquinone radical and fully reduced form of alloxazine. The excited state of alloxazine reacting with water is likely to be a singlet excited state. It should be also kept in mind that the possibility of the presence in aqueous solution of equilibria involving tautomeric triplet states of alloxazines has been reported [25,28]. Some future studies on this matter are planned.

Different processes mediated by singlet oxygen continue to attract the attention of the scientific community owing to their application in various areas e.g. in photodynamic therapy, purification of wastewaters and photodegradation of polymers. For a large number of compounds including alloxazines, considerable interest has been devoted to the determination of their ability to act as $O_2(^1\Delta_g)$ photosensitisers. This is reflected in the reviews [17,29]. For general interest we performed some experiments concerning alloxazine photosensitised production of singlet oxygen in D_2O. It has been shown previously by us that lumichrome can be used as a photosensitiser of singlet oxygen in water at pH 6 [9]. In contrast to isoalloxazines, alloxazines are rather photochemically stable in water [11,13]. For example, under aerobic conditions riboflavin easily decompose after several minutes of illumination with simulated sunlight. In contrast, lumichrome has been found extremely stable toward sunlight, and was found to be a major flavin photoproduct in natural water [13]. In the present work the quantum yields and lifetimes of singlet oxygen, ϕ_Δ and τ_Δ, formed by triplet photosensitisation were determined by exciting air-saturated alloxazine samples in D_2O at 355 nm. The data are presented in Table 2. The intensities of singlet oxygen emission at 1270 nm were determined relative to that of the standard, riboflavin in D_2O, for which a value of 0.3 has been measured [17]. The emission intensity at 1270 nm increased in samples with higher concentrations of oxygen and was extinguished by bubbling N_2 through the solution for a few minutes. These observations confirm that all the compounds used in this study acted as photosensitisers, and that $^1O_2$ is responsible for the emission at 1270 nm. For each of the compounds examined here, the values of ϕ_Δ are similar to those reported by us for lumichrome and 1-methyllumichrome in air-equilibrated D_2O solutions ($\phi_\Delta = 0.36$ for lumichrome, and $\phi_\Delta = 0.41$ for 1-methyllumichrome) [9] and much lower than those measured previously by us in air-equilibrated acetonitrile solutions ($\phi_\Delta = 0.73$ for lumichrome, and $f_\Delta = 0.67$ for 1-methyllumichrome) [10]. The lifetimes of singlet oxygen reported in Table 2 are shorter then typical lifetime of singlet oxygen in D_2O. On the other hand, for the concentration range of alloxazines from 6×10^{-5} to 5×10^{-4} mol dm^{-3} no measurable changes in the singlet oxygen lifetime have been observed. In the concentration range used we were limited by solubility of alloxazines on the one side and the signal of phosphorescence from singlet oxygen on the other. The results may suggest that singlet oxygen reacts with these alloxazines in their ground-state, but we did not succeed in determining the rate constant for such reactions. The values of ϕ_Δ reported in Table 2 for alloxazines in D_2O are smaller than our recently reported values for ϕ_Δ a set of alloxazines, including lumichrome and 1-methyllumichrome, in acetonitrile solution [10]. In acetonitrile it was shown that the efficiency of singlet oxygen production from quenching of the triplet state was unity within the error of the experiment. Further experiments are required to elucidate triplet quantum yields in water in order for a comparison of this efficiency to be made.

4. Conclusions

The photophysics of alloxazines in water has been shown to be dominated by hydrogen bonding effects, with

non-radiative deactivation of the singlet state being a dominant pathway. Triplet–triplet absorption is also observed, along with other transient species in solution which may include protonated and radicaloid species. In the presence of oxygen, triplet quenching leads to production of singlet oxygen, with a quantum yield lower than observed in acetonitrile. The absolute efficiency of production following triplet state production is the subject of current research.

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