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Photochemistry of flutamide in various media: Investigation of the reaction mechanism as revealed by external magnetic field effects on product yields

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ABSTRACT

Photochemistry of an anticancer drug flutamide, 2-methyl-*N*-[4-nitro-3-(trifluoromethyl) phenyl] propanamide has been investigated in various media in the absence and presence of 0.1 T magnetic field. On photoexcitation flutamide gives rise to a phenol derivative through nitro-to-nitrite rearrangement in homogeneous as well as in inhomogeneous solutions. Magnetic field effects on the product yields revealed that a radical pair is involved in the photoreduction, where the excited triplet state of flutamide is the precursor of the pair. It undergoes hydrogen abstraction reaction in inhomogeneous solutions where solvent molecules act not only as component of solvent cage but also as hydrogen donor.

1. Introduction

Flutamide, 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl] propanamide, is a non-steroidal anticancer drug, commonly used in advanced prostate cancer [1–3]. Some reports have shown the capability of flutamide to induce *in vivo* phototoxic and photoallergic effects [4–9]. To clarify the mechanism of the drug-induced disorders, it is necessary to investigate its photophysical and photochemical properties. Some of preceding studies are concerned with the photochemistry of flutamide in various media [10–14]. Flutamide gave rise exclusively to a phenol derivative through the nitro-to-nitrite photo-rearrangement followed by cleavage of the O–N bond [10,12–14] not only in homogeneous solution such as phosphate buffer but also in inhomogeneous solutions containing cage-forming compounds, β-cyclodextrin or vesicles. Only in inhomogeneous solution, flutamide gave forth photo-reduction product [10,12–14].

Magnetic field effects (MFEs) can be used as a simple and useful tool to reveal mechanism and microenvironment for chemical reactions involving radical pair [15]. The MFEs on reaction rates and product yields have been rationalized in terms of the radical pair model developed for interpretation of CIDNP [16,17]. The MFEs observed for biradicals can be dealt with in a similar manner [17].

Since a radical pair is composed of two radicals, it has two different spin states, i.e., singlet (S) and triplet (T). In the absence of magnetic fields, S and three triplet sublevels (T_+, T_0, T_-) of a radical pair are degenerate and the intersystem crossing (ISC) between S and three triplet sublevels is induced by the electron–nuclear hyperfine coupling. The singlet radical pair recombines, resulting in a cage product, whereas the triplet radical pair cannot recombine to form stable species. Because almost all stable organic compounds are in the ground singlet state, the triplet radical pair cannot be transformed into stable compounds and separates into two free radicals to yield escape products. In the presence of magnetic fields the ISC rate is reduced because the degeneracy is removed due to the Zeeman splitting of sublevels. Consequently, product yields can be modulated on application of a magnetic field.

The spin multiplicity of the radical pair is the same as that of its precursor, for example, excited singlet or triplet state. If the excited triplet state acts as the precursor, the cage product yield decreases due to the reduced intersystem crossing between the triplet and singlet radical pair induced by hyperfine coupling. The decrease in the cage product yield is concomitant with the increase in the escape product yield by applying a magnetic field. Thus, we can easily determine the initial spin multiplicity of the radical pair precursor by observing and analyzing MFEs.

From the analysis of MFEs we can speculate about the nature of microenvironment surrounding the solute molecules. The lifetime of the radical pair is controlled by the environmental properties,

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for instance, viscosity. Since the radical pair is short-lived in non-viscous homogeneous solutions, no MFEs or very small MFEs are expected to be observed on reaction rates or yields. On the other hand, the radical pair lifetime is long in inhomogeneous media, and therefore appreciable or significant MFEs are observable.

In order to clarify the reaction mechanism in detail, we elucidated the photochemistry of flutamide by comparing reactivity in various media, such as phosphate buffer (pH 7.4), micelle such as sodium dodecyl sulfate or polyoxyethylene(23)lauryl ether (Brij 35), β-cyclodextrin (β-CD), and bovine serum albumin (BSA) solution in the absence and presence of a magnetic field (ca. 0.1 T). These media were selected for the sake of modeling biological environments with increasing complexity and physiological pH.

2. Materials and methods

2.1. Chemicals

Flutamide was purchased from Tokyo Chemical Industry Co. and used as received. Phosphate buffer 10^{-2} M pH 7.4 (PB) was prepared from reagent grade products. The pH values were measured for buffered solutions with a glass electrode. The following solutions were prepared from reagent grade products by dissolving in the buffer solution: 10^{-2} M β-cyclodextrin (β-CD), 10^{-1} M sodium dodecyl sulfate (SDS), 1.6×10^{-2} M polyoxyethylene (23)lauryl ether (Brij35) and 1 mg/mL bovine serum albumin (BSA). Solvents of HPLC and analytical grade were used in the photochemical experiments.

2.2. Instrumentation

Absorption spectra were recorded on a spectrophotometer (USB4000 and DT-NIMI-2-GS, Ocean Optics Inc.). Proton magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL ECS-400. Gas chromatography–Mass spectrometry (GC–MS) was performed on a Hewlett-Packard HP6890/HP5973. High performance liquid chromatograms were obtained on a Shimadzu LC-20 series. The quantitative analysis of flutamide and its photoproducts were achieved on a Hiber LiChrosorb RP-18 eluting with a mixed solvent of CH_3CN and 0.01 M phosphate buffer (pH 7.4) of a volume ratio of 1–1. Preparative scale HPLC was achieved on a Wakosil-II 5C18 HG-Prep using eluting with a mixed solvent consisting of CH_3CN and water of a volume ratio of 1 to 1. The retention times of the starting species, Product 1 (*N*- [4-hydroxy-3-(trifluoromethyl) phenyl]isobutylamide) and Product 2 were found to be 12, 4.8 and 13.5 min, respectively. These retention times in HPLC analysis were essentially the same as those observed in various solvents.

2.3. Irradiation conditions

Irradiation of flutamide was performed by using a Xe arc (Ushio Optical Modulex UXL-300SX) filtered by a glass filter UTVAF-50S-34U (Sigma-Koki) and a solution filter K_2CrO_4 (7.15 mg/100 mL; path length, 2 cm), its peak wavelength being 313 nm. The incident photon flux on a 2.2 mL quartz cuvettes was determined to be ca. 1×10^{16} quanta s^{-1} by means of ferric oxalate actinometry [18,19]. Flutamide solutions of 10^{-4} M in various media were degassed by freeze–pump–thaw cycles and irradiated for periods up to 2 h. The absorption spectra were measured at regular time intervals. After irradiation, reaction mixtures were analyzed by HPLC.

The quantum yields related to the flutamide photo-degradation and to the formation of its photoproducts were calculated for photolysis at 313 nm, below the 15% conversion of the starting species, on the basis of the following relation [10]:

$$\Phi = \left(\frac{d[X]}{dt}\right)\left(\frac{v}{FI}\right) \quad (1)$$

where $d[X]/dt$ is the initial rate of disappearance of flutamide or the initial rate of formation of the single photoproducts, v is the volume of the irradiation sample, $F = 1–10^{-A}$ is the fraction of photons absorbed by flutamide at the excitation wavelength and I is the light intensity (mol of photons min^{-1}). F is evaluated from absorbance (A) at 313 nm.

2.4. Magnetic field effects on the photochemistry of flutamide

A pair of rare-earth permanent magnets (30 mm × 20 mm × 8 mm, Tokin LM-30) was placed on the side surface of a cell holder to generate 0.1 T.

2.5. Synthesis and characterization of the photoproducts

After irradiation, irradiated solutions (2 mL × 3) were extracted with diethyl ether. Extracted solutions were evaporated and dissolved in an eluting solution for preparative scale HPLC. Product 1 and Product 2 were obtained on a preparative scale HPLC. On the other hand, the Product 1 and Product 2 were synthesized according to the method described below. The photoproducts separated by HPLC were identified in comparison with absorption spectra, HPLC retention times and GC–MS fragmentation patterns obtained for the synthesized authentic samples.

2.5.1. Product 1: N-[4-hydroxy-3-(trifluoromethyl)phenyl] isobutyramide

2.5.1.1. Preparation of 4-nitro-2-(trifluoromethyl)phenol. To 4-nitro-2-(trifluoromethyl) aniline (502 mg, 2.43 mmol) in concentrated sulfuric acid (2.5 mL), a solution of sodium nitrite (190 mg of sodium nitrite in 1.3 mL of concentrated sulfuric acid) was added gradually and stirred for 1 h. The reaction mixture was poured into ice-cold water and vigorously stirred for 5 min. The solution was heated gradually to 100 °C for 1 h. After cooling at room temperature, aqueous ammonia was added for neutralization and extracted with ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated, affording the corresponding phenol (46.5 mg, 9%) as an orange solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.49 (d, J = 2.6 Hz, 1H), 8.34 (dd, J = 2.7, 7.9 Hz, 1H), 7.26 (brs, 1H), 7.09 (d, J = 9.0 Hz, 1H).

2.5.1.2. Preparation of 4-amino-2-(trifluoromethyl)phenol. To mixture of 4-nitro-2-(trifluoromethyl)phenol (25 mg, 0.12 mmol) and ammonium chloride (50 mg, 0.94 mmol) in methanol/water 1:1 solution, zinc powder (933 mg, 14.3 mmol) was added and stirred for 1 h. The mixture was filtered off, washed with hot water, and extracted three times with ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated, affording the corresponding amine (18.2 mg, 85%) as an orange solid.

2.5.1.3. Preparation of N-[4-hydroxy-3-(trifluoromethyl)phenyl] isobutyramide. The amine 4-amino-2-(trifluoromethyl)phenol (5.7 mg, 32.2 μmol) was treated with an excess of isobutyryl chloride (27.5 μL, 177 μmol) and pyridine at room temperature for 19 h. The reaction mixture was extracted with ethyl acetate and washed with aqueous cupric sulfate. After concentration, the crude (9.9 mg) was purified by column chromatography (Al_2O_3, hexane–ethyl acetate = 3:2) to give almost pure hydroxy form as a white solid. HR–MS (EI) m/z: 247.0819 (Calcd. for $C_{11}H_{12}F_3NO_2$: 247.0820). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.72 (d, J = 2.7 Hz, 1H),

7.54 (dd, J = 8.8, 2.7 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 2.59(sep, J = 6.8 Hz, 1H), 1.09 (d, J = 6.8 Hz, 6H).

2.5.2. Product 2: N-[4-nitroso-3-(trifluoromethyl)phenyl] isobutyramide

2.5.2.1. Preparation of N-[4-amino-3-(trifluoromethyl)phenyl] isobutyramide. To mixture of flutamide (100.7 mg, 365 μmol) and ammonium chloride (0.15 g, 2.80 mmol) in methanol/water 1:1 solution, zinc dust (3.76 g, 57.43 mmol) was added and stirred for 1.5 h. The mixture was filtered off, washed with hot water, and extracted three times with ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated, affording the corresponding amine (79.9 mg, 84%) as an orange–yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.55 (d, J = 2.4 Hz, 1H), 7.45 (dd, J = 8.7, 2.4 Hz, 1H), 7.35 (brs, 1H), 6.68 (d, J = 8.7 Hz, 1H), 4.08 (brs, 2H), 2.51 (sep, J = 6.9 Hz, 1H), 1.22 (d, J = 6.9 Hz, 6H).

2.5.2.2. Preparation of N-(4-nitroso-3-(trifluoromethyl)phenyl) isobutyramide. To ice-cooled dichloromethane solution of N-[4-amino-3-(trifluoromethyl)phenyl]isobutyramide (40.2 mg, 0.163 mmol) was added dropwise with stirring two molar amount of meta-chloroperoxybenzoic acid (56.4 mg) in the same solvent. After being kept at 0 °C overnight, the reaction mixture was shaken with aqueous sodium carbonate to remove acid. The organic layer was dried over anhydrous Na_2SO_4, and then the solvent was evaporated. The crude product was purified by means of column chromatography (SiO_2; EtOAc/hexane, 4:6) to afford nitroso compound as a greenish yellow solid (21.4 mg, 50%). HR–MS (EI) m/z: 260.07726 (Calcd. for $C_{11}H_{11}F_3N_2O_2$: 260.07726). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.25 (d, J = 2.2 Hz, 1H), 7.77 (dd, J = 8.9, 2.3 Hz, 1H), 7.26 (brs, 1H), 6.38 (d, J = 8.9 Hz, 1H), 2.59 (sep, J = 6.9 Hz, 1H), 1.30 (d, J = 6.9 Hz, 6H).

3. Results and discussion

3.1. Photoreactions in various media

In 0.1 M SDS and 1.6×10^{-2} M Brij35 solutions, micelles were formed as their critical micellar concentrations in phosphate buffer solution were 4.5×10^{-3} and 4×10^{-5} M, respectively. From solubility measurements of flutamide in SDS and Brij35 solutions, the binding constants of flutamide to SDS and Brij35 micelles were estimated to be about 1.9×10^4 and 5.8×10^4 M^{-1}, respectively [20]. The ratio of flutamide bound to the respective micelle to that in water was 30:1 under the present experimental conditions, indicating that the photoreaction of flutamide takes place almost exclusively in micelle cages and not in buffer solution. In the case of 10^{-2} M β-CD solution, the ratio of flutamide bound to β-CD to that in water was estimated as 3:1 from the reported binding constant (300 ± 50 M^{-1}) [10], indicating photoreaction takes place mainly flutamide bound to β-CD. BSA is used as biological environment and flutamide is supposed to bound partly to BSA.

Fig. 1 shows the spectral changes observed for a degassed solution of flutamide 10^{-4} M in phosphate buffer pH 7.4 at different irradiation times upon 313 nm light excitation. The absorption spectrum of flutamide in phosphate buffer solution is characterized by a broad band with maximum at 300 nm. The peak intensity at 300 nm decreased with irradiation time, while the absorbance at 260 nm increased.

Fig. 2 shows the spectral changes observed for flutamide in 0.1 M SDS solution. The photo-induced spectral changes observed for SDS solution were entirely different from those observed for the phosphate buffer solution. The spectral changes observed for the solutions containing SDS, Brij35, β-CD and BSA exhibit isosbestic points in the wavelength region 350–370 nm, suggesting formation of species with an absorption maximum around 360 nm.

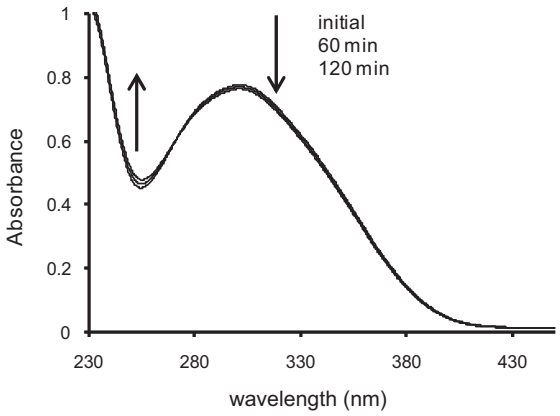


Fig. 1. Spectral changes observed for degassed 10^{-4} M flutamide solution in phosphate buffer (pH 7.4) upon regular irradiation time intervals of 60 min.

Reaction mixtures were analyzed by HPLC on detecting absorbance at 254 nm. All chromatograms recorded for photolyzed solutions (PB, SDS, Brij35, β-CD and BSA) had a peak with retention time of 4.8 min, whose intensity increased with irradiation time. This peak was assigned to Product 1 by comparing UV absorption spectrum, HPLC chromatogram, and GC–MS fragmentation pattern with the separately synthesized authentic sample. Except for the PB solution, a HPLC peak with a retention time of 13.5 min was detected by monitoring absorbance at 360 nm, whose intensity augmented on prolonged irradiation. This peak corresponded to Product 2 by comparing the results of absorption spectra, HPLC and GC–MS obtained for the synthesized authentic Product 2. In both of the homogeneous and inhomogeneous media, photolysis of flutamide gave forth the phenolic Product 1 through the photo-induced nitro-to-nitrite rearrangement. Flutamide underwent photoreduction in SDS, Brij35, β-CD and BSA solutions, yielding Product 2.

3.2. Photoreactivity in various media

The quantum yields for photodegradation (Φ_{-FM}), photorearrangement (Φ_1) and photoreduction (Φ_2) of flutamide were evaluated through HPLC analysis (Table 1).

Photolysis of flutamide in phosphate buffer solution yielded exclusively Product 1. No other photoproducts were identified by HPLC. The photo-degradation quantum yield of flutamide was $\Phi_{-FM} = 1.1 \times 10^{-4}$ and photo-production quantum yield of Product 1 and Product 2 were $\Phi_1 = 4.0 \times 10^{-5}$ and $\Phi_2 = 0$, respectively. In 0.1 M SDS solution, the photo-degradation

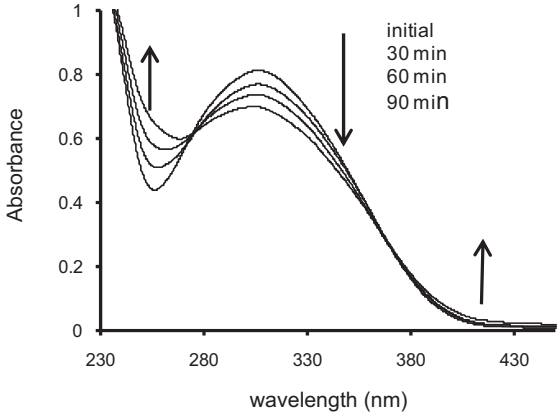


Fig. 2. Spectral changes observed for degassed 10^{-4} M flutamide solution in phosphate buffer (pH 7.4) in the presence of 10^{-1} M SDS upon regular irradiation time intervals of 30 min.

Table 1
Quantum yields for the photodegradation of flutamide (Φ_{-FM}) and the formation of photoproducts (Φ_1 and Φ_2) in various media.

	Φ_{-FM}	Φ_1	Φ_2
PB[a]	1.1×10^{-4}	4.0×10^{-5}	0
0.1 M SDS/PB	5.5×10^{-4}	2.0×10^{-4}	7.0×10^{-5}
0.016 M Brij35/PB	5.1×10^{-3}	5.8×10^{-4}	2.1×10^{-3}
0.01 M β-CD/PB	6.0×10^{-4}	2.5×10^{-4}	7.6×10^{-5}
1 mg BSA/mL PB	1.0×10^{-3}	8.8×10^{-5}	8.8×10^{-5}

[a] PB: phosphate buffer solution (pH 7.4).

quantum yield of flutamide was $\Phi_{-FM} = 5.5 \times 10^{-4}$ and photoproduction quantum yield of Product 1 and Product 2 were $\Phi_1 = 2.0 \times 10^{-4}$ and $\Phi_2 = 7.0 \times 10^{-5}$, respectively. The value of Φ_{-FM} in the SDS solution was about 5 times the value of Φ_{-FM} in phosphate buffer solution. The nitro-to-nitrite photo-rearrangement was accelerated in the SDS solution compared with in the phosphate buffer solution. In addition, the photo-reduction took place in the SDS solution. The value of Φ_2 was about 1/3 of the value of Φ_1 in the SDS solution. The nitro-to-nitrite photo-rearrangement is dominant compared to the photo-reduction in the SDS solution. SDS micelles provide a hydrophobic environment suitable for the photo-induced rearrangement.

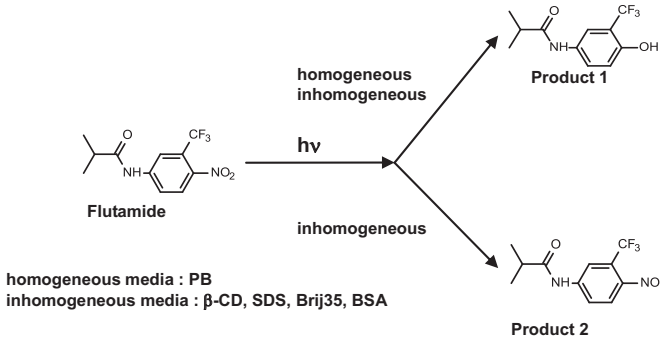
The value of Φ_{-FM} in the 0.016 M Brij35 solution was about 50 times higher than that of Φ_{-FM} in phosphate buffer solution. In the Brij35 solution, the value of Φ_2 was larger than the value of Φ_1. Since the value of Φ_1 in the Brij35 solution was about 3 times the value in the SDS solution and the value of Φ_2 in the Brij35 solution was about 30 times the value in the SDS solution, the photo-reduction was overwhelmingly accelerated compared with the photo-rearrangement. This is in accordance with the fact that the polyoxyethylene chain of Brij35 can act as a better reductant than the methylene groups in SDS molecule on supplying hydrogen atoms to the nitro group in the excited flutamide.

The values of the Φ_{-FM}, Φ_1 and Φ_2 in the β-CD solution were almost the same as those in the SDS solution, suggesting that the macrocyclic environment of the β-CD was similar to that of the SDS micelle for the photoreaction of flutamide. At the present stage of investigation we have no reasonable explanation for the similarity observed in two environments.

The value of Φ_{-FM} in the BSA solution was about 10 times the value of Φ_{-FM} in phosphate buffer solution. The value of Φ_1 in the BSA was about 2 times larger than the value in the phosphate buffer. The value of Φ_2 in the BSA was almost the same as those in the SDS and β-CD solutions. These facts indicate that flutamide was bound to BSA. The quantum yield for flutamide disappearance (Φ_{-FM}) is much larger than those for product formation (Φ_1 and Φ_2). The discrepancy in the magnitude of quantum yields suggests that other unknown reaction pathways become dominant in the BSA solution.

In SDS, Brij35, β-CD and BSA solutions the degradation yield of flutamide becomes larger than that in phosphate buffer solution. The photochemistry of flutamide in β-CD and BSA solution is assumed to be the reaction of flutamide-β-CD and flutamide-BSA complexes. However, the photoreaction in β-CD and BSA solutions is similar to that in micellar solutions, implying that photochemical behavior of flutamide complexes is analogous to that in micellar solutions. It seems that macromolecules, β-CD (mol. wt., 1135) and BSA (mol. wt., 66,000) provide locally hydrophobic environment for flutamide bound to them, as interior of SDS and Brij35 micelle is hydrophobic or nonpolar.

According to semi-empirical quantum mechanical calculations [10], the lowest excited triplet state and second lowest triplet state of flutamide are characterized as π,π* and n,π* transition, respectively. Probably the n,π* triplet state is mixed to some extent with the lowest π,π* triplet state by vibronic coupling. On from the phosphate buffer solution to miceller media, the n,π* state of flutamide, having less polar electronic structure, is stabilized. This leads to the increase in the n, π* nature in the lowest π,π* triplet state. Thus, the reactivity of flutamide triplet presumably increases in SDS and Briji35 micellar solutions. Similarly, reactivity of flutamide bound to β-CD and BSA increases in a hydrophobic environment compared to that in phosphate buffer.



Scheme 1. Photoproducts formed on photolysis of flutamide.

We propose Scheme 1 for the photochemistry of flutamide on the basis of above-mentioned results. In conclusion, at least two different photoreactions (the photoinduced rearrangement and redox process) compete in inhomogeneous media. Two different reaction pathways in this scheme can be further rationalized by analyzing MFEs on photochemical yields for Product 1 and Product 2 (vide infra).

3.3. Magnetic field effects on the photoreaction of flutamide

MFEs on the product yields were studied in order to identify the excited state of flutamide responsible for the present photochemical reaction. Flutamide was irradiated with UV light in the absence or in the presence of a magnetic field (0.1 T). Fig. 3 shows the UV spectral changes induced by photolysis of flutamide solutions in Brij35, (a) in the absence and (b) in the presence of 0.1 T. Eq. (2) is introduced and employed for evaluating the relative quantum yield (Q) of disappearance or formation for photoinduced reduction of the nitro-aromatic species [21,22]:

$$\ln\{\exp(A) - 1\} - \ln\{\exp(A_0) - 1\} = Qt \quad (2)$$

where the A and A_0 values are absorbance at the time $t = t$ and $t = 0$, respectively. The yield for the disappearance of flutamide was determined by the use of Eq. (2) and absorbance changes at 306 nm shown in Fig. 3. The formation yield of Product 2 was calculated in a similar manner by analyzing the absorbance increase at 380 nm shown in Fig. 3. Fig. 4 illustrates the plots of absorbance changes based on Eq. (2) at (a) 306 nm and (b) 380 nm. While the disappearance of flutamide exhibits no MFEs, the formation yield of Product 2 is remarkably lowered on application of 0.1 T. The deviation from a linear relationship observed for the absorbance change at 380 nm may be related to subsequent processes involving Product 2 [23]. There were no MFEs on the absorbance change at 260 nm. However it was difficult to conclude that there were no MFE on the yield of Product 1, as absorption bands of Product 1, flutamide and Product 2 were overlapped in this wavelength region.

Table 2 lists the magnitude of MFEs on the relative yield of photoreaction calculated from absorbance at the time of 10% degradation of the starting species in SDS, Brij35 and β-CD solutions, except for a BSA solution. The reaction mixture obtained after 15 min irradiation was analyzed for the BSA solution. The magnetic field of 0.1 T suppressed appreciably the yield of Product 2 in inhomogeneous media.

Table 2
Magnetic field effects on relative yield Q^H/Q^0, where Q^H and Q^0 were calculated on the basis of equation (2) for the absorbance changes in the absence and presence of magnetic field (0.1 T). Photolysis time is set at 10% decrease in the peak absorbance for β-CD, SDS, Brij35 solutions or 15 min for BSA solution.

	Q^H/Q^0 of 300–306 nm[b]	Q^H/Q^0 of 380–400 nm[b]
PB[a]	–	–
0.1 M SDS/PB[a]	0.95	0.75
0.016 M Brij35/PB[a]	0.97	0.54
0.01 M β − CD/PB[a]	0.94	0.67
1 mg BSA/mL PB[a]	(0.82)	(0.68)

[a] PB: phosphate buffer solution (pH 7.4).
[b] $Qt = \ln\{\exp(A) - 1\} - \ln\{\exp(A_0) - 1\}$, where A and A_0 refer to the absorbance at the time $t = t$ and $t = 0$, respectively.

Table 3
Magnetic field effects on relative yield, A^H/A^0, where A^H and A^0 refer to the integrated peak intensity of flutamide and photoproducts (Product 1 or Product 2) in HPLC chromatograms for sample solutions photolyzed in the absence and presence of magnetic field (0.1 T), respectively. Photolysis time is set at 10% decrease in the peak absorbance for β-CD, SDS, Brij35 solutions, 180 min for PB solution, and 15 min for BSA solution.

	A^H/A^0 for flutamide	A^H/A^0 for Product 1	A^H/A^0 for Product 2
PB[a]	1.01 ± 0.02	1.02 ± 0.02	No product
0.1 M SDS/PB	1.01 ± 0.03	1.01 ± 0.03	0.51 ± 0.04
0.016 M Brij35/PB	1.02 ± 0.01	0.99 ± 0.06	0.70 ± 0.18
0.01 M β-CD/PB	0.98 ± 0.05	0.94 ± 0.04	0.89 ± 0.05
1 mg BSA/mL PB	1.00 ± 0.01	1.10 ± 0.32	0.66 ± 0.07

PB: phosphate buffer solution (pH 7.4).

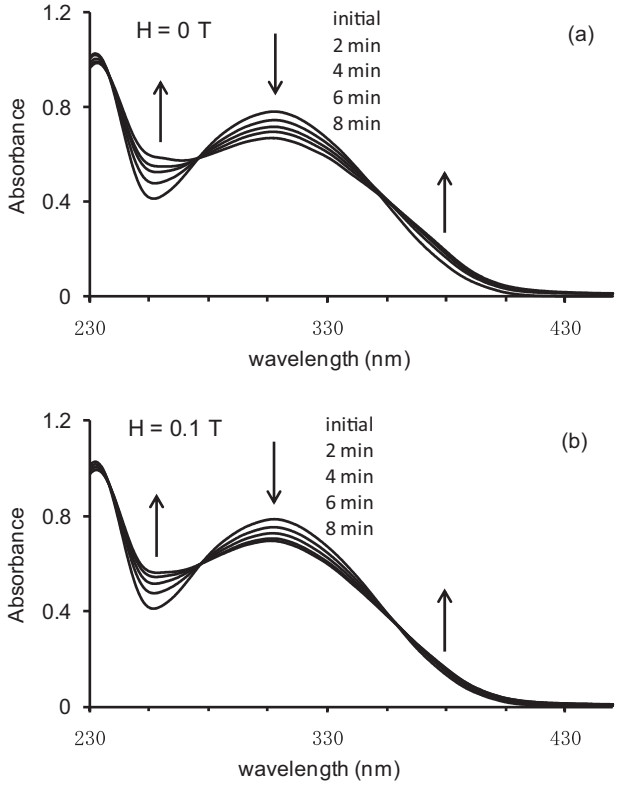


Fig. 3. Magnetic field effects on the UV-spectral changes induced by the photolysis of the 0.016 M Brij35 solution of 10^{-4} M flutamide. (a) In the absence of magnetic field (b) In the presence of magnetic field (0.1 T).

HPLC analysis of the irradiated solution revealed the magnitude of MFEs on the photoreaction of flutamide in a more accurate manner. Table 3 shows the MFEs on the degradation of flutamide and on the formation yield of Product 1 and Product 2 in various media. The yield of Product 2 was suppressed by the external magnetic field in all media. In contrast, neither the consumption of flutamide nor the formation of Product 1 was affected in the presence of 0.1 T.

The magnitude of MFEs is related to the lifetime of radical pair, since the lifetime is the inverse of the sum of rate constants for ISC (magnetic field dependent) and the escape process (magnetic-field independent) of radicals from a micelle in the case of micellar solution. The observed MFEs in Brij35 solutions are smaller than that in SDS solution, whereas the yield of hydrogen abstraction in Brij35 solution is larger than that in SDS solution (Table 1). These facts suggest that in SDS micellar solution flutamide was presumably solubilized in viscous hydrocarbon core of the micelle, whereas in Briji35 (polyoxyethylene(23)lauryl ether) solution it was solubilized in fluid polyoxyethylene outer core and not in hydrocarbon inner one. Similarly, in the case of photoreaction of flutamide-β-CD and flutamide–BSA complexes the binding of flutamide to β-CD does not seem to be strong compared to that of BSA. Radicals formed in a cage provided by β-CD (mol. wt. 1135) could easily escape from the cage, as a β-CD cage with a doughnut-shape is small compared with micelle cages and has two open exits to bulk solution. In the case of BSA, flutamide is bound presumably in an inner pocket of giant BSA molecule (mol. wt. 66,000), though no detailed information of binding cite is available at present.

As has been described in Introduction, when the radical pair is involved in the photochemistry, the product yields may be affected by applying a magnetic field. The fact that no MFEs were observed on the degradation of flutamide indicates that no flutamide was regenerated via radical pair intermediate. Since no MFEs were observed on Product 1 formation, the photoinduced rearrangement into Product 1 does not occur through the radical pair mechanism.

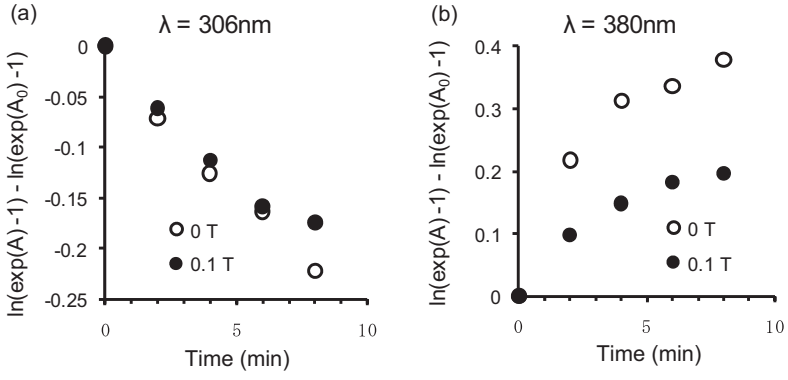
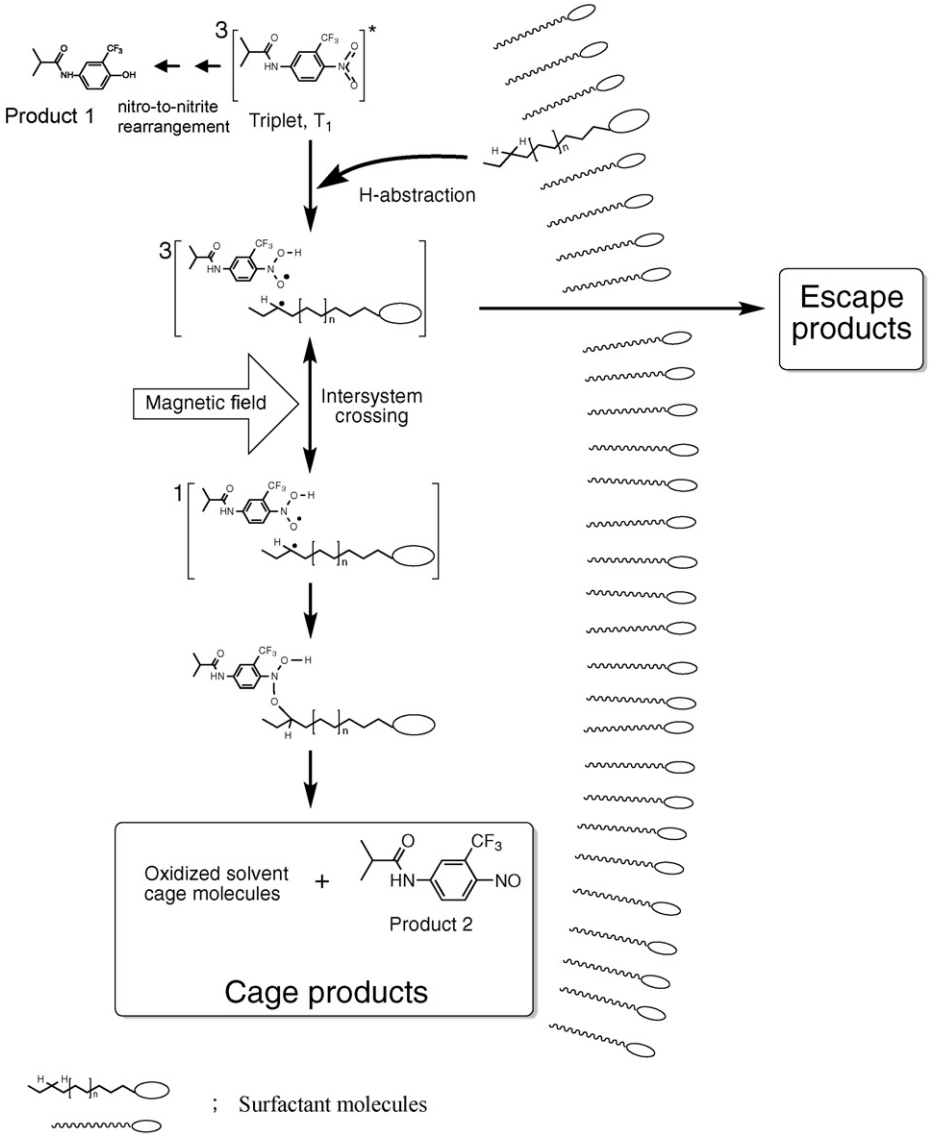


Fig. 4. Plots based on Eq. (2) for the absorbance changes (a) at 306 nm and (b) at 380 nm in Fig. 3.



Scheme 2. Photoreduction mechanism of flutamide in micellar solutions.

If the triplet radical pair acts as the precursor, the yield of cage product decreases in the presence of a magnetic field of 0.1 T, since T–S ISC of the radical pair, which is induced by electron–nuclear hyperfine interaction, is reduced on application of the magnetic field. Product 2 can be characterized as cage product, which is formed from the singlet radical pair derived from hydrogen-acceptor (aromatic nitro group) and hydrogen-donor (cage-forming compounds, i.e., SDS, Brij35, β-CD and BSA). From the fact that the magnetic field lowered the formation yield of Product 2, it is concluded that the initial spin state of radical pair is triplet and that hydrogen abstraction reaction takes place at the nitro group in the excited triplet state of flutamide, because the spin multiplicity is conserved during the photochemical reaction.

The radical pair involved in photoredox reaction of the nitro group is not a direct precursor of photoinduced nitro-to-nitrite rearrangement. In other words, the photo-rearrangement reaction occurs in the upstream region of radical pair formation from flutamide in the excited state. Flutamide is non-fluorescent or weakly fluorescent, though its degradation quantum yield is as small as 1.1×10^{-4} in phosphate buffer solution. This fact suggests that ISC from the excited singlet state to the triplet state may be very rapid for the excited flutamide and therefore the photoinduced rearrangement reaction takes place in the triplet manifold of the nitro-aromatic species, which has been generally discussed in the literature [24,25].

We propose Scheme 2 for the photoreduction of flutamide in micellar solutions. SDS and Brij35 surfactant molecules can act as a good hydrogen-donor. The nitro group of aromatic species in the triplet state abstracts hydrogen atom from the cage-forming compound and produce a triplet radical pair. The triplet radical pair undergoes the ISC to the singlet radical pair, which can be transformed into stable photoredox end-products in the ground singlet state. The precursor of stable singlet products is composed of an aromatic-ring-N-O-CH moiety (see Scheme 2) where CH group is a part of cage-forming compound and subsequently decomposes into the nitroso-aromatic cage product (Product 2) and the oxidized cage molecule containing a carbonyl group [23]. The magnetic field modulates the magnitude of ISC between the triplet and singlet radical pairs. The presence of the cage-forming compounds such as SDS and Brij35 enhances the lifetime of the triplet radical pair and the transition probability of the ISC to the singlet radical pair may become comparable to or larger than the probability of diffusion from the solvent cage. The singlet radical pair readily recombines to form the direct precursor, which yields cage products, namely,

the nitroso-aromatic species (Product 2) and the oxidation product derived from the solvent molecule. In the absence of cage-forming compounds, the triplet radical pair diffuses away immediately after its generation by hydrogen abstraction. The photoreaction of flutamide bound to β-CD and BSA which are cage-forming compound, can be explained in a similar manner. On the other hand, in a homogeneous medium such as phosphate buffer solution, the triplet radical pair may not undergo efficiently ISC due to rapid diffusion from the solvent cage, and therefore no cage products are formed in the buffer solution. The role of cage-forming compounds is to provide hydrogen atoms as reductant and to prevent the diffusion of the triplet radical pair. In other words, micellar media slows down the escape process and therefore ISC induced by HFC mechanism can compete with the diffusion.

In some of preceding papers [10,12–14] the photoreactivity of flutamide is accounted for in terms of the structural changes caused by "steric constraints" in the solvent cage or environment. Sortino and co-workers explained that the increased planarity of flutamide in inhomogeneous media decreases the overlap of the p atomic orbital of nitro-oxygen with that of aromatic ring carbon necessary for the nitro-to-nitrite rearrangement [10,12–14]. They argued that the increase in torsional angle of the nitro group with respect to the aromatic ring favors the nitro-to-nitrite rearrangement and at the same time disfavors photo-reduction. The results obtained in the present study clearly shows that the nitro-to-nitrite rearrangement is accelerated in inhomogeneous media. It can be interpreted that the hydrophobic environment provided by the solvent cage is more suitable for the nitro-to-nitrite rearrangement.

4. Conclusion

The mechanism of flutamide photochemistry can be summarized below.

The nitro group in excited flutamide undergoes the nitro-to-nitrite rearrangement both in homogeneous and inhomogeneous media. The photo-induced rearrangement is accelerated in inhomogeneous media (SDS, Brij35, β-CD and BSA solutions), which provide hydrophobic environment. The solvent cage plays an important role in photo-reduction with SDS, Brij35, β-CD and BSA. The photo-reduction is significantly accelerated in the presence of hydrogen donors, thereby increasing the yield of Product 2. From the analysis of MFEs it is clearly demonstrated that hydrogen abstraction reaction takes place in the triplet excited state of flutamide. In homogeneous buffer solution, the hydrogen abstraction reaction does not occur because no hydrogen donors are dissolved in the solution.

The results obtained in the present study may contribute to a better understanding of photoreactions of flutamide in the biological condition by taking account of the similarity of inhomogeneous media to biological environment.

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