Laser flash photolysis study of the reactivity of β-naphthoflavone triplet: Hydrogen abstraction and singlet oxygen generation

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ARTICLE INFO

ABSTRACT

The absorption spectra for β-naphthoflavone (**1**) reveal a solvatochromic red shift in polar solvents which is consistent with the π,π^* character of the $S_0 \rightarrow S_1$ electronic transition. The laser flash photolysis technique has been used to characterize and study the reactivity of the triplet excited state of **1**. Excitation (355 nm) of degassed solutions of **1**, in acetonitrile, resulted in the formation of its corresponding triplet excited state. Addition of hydrogen donors, such as 2-propanol and 1,4-cyclohexadiene, led to triplet quenching and formation of a new transient, which was assigned to the corresponding ketyl radical obtained from a hydrogen abstraction reaction by triplet **1**. This ketyl radical was characterized by experiments with methylviologen. The triplet excited state of **1** was efficiently quenched by phenols and N-acetyl L-tryptophan methyl ester. In all cases new transients were formed in the quenching process, which were assigned to the corresponding radical pair resulting from an initial electron transfer from the quencher to the excited naphthoflavone, followed by a fast proton transfer. Singlet oxygen ($^1O_2$) is formed from the triplet of **1**, and a quantum yield of 0.51 was measured. TDDFT calculations with implicit solvation (IEF-PCM) were used to calculate the ground state UV–vis absorption spectrum, from which the nature of the lowest energy transitions were characterized, and the triplet–triplet absorption spectrum consistent with the triplet transient generated by LFP. Excellent correlation of the calculated and experimental spectra was achieved using the conventional PBE0 hybrid functional.

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1. Introduction

Flavones are a large class of natural pigments which contain a 2-phenyl-4-benzopyrone unit and belong to the general class of flavonoids. A large number of derivatives result from various combinations of multiple hydroxyl and/or methoxyl substituents bonded to the flavonoid skeleton, which gives them potential antioxidant activity toward free radical species. These compounds are usually found in the tissues of higher plants, mainly in tropical regions [1]. Photocontrol of their production is well documented and seems to operate at many different points along the biosynthetic pathway [2]. The efficient absorbance of UV irradiation has implied their possible involvement in the protection against UV-induced damage in plants [3,4].

Photophysical and photochemical studies on flavonoids have revealed important characteristics which may be related to their photobiological behavior. Photodimerization is the main photoreaction of the parent flavone in 2-propanol [5]. Time-resolved spectroscopy experiments suggest that flavone photochemistry is mainly mediated by the triplet excited state [6,7], as confirmed by its high ISC quantum yield (∼0.9) [8]. The transient absorption spectrum of triplet flavone shows bands at 365–370 nm and 640–650 nm, and this transient has been shown to be reactive toward hydrogen donors [2,6–9].

The phosphorescence spectra and lifetime measured for flavone at 77 K indicates a mixed n,π^*–π,π^* character for the lowest triplet excited state irrespective of the solvent polarity [10] and a triplet energy of 62 kcal mol^{-1} [8,10].

β-Naphthoflavone (**1**) or 5,6-benzoflavone is a synthetic derivative of a naturally occurring flavonoid and has the potential to strongly induce cytochrome P450 1A enzymes via activation of aryl hydrocarbon receptor [11,12]. It has been shown that **1** has the property to increase the liver tumor-promoting activity [13].

Recently Mitsumori et al. shows that **1** induced the sustained production of oxidative stress in the livers of rats [14]. The authors suggest that secondary effects of **1**, which include enhancement of reactive oxygen species (ROS), oxidative DNA damage and lipid peroxidation, may contribute to its liver tumor promotion activity [14–16].

Oxidative damage to biological systems, either accidental or intentional, is a major cause of cell death [17]. In particular, apoptotic or necrotic signaling pathways to cell death can be induced by the combined use of a photo-activated drug, called the photosensitizer, and per se harmless visible light. This process involves the generation of reactive oxygen species (ROS) capable of inflicting damage to susceptible cell components such as proteins [18,19], membrane lipids [20], and nucleic acids [21].

Several mechanisms account for the photosensitization process toward biomolecules [22,23]. More general photosensitizing mechanisms can involve either photo-oxidation of nucleic acid components by the sensitizer, yielding the corresponding radical pair and ultimately leading to sensitizer-protein photobinding (type I photosensitizers), or a triplet–triplet energy transfer to molecular oxygen, resulting in formation of singlet oxygen O_2 ($^1\Delta_g$) and other reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical (type II photosensitizers). Aromatic amino acid residues in proteins, especially tryptophan (Trp) and tyrosine (Tyr), are easily oxidized [24,25]. Thus, the reaction of Trp and Tyr with photosensitizers has received considerable attention in the field of proteins photo-oxidation.

Recently, characterization and reactivity of triplet α-naphthoflavone (**2**), an isomer of **1**, was studied by us via laser flash photolysis and theoretical calculations with density functional theory (DFT) and atoms in molecules (AIM) [26].

In this sense, a study of the photochemistry of β-naphthoflavone (**1**), characterizing its triplet excited state and explaining its reaction mechanisms of deactivation could aid in the understanding of the pharmacological action of this class of molecule.
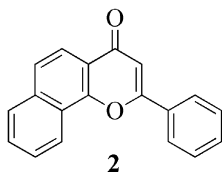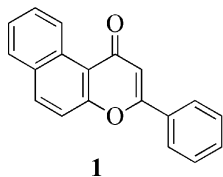
2. Materials and methods

2.1. General

Acetonitrile, 2-propanol, cyclohexane and 1-methylnaphthalene (all spectroscopic grade), were provided by Sigma–Aldrich. Water was milli-Q grade. Phenol, 4-methoxyphenol, 4-cyanophenol, 1,4-cyclohexadiene and DABCO were used as received from Aldrich. β-naphthoflavone (Aldrich) was recrystallized from hexane:ethyl acetate 1:1.

Perinaphthenone, β-carotene, indole, methylviologen and L-tryptophan methyl ester, from Aldrich, were used as received (purity >99%).

N-Acetyl L-tryptophan methyl ester (NATME) was synthesized by a standard procedure (mp = 150–152 °C, Lit = 152 °C; yield: 88%) [27].

2.2. Absorption spectra

Optical spectra in different solvents were measured on a Shimatzu model UV-2450 UV–vis spectrophotometer.

2.3. Laser flash photolysis

The laser flash photolysis (LFP) experiments were carried out on a LuzChem Instrument model mLFP122. Samples were contained in a 10 mm × 10 mm cell made from Suprasil tubing and were deaerated by bubbling with argon for about 20 min. The samples were irradiated with the third harmonic of a Nd/YAG Surelite laser (λ = 355 nm). All laser flash photolysis experiments were performed in acetonitrile solution, unless otherwise indicated in the text. The concentration of **1** was adjusted to yield an absorbance of ∼0.3 at the excitation wavelength (355 nm). Stock solutions of quenchers were prepared so that it was only necessary to add microliter volumes to the sample cell in order to obtain appropriate concentrations of the quencher. The rate constants for the reaction of triplet **1** with the different quenchers employed in this work were obtained from Stern–Volmer plots, following Eq. (1).

$$k_{obs} = k_o + k_{q[Q]} \tag{1}$$

where k_o is the triplet decay rate constant in the absence of quencher; k_q is the triplet decay rate constant in the presence of the quencher and [Q] is the quencher concentration in mol L^{-1}. The decay trace at 500 nm was used to determine the quenching rate constants.

2.4. Singlet oxygen measurements

The quantum efficiency of singlet oxygen ($^1O_2$) formation was determined using a spectrofluorometer (FS920 Edinburgh Instruments) with a TMS300 monochromator. The detection system was equipped with a NIR Hamamatsu model H1033-45 photomultiplier. The excitation source (355 nm) was provided by a CryLas Nd-YAG HP 355-50 laser (pulse width of 1.0 ns and energy <150 μJ. Singlet oxygen formation was observed by monitoring the phosphorescence at 1270 nm upon laser excitation of optically matched solutions (A = 0.3 in air-saturated acetonitrile) of **1** relative to a standard solution of perinaphthenone, which shows singlet oxygen quantum yield of 1.0 [28]. The quantum yield for singlet oxygen formation was determined from the slope of the plots of signal intensity at zero time in 1270 nm versus laser light intensity, using a set of neutral density filters, employing Eq. (2), where I_{sample} is the emission intensity recorded for the sample, I_{PER} is the perinaphthenone emission intensity, used as standard, and Φ_{PER} is the quantum yield of singlet oxygen formation from perinaphthenone.

$$\Phi_{sample} = \frac{I_{sample}}{I_{PER}} \times \Phi_{PER} \tag{2}$$

2.5. Theoretical calculations

Geometry and energy calculations were performed at (U)B3LYP/6-311++G**//(U)B3LYP/6-31+G* computational level for all structures unless otherwise stated [29–31]. The calculations were performed using Gaussian 09 [32]. The gas phase and solvated phase geometries were optimized using standard techniques [33,34], and vibrational analysis was performed to confirm that the geometries were true minima on the potential energy surface, as shown by the absence of imaginary frequencies. The DFT method was used as it reasonably estimates spin contamination. Values for $\langle S^2 \rangle$ were typically consistent with two unpaired electrons for all triplets. Implicit solvated structures were optimized by use of IEF-PCM as implemented in Gaussian 09 [35–37]. UV–vis spectra were calculated with implicit solvation (IEF-PCM) by TD-DFT B3LYP, CAM-B3LYP [38] and PBE0 [39,40] (6-311++G**//6-31+G* (nstates = 20)) using solvent optimized geometries (IEF-PCM).