Molecular Structure of the Photo-Oxidation Product of Ellagic Acid in Solution

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ABSTRACT: The photoreaction of the antioxidant ellagic acid (EA) elicits a drastic coloration in solution from colorless to yellow in aerated tetrahydrofuran, which appears as a new absorption band at 405 nm. Analysis of the X-ray crystal structure suggests that the photo-oxidation product of EA is a multiple cleavage π-structure (Ox-EA) that results from the interaction of EA with singlet oxygen followed by sequential cleavage and rearrangement steps.

INTRODUCTION

Among a variety of π-conjugated hydrogen-bonded molecules, ellagic acid (EA) has an interesting molecular structure comprising a planar biphenyl moiety bridged by two lactone rings and four hydrogen-bonding −OH groups.1 EA is mainly found in raspberries, blackberries, strawberries,2,3 nuts, seeds, and fruit liqueurs4,5 as a polyphenol derivative. Its planar π-structure also has both hydrogen-bonding acceptor (lactone) and donor (−OH) sites, and its antioxidant and anticancer effects have attracted much attention. For instance, the high reactivity of EA with the strong carcinogen benzo[a]pyrene, a metabolic product from benzo[a]pyrene, inhibits DNA mutation through its binding at guanine.6,7 Although several reports about the antioxidant effects of EA have emerged, a molecular-level understanding of the reaction mechanism is absent.8

The antioxidant effects of EA have been reported in terms of active radical oxygen species such as O2· and OH· as scavengers.9 However, relatively little has been reported on its antioxidant effects against nonfree radical oxygen species such as singlet oxygen (1O2), triplet oxygen (3O2), superoxide (O2−), and peroxide (H2O2). Clearly, understanding the reaction mechanisms of EA with various O2 species is valuable to more fully understand the antioxidant effect of π-molecules. To this end, photocoloration phenomena of EA in aerated tetrahydrofuran (THF) from colorless to yellow following photo-irradiation were observed.

RESULT AND DISCUSSION

The π-planar structure of EA was drastically changed to a twisted Ox-EA structure (Scheme 1), as confirmed by single-crystal X-ray diffraction analysis. Broadly, evaluating the photoreaction of EA has the potential to provide new insight into the antioxidant effects of EA.
concomitant photo coloration behavior. Notably, such photo-
coloration behavior of EA was not observed in highly polar
solvents like dimethylformamide (DMF) and CH$_3$OH (Figure
S3).

Two types of optical filters—I ($\lambda_{ex} = 280$–400 nm) and II
($\lambda_{ex} > 400$ nm)—were used for the photoreaction in order
to evaluate the wavelength dependence of the irradiation light
(Figure S4). Utilization of filter I induced a complete
conversion of EA to Ox-EA, with saturation of the absorption
intensity at 403 nm within 300 min. By comparison, the
photoreaction progressed much more slowly with filter II, at
approximately 10% conversion under the same condition.
Therefore, the optical absorption of EA can effectively indicate
the extent of photocoloration and the consequent photo-
reaction.

Yellow-colored single crystals isolated from the photo-
reaction products were utilized for the X-ray single-crystal
structural analysis (Figures S5 and S6). The relatively low yield
of 26% was due to a loss of the reaction product during the
purification. Both the time-dependent UV–vis and $^1$H NMR
spectra supported the existence of clear and complete
photoreaction (Figures 1a, S2, and S7). The planar
$\pi$-conjugated structure of EA was transformed to an asym-
metrical $\pi$-structure (Ox-EA) by multiple bond cleavages
(Figure 2a). The absorption spectrum of the yellow crystals in
THF was completely consistent with that of the photoreaction
products caused by the presence of O$_2$. Therefore, the
absorption intensity at 254 nm was consistent with the formation of Ox-EA in the absence of a
biphenyl $\pi$-core. DFT calculations of EA and Ox-EA at the
B3LYP/6-31G(d,p) level of theory also supported their
HOMO–LUMO gaps of 4.21 eV (EA) and 3.33
eV (Ox-EA), where the LUMO of EA was approximately 1.2
eV higher in energy than that of Ox-EA (Figure S10).

The molecular formula of Ox-EA could be formally
determined by the addition of one oxygen molecule to the
EA, suggesting that molecular oxygen in THF served as a
necessary input for this photoreaction. To this end, the
photoreaction of EA was examined in both aerated and
nitrogen-purged THF (Figure 3a). Notably, no photo-
reaction of EA occurred in the absence of O$_2$ in THF.
Therefore, dissolved O$_2$ is needed for the photo-oxidation
reaction of EA in THF. Because EA itself is stable under
ambient conditions (Figure 3a), its reaction with O$_2$ should
initiate this photoreaction.

Two types molecular oxygen, either singlet O$_2$ or triplet
$^3$O$_2$, possibly contributed to the photoreaction of EA in THF.
Di(tert-butyl)hydroxytoluene (BHT) has previously been used as
a reaction inhibitor of $^3$O$_2$ in THF. On this basis, the
photoreaction efficiency of EA was examined in photoreaction
solvents with and without BHT in THF. A slight broadening
of the absorption band at 403 nm of Ox-EA was confirmed in the
THF solution without BHT during a 24 h photoirradiation
period (Figure S12), suggesting decomposition of the reaction
products caused by the presence of $^3$O$_2$. Therefore, the

photoreaction proceeds in the presence of $^{1}$O$_2$ as EA + $^{1}$O$_2$ = Ox-EA. Photoreaction of EA was not observed in either DMF or CH$_3$OH. A similar solvent-dependent inactivation of the photoreaction of the bacteriorhodopsin dimer (BC1) has been reported in $^{1}$O$_2$-containing DMF and CH$_3$OH. The formation of $^{1}$O$_2$ species by the excited-state BC1$^*$ was suppressed in highly polar solvents. These results also support our observations that singlet oxygen generated by the photosensitizing reaction of EA promoted this photoreaction.

The reaction rate of EA + $^{1}$O$_2$ = Ox-EA in THF was determined by a spectroscopic method with an initial [EA$_0$] concentration of 500 μM, where the photoreaction, which occurs under ambient conditions of the instrument, is supplied with a sufficient amount of O$_2$. Time-dependent spectral changes of the absorption band at 403 nm by the photo-irradiation decrease the concentration of the photoreaction product [Ox-EA], which gives a reaction rate of $k = 7.8 \times 10^{-5}$ s$^{-1}$ assuming a first-order reaction.

The photoreaction of π-molecules with $^{1}$O$_2$ has been identified as [4 + 2] or [2 + 2] cyclization reactions (Scheme 2). The stabilities of possible [4 + 2] and [2 + 2] cyclization products of EA with oxygen were evaluated using DFT calculations based on the B3LYP/6-31G(d,p) level of theory (Scheme 2 and Figure S13). The most stable $^{1}$O$_2$ adduct for EA was the reaction intermediate of the [2 + 2]-1 structure, whose energy was 61.0 and 73.6 kJ mol$^{-1}$ lower than those of the [2 + 2]-2 and [4 + 2] cyclization products, respectively (Scheme 2). Effective conjugation in [2 + 2]-1 imparts it with relatively high stability, whereas destruction of this π-conjugation in the [4 + 2] cyclization process destabilizes the intermediate structure. Although no reaction intermediates were detected, a plausible mechanism for this photoreaction is proposed in Scheme 2b. Specifically, the photogenerated dioxetane intermediate [2 + 2]-1, which is unstable, is easily cleaved to form a tricycle intermediate A with a terminal conjugated enol carboxylic acid group. Sequential ring-rearrangement reactions between the terminal enol and the reactive acid anhydride then produce the stable Ox-EA product. Overall, then, selective formation of dioxetane followed by structural rearrangements affords Ox-EA.

In conclusion, the photoabsorption of EA in aerated THF generated excited-state $^{3}$EA$^*$, which was transformed from the singlet to the triplet state by intersystem crossing. The reaction of triplet $^{3}$EA$^*$ with O$_2$ generated dissolved singlet $^{1}$O$_2$ in

**Scheme 2. Photoreaction Mechanism of EA in THF; (a) Relative Stability of Three Possible Intermediate EA–Oxygen Adducts; (b) Photoinduced Oxidation Mechanism of EA to Form Ox-EA**

![Scheme 2](image-url)
THF, which reacted with the π-planar structure of EA and formed an unstable dioxetane intermediate by a [2 + 2] cyclization reaction. Stepwise cleavages of the biphenyl π-core formed a dioxetane structure with concomitant intramolecular ring rearrangement between the enol oxygen and acid anhydride, which drastically modified the molecular and electronic structure of EA to form the photo-oxidation product Ox-EA. A possible antioxidant reaction mechanism of EA via its photoreaction in THF is proposed.

**EXPERIMENTAL SECTION**

**General Methods.** Commercially available reagents and solvents were used without further purification. Chemical shifts (δ) in ppm were referenced to residual nondeuterated solvent (1H 7.22 ppm and 13C 67.24 ppm for THF-d8). Mass spectra were obtained in the FAB negative mode with a magnetic sector mass spectrometer. Infrared (IR, 400–4000 cm⁻¹) spectra were measured with a resolution of 4 cm⁻¹.

**Photocoloration of EA.** Photocoloration of EA was monitored every 30 min after dilution of the photocatalytic solution with CH2Cl2 to give an orange solid (28.3 mg), which contains Ox-EA. The authors declare no competing financial interest.

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