Ultrafast Photoprotecting Sunscreens in Natural Plants

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Supporting Information

ABSTRACT: We explore the ultrafast photoprotective properties of a series of sinapic acid derivatives in a range of solvents, utilizing femtosecond transient electronic absorption spectroscopy. We find that a primary relaxation mechanism displayed by the plant sunscreen sinapoyl malate and other related molecular species may be understood as a multistep process involving internal conversion of the initially photoexcited 1\(^{π}\pi^*\) state along a trans–cis photoisomerization coordinate, leading to the repopulation of the original trans ground-state isomer or the formation of a stable cis isomer.

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electronic state via a trans→cis isomerization of the aliphatic C=C bond.

The TEAS setup,27,28 used throughout this work employed \( \sim 320-330 \) nm, 1–2 mJ cm\(^{-2}\) pump pulses with probe pulses drawn from a broad-band white-light continuum (335–675 nm), with polarization set to the magic angle (54.7°) relative to the pump pulses. Transient absorption spectra (TAS) were taken of 1 mM SA (≥98%, Sigma-Aldrich), MS, and SM (synthesized as described previously\(^{29,30}\)), in solution with either the nonpolar aprotic solvent dioxane (≥99%, Fisher Scientific), the polar aprotic solvent acetonitrile (ACN; ≥99%, Sigma-Aldrich), or the polar protic solvent methanol (≥99.6%, Sigma-Aldrich) for a range of pump–probe time delays, \( \Delta t \), up to a maximum of 2 ns. Each molecule was excited at its UV-R absorption maximum (see the Supporting Information (SI)). All TAS were chirp-corrected using the KOALA package, and reported lifetimes were determined using a global fitting procedure\(^{7-9}\) with uncertainties reported to a 95% confidence interval (2\( \sigma \)) using asymptotic standard errors; see the SI for details.

Continuous-wave UV irradiation studies were performed on all molecules using the following procedure. A static UV–visible spectrum of each sample was taken (Cary 300 spectrometer), to obtain a “before” spectrum. Samples were then irradiated with continuous-wave radiation from an arc lamp (OBB, Tunable KiloArc) for 10 min. The central wavelength used for irradiation was the same as the pump wavelength used in the TEAS measurements. The bandwidth was set to 10 nm with a power of 3 W. A second UV–visible spectrum was taken following irradiation, referred to as the “after” spectrum. The before spectrum was subtracted from the after spectrum, resulting in the reported “difference spectrum”.

Considering first the biological precursor, SA, in solution with dioxane, ACN, or methanol, the TAS are shown in Figure 1 for photoexcitation at 325, 323, and 318 nm, respectively. For SA–dioxane (Figure 1A), the TAS is dominated by three features. First is an intense absorption centered at \( \sim 420 \) nm, which decays away to the baseline by \( \sim 50 \) ps. Second, there is a broad absorption spanning the spectral region of \( \sim 420–650 \) nm. Finally, a negative signal is observed below \( \sim 350 \) nm. Because photoexcitation at around \( \sim 320 \) nm (\( \sim 4 \) eV) promotes a \( 1^1\pi\pi^{\ast} \leftarrow S_0 \) transition,\(^{26}\) the first two features are attributed to excited-state absorption (ESA) of the \( 1^1\pi\pi^{\ast} \) state (i.e., \( S_n \leftarrow 1^1\pi\pi^{\ast} \)). The negative feature, which grows in with increasing pump–probe time delays, with the decay of the ESA, is assigned to a ground-state bleach (GSB) through comparison with the static UV–visible absorption spectrum (see the SI), which does not fully recover at the maximum available pump–probe time delay of 2 ns. The TAS for SA–ACN and SA–methanol (Figure 1B and C, respectively) are also dominated by the three features seen in the SA–dioxane TAS with these addenda: the intense absorption of the \( 1^1\pi\pi^{\ast} \) ESA is blue-shifted, centered instead on \( \sim 370 \) nm, and an additional feature is observed; there is also a strong negative signal centered at around \( \sim 460 \) nm that we attribute to stimulated emission.\(^{26,33}\)

Quantitative insight into the dynamical processes observed in the TAS can be obtained by employing a global fitting procedure (see the SI).\(^{7,27,32}\) The lifetimes of the available processes are summarized in Table 1 for all of the systems.

**Table 1. Summary of the Lifetimes of Dynamical Processes of SA, MS, and SM**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>dioxane</th>
<th>ACN</th>
<th>methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \tau_1/\text{fs} )</td>
<td>93 ± 17</td>
<td>52 ± 5</td>
<td>572 ± 87</td>
</tr>
<tr>
<td>( \tau_2/\text{ps} )</td>
<td>0.90 ± 0.19</td>
<td>0.57 ± 0.04</td>
<td>3.79 ± 0.72</td>
</tr>
<tr>
<td>( \tau_3/\text{ps} )</td>
<td>12.2 ± 1.1</td>
<td>17.0 ± 0.66</td>
<td>25.5 ± 1.6</td>
</tr>
<tr>
<td>MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \tau_1/\text{fs} )</td>
<td>115 ± 49</td>
<td>53 ± 5</td>
<td>647 ± 114</td>
</tr>
<tr>
<td>( \tau_2/\text{ps} )</td>
<td>1.32 ± 0.16</td>
<td>0.54 ± 0.05</td>
<td>4.26 ± 0.90</td>
</tr>
<tr>
<td>( \tau_3/\text{ps} )</td>
<td>12.8 ± 1.3</td>
<td>18.0 ± 0.8</td>
<td>24.2 ± 1.5</td>
</tr>
<tr>
<td>SM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \tau_1/\text{fs} )</td>
<td>119 ± 28</td>
<td>51 ± 4</td>
<td>619 ± 101</td>
</tr>
<tr>
<td>( \tau_2/\text{ps} )</td>
<td>1.62 ± 0.15</td>
<td>0.63 ± 0.04</td>
<td>4.81 ± 0.77</td>
</tr>
<tr>
<td>( \tau_3/\text{ps} )</td>
<td>22.4 ± 1.9</td>
<td>27.3 ± 0.77</td>
<td>33.5 ± 1.7</td>
</tr>
</tbody>
</table>
studied herein, and we return to this table throughout our ensuing discussion. Following this, continuous-wave irradiation was used to investigate the incomplete GSB recovery and assist in our analysis of these dynamical processes. The resulting difference UV-visible spectra are shown in the bottom panels of Figure 1 (black lines), overlaid with the absorption spectrum obtained for \( \Delta t = 2 \) ns from the corresponding TAS (red lines). For SA-dioxane, the difference spectrum and the \( \Delta t = 2 \) ns spectrum (Figure 1G) match closely, with a positive absorption appearing at \( \sim 370 \) nm in both spectra. This \( \sim 370 \) nm absorption is also seen in SA-ACN, and its red-wavelength shoulder is spectrally broadened. Finally, for SA-methanol, there are large discrepancies between the difference spectrum and the absorption spectrum obtained for \( \Delta t = 2 \) ns (Figure 11). A \( \sim 20 \) nm “gap” between the two absorption features is observed. Once again, there is a shoulder to the red of the absorption feature in the \( \Delta t = 2 \) ns spectrum, which appears broader than that seen in SA-dioxane and SA-ACN. We note that the results for MS closely agree with those of SA and are presented in the SI and Table 1 for completeness.

We now consider the biological sunscreen deposited in the upper epidermis of plant leaves, SM. The TAS are shown in Figure 2A–C for SM-dioxane, SM-ACN, and SM-methanol for photoexcitation at 329, 328, and 326 nm, respectively. As described for both SA and MS studies, similar solvent-dependent spectral features are observed in the TAS. Once again employing a global fitting procedure, we determine the lifetimes of the dynamical processes, and these are summarized in Table 1. Continuous-wave studies also reveal similar patterns as those observed for SA and MS (Figures 2D–F), specifically, an increasing shoulder appearing to the red of the absorption feature at \( \sim 370 \) nm in the more protic and hydrogen-bonding solvent methanol.

We now discuss the implications with regards to photoprotection, drawing on the different aspects of the experimental results. First, considering the continuous-wave irradiation studies, we note very good agreement between the difference spectrum and the \( \Delta t = 2 \) ns spectrum for SA, MS, and SM in the aprotic, weakly hydrogen bonding solvent dioxane. In these measurements, the trans isomer and any photoproduct will have different static UV-visible spectra, and as such, any appreciable formation of photoproducts can be identified. An intense positive peak centered at \( \sim 370 \) nm is attributed to a long-lived photoproduct, which we assign to be the cis isomer of each molecule, drawing confidence from the observation of photoisomerization in similar molecules.\(^{16,17}\) This feature is also seen for SA, MS, and SM in ACN and, to a lesser extent, methanol. In addition, a weak absorption is observed, convoluted with the \( \sim 370 \) nm peak, which we have referred to as the red-wavelength shoulder. In ACN, an aprotic, mild hydrogen bonding solvent, for all three molecules, we observe a broadening of this red-wavelength shoulder. In methanol, a protic strongly hydrogen bonding solvent, the three molecules display this signal broadening as with ACN, but the absorption feature of the difference spectrum is spectrally red-shifted \( \sim 20 \) nm relative to the \( \Delta t = 2 \) ns spectrum for all three molecules. The absorption feature in the \( \Delta t = 2 \) ns spectrum has characteristics similar to the UV-visible absorption spectrum of the SA radical.\(^{25}\) We attribute the disparity seen for all methanol measurements (and to some extent in ACN) between the \( \Delta t = 2 \) ns absorption spectrum and the difference spectrum to two processes: (1) a two-photon (at least) ionization process that generates the radical (see the power dependency measurements in the SI for SA as exemplar)\(^{34-36}\) and (2) possible triplet-state absorption given the characteristic “tail” in the absorption toward the red end of the TAS.\(^{37}\) We suggest that (1) arises due to methanol’s (and again, to some extent ACN) apparent propensity to alter the electronic structure of the molecules through perturbative interactions.

Through due consideration of the data provided by the TEAS measurements, drawing on ab initio calculations on isolated “gas-phase” hydroxicinnamic acids\(^{38}\) and experimental results on related systems,\(^{26,34}\) we attempt to rationalize the dynamical processes in operation. We do however note that additional theory is required to fully comprehend the dynamical processes evidently in operation. Following excitation to the \( 1\pi\pi^* \) state by the pump pulse, we propose that SA, MS, and SM undergo numerous processes that are convoluted together and described by the lifetimes \( t_1 \) and \( t_2 \), thus making distinct assignment of any one process with a lifetime difficult. In particular, we suggest that a coherent artifact of the instrument response function and an evolution out of the Franck-Condon window contribute to \( t_1 \). Along with any solvent rearrangement, IC ensues from this state to the intermediary \( 2\pi\pi^* \) state via a \( 1\pi\pi^*/2\pi\pi^* \) conical intersection (CI), which we suggest contributes to the lifetime \( t_2 \). The time scale for these processes sensibly compares with previous dynamical studies in related molecular systems.\(^{35,33,35,37,39-41}\) Both of these lifetimes will inevitably be effected by the formation of the radical species (see Table 1). From here, isomerization may occur along the

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Figure 2. TAS for SM in solution with dioxane (A), ACN (B), and methanol (C) in the form of a color map indicating the change in optical density (ΔOD). Complementary UV irradiation difference spectra for SM-dioxane (D), SM-ACN (E), and SM-methanol (F) are shown as a black line in comparison to the absorption spectrum at the maximum available pump–probe time delay of 2 ns (red line).
$2^1\pi\pi^*$ state to generate the cis isomer in $S_0$, mediated through a $2^1\pi\pi^*/S_0$ CI, with the remaining population reverting back to the original ground-state trans isomer. These final steps account for the lifetime $\tau_S$. The overall relaxation dynamics are depicted in the schematic shown in Figure 3. An alternative relaxation mechanism to that proposed in Figure 3 consistent with the data presented should be noted. The dynamics may ensue from a vertical excitation to the $1^1\pi\pi^*$ state and IC to a $2^1\pi\pi^*$ state in the adiabatic limit before nonadiabatic transfer to the ground electronic ($S_0$) state, with associated time scales $\tau_1$, $\tau_2$, and $\tau_3$ as discussed in the text. The $1^1\pi\pi^*$ state has been omitted for simplicity. Note that an alternative relaxation mechanism involves dynamics along a single excited ($1^1\pi\pi^*$) state; see the main text for details.

![Figure 3: Schematic of the relaxation scheme proposed in this work adapted from the calculated potential energy surfaces for similar systems; $^a$ a vertical excitation to the $1^1\pi\pi^*$ state and IC to a $2^1\pi\pi^*$ state in the adiabatic limit before nonadiabatic transfer to the ground electronic ($S_0$) state, with associated time scales $\tau_1$, $\tau_2$, and $\tau_3$ as discussed in the text. The $1^1\pi\pi^*$ state has been omitted for simplicity. Note that an alternative relaxation mechanism involves dynamics along a single excited ($1^1\pi\pi^*$) state; see the main text for details.](image)

In summary, we have explored the photoprotection mechanisms in operation in SA, MS, and the plant sunscreen SM. In all three systems, excited-state relaxation occurs on an ultrafast time scale, involving, in part, IC from $1^1\pi\pi^*$ to $2^1\pi\pi^*$ to $S_0$ mediated by the appropriate $1^1\pi\pi^*/2^1\pi\pi^*$ and $2^1\pi\pi^*/S_0$ CIs. Importantly, at the $2^1\pi\pi^*/S_0$ CI, the photoexcited molecule can either re-form the original ground-state trans isomer or generate the cis isomer. We also suggest a combination of other processes in operation, notably the formation of a radical species and possibly intersystem crossing. Crucially, however, and with the exception of the radical species that is generated through consequence of the experiment itself (at least a two-photon absorption process that is unlikely to occur in nature), the present work serves to highlight the efficiency in which the plant sunscreen, SM, is able to undergo ultrafast relaxation in order to bypass the deleterious effects of UV radiation in the biosphere. This study also further highlights that there may be other reasons why SM is selected as a sunscreen molecule in plants, given that there is little difference in the excited-state dynamics between the biological precursor (SA) through to plant sunscreen (SM) in the solution phase.

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**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.5b02474.

TAS and difference spectra of MS in dioxane, ACN, and methanol; UV–visible absorption spectra of MS, SA, and SM in a range of solvents; TEAS experimental, global fitting, and asymptotic standard error details; decay associated spectra, fitting residuals, and infrared spectra of SA; selected spectra of MS and SM for specific delay times; and power-dependent measurements for SA (PDF).
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Prof. Peter J. Sadler (University of Warwick) for the use of the GriloArc and Prof. Alison Rodger (University of Warwick) for the use of the infrared spectrometer. The authors also thank Prof. Timothy S. Zwier (Purdue University) and Dr. Tolga T. V. Karsili (Technische Universität München) for useful discussions. L.A.B. thanks the Engineering and Physical Sciences Research Council (EPSRC) for providing a studentship under Grant EP/F500378/1 through the Molecular Organisation and Assembly in Cells Doctoral Training Centre and the Centre for Scientific Computing at the University of Warwick for providing computational resources. M.D.H. thanks the University of Warwick for an EPSRC studentship. S.E.G. thanks the Warwick Institute of Advanced Study for postdoctoral funding. V.G.S. thanks the EPSRC for an equipment grant (EP/J007153) and the Royal Society for a University Research Fellowship.

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