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Photosensitized Amino Acid Degradation in the Presence of Riboflavin and Its Derivatives

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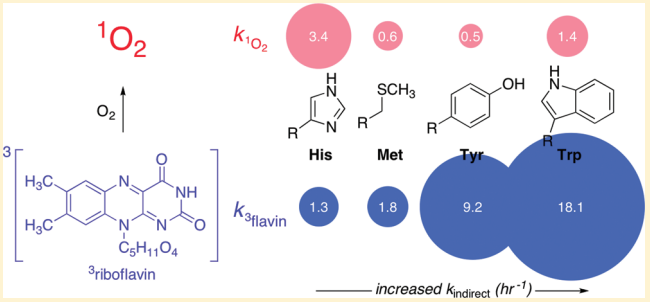
Supporting Information

ABSTRACT: The addition of photosensitizers to water can accelerate disinfection in sunlight-based systems by enhancing oxidation of target compounds through direct reaction with the excited sensitizer or through production of another oxidant, such as singlet oxygen ($^1O_2$). The kinetics of the oxidation of selected amino acids in the presence of the sensitizer riboflavin (Vitamin B2), its primary photoproduct lumichrome, and its derivative riboflavin tetraacetate (2′,3′,4′,5′-tetraacetylriboflavin; RTA) were quantified and the mechanisms of reaction were determined during exposure to 365 ± 9 nm light. $^1O_2$-mediated reactions contributed to the rapid photodegradation of the four amino acids, but its contribution was sensitizer-dependent and varied from 5.4−10.2% for tyrosine, 7.1−12.4% for tryptophan, 18.7−69.0% for methionine, and 64.7−100.2% for histidine. Riboflavin was subject to rapid photodegradation ($t_{1/2}$ <8 min), while the half-lives of lumichrome and RTA were 100 and 30 times longer, respectively. Lumichrome and RTA also were more efficient $^1O_2$ sensitizers (quantum yield (Φ) = 0.63 and 0.66) compared to riboflavin (Φ = 0.48). Of the three flavin-based compounds, RTA shows the most promise as a sensitizer in sunlight-based disinfection systems because it absorbs both visible and UV light, is an efficient $^1O_2$ sensitizer, is a strong oxidant in its triplet state, and exhibits greater photostability.



INTRODUCTION

The ability of light to inactivate pathogens is the basis for numerous disinfection systems, including UV-based water disinfection systems,[1] disinfection of blood components,[2] and photodynamic therapy to treat localized infections or tumors.[3,4] Photoinactivation of organisms by sunlight has been observed in natural systems[5] and is the basis of point-of-use treatment systems, such as solar water disinfection (SODIS), that provide a simple and cost-effective approach for water disinfection.[6] However, sunlight-based processes are considerably less efficient than systems that rely on UV light (e.g., refs 1 and 2). For example, SODIS may require up to 2 days of sunlight exposure to achieve complete disinfection during cloudy weather.[7]

Light inactivates organisms through three mechanisms: direct damage, endogenous photoinactivation, and exogenous photoinactivation. Ultraviolet (UV) light can directly damage cell components, such as nucleic acids.[8,9] However, endogenous and exogenous mechanisms are likely to dominate pathogen inactivation in solar-based treatment systems due to the efficient absorption of UVB light in natural waters. During these indirect processes, light-absorbing sensitizers are excited by sunlight and undergo energy or electron transfer reactions directly with the target compounds via a Type I mechanism (reaction 4 in Scheme 1) or transfer energy to oxygen to form singlet oxygen ($^1O_2$; reaction 5), which reacts with the target compound via a Type II mechanism (reaction 6).[10] Sensitizers may be present inside the organism (e.g., flavins)[8] or in the water (e.g., dissolved organic matter),[9,11] leading to endogenous or exogenous inactivation, respectively.

The addition of exogenous sensitizers that accelerate disinfection could improve the efficiency of photodisinfection systems, particularly those that rely on natural sunlight. Riboflavin-based compounds (Figure 1) are ideal additives to these systems because the vitamin is nontoxic to humans and aquatic organisms, absorbs strongly in the solar spectrum (Figure 2), and is widely available at low cost.[12] Riboflavin has been used as a photosensitizer to degrade a range of contaminants (e.g., anilines, phenols, herbicides, PAHs)[15−20] through both Type I[15−18] and Type II[19,20] processes. Riboflavin has also been applied as photosensitizer in blood component[2] and dental disinfection systems.[4] The vitamin has been proven effective at increasing pathogen inactivation under simulated solar irradiation,[21] although the mechanisms of photoinactivation under these conditions were not investigated.

When the flavin moiety of riboflavin absorbs a photon (reaction 1), the molecule undergoes rapid intersystem crossing (reaction 2)[13] and is an efficient $^1O_2$ sensitizer (reaction 5).[14] Riboflavin is susceptible to photodegradation, likely due to the instability of the ribose chain,[13] and forms the $^1O_2$ sensitizer

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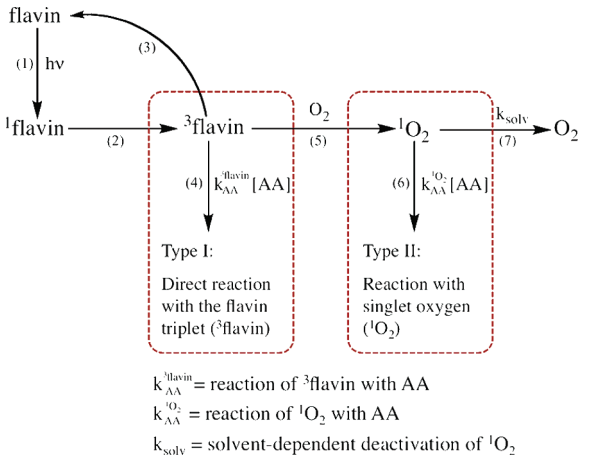
lumichrome as a photoproduct.[22] Modification of the ribose chain of the molecule may change the yield[23] and lifetime[24] of the triplet state and result in a more photostable molecule. For example, the derivative riboflavin tetraacetate (2′,3′,4′,5′-tetra-acetylriboflavin; RTA) is more photostable than riboflavin[25] and is more efficient at oxidizing anilines and phenols.[15]

This study investigates the ability of riboflavin, lumichrome, and RTA to serve as photosensitizers for the degradation of selected amino acids (AAs). AAs were chosen as model target molecules because they are the building blocks of proteins, which are likely targets for $^1O_2$ oxidation in pathogens due to their abundance and reactivity.[26] $^1O_2$ is capable of causing site-specific amino acid oxidation in virus protein capsids,[27] and damage to membrane-bound proteins by oxidative stress and carbonylation is known to play a role in bacteria inactivation during solar irradiation.[28,29] Furthermore, the use of simple target compounds enables the elucidation of complex reaction mechanisms using well-defined reaction kinetic parameters, which are complex and incompletely understood in whole proteins. The four amino acids that are included, histidine, methionine, tyrosine, and tryptophan, are susceptible to photochemical oxidation due to their sulfur-residues or aromatic groups.[30] Although several previous studies have reported reaction pathways for AA oxidation by riboflavin,[31−34] contradictory AA transformation mechanisms have been proposed. This study establishes the kinetics and reaction mechanisms for the photodegradation of these AAs in the presence of riboflavin derivatives and provides the basis for understanding sensitized protein oxidation mechanisms in more complex systems and for employing these sensitizers in photoinactivation systems.

Scheme 1. Flavin-Mediated Amino Acid (AA) Photodegradation Pathways



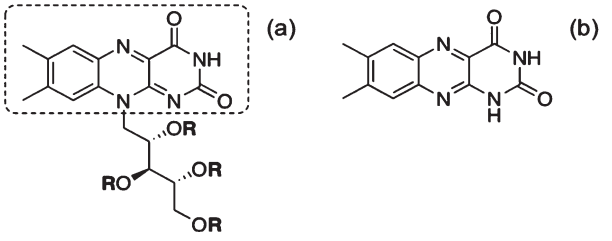


Figure 1. Structure of (a) riboflavin (**R** = H), RTA (**R** = $COCH_3$), and (b) lumichrome. The dashed box indicates the flavin moiety of the molecule.

MATERIALS AND METHODS

Materials. Riboflavin, lumichrome, all L-amino acids, perinaphthenone, and deuterium oxide (D_2O) were obtained from commercial suppliers and used as received. Furfuryl alcohol (FFA) was distilled prior to use. Details on the synthesis of RTA and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) are provided in Supporting Information. All solutions were prepared using 18 MΩ water (Barnstead Nanopure Diamond Water Purification System).

Experimental Setup. All photolysis experiments were conducted in borosilicate glass test tubes on a turn-table apparatus inside a photochemical reactor (Rayonet) equipped with two 365-nm bulbs (Southern New England Ultraviolet Co. RPR-3500 Å; Figure 2). Experiments were conducted with air-saturated solutions containing 0−20 μM flavin, 40 μM target compound, and 1−5 mM sodium phosphate (pH 7.4) at room temperature (23 ± 1 °C). Solution temperatures increased by ≤3 °C during photolysis. The solutions containing 0 μM flavin were monitored to determine the direct photodegradation rate of the target compounds. At specified sampling times, the bulbs were switched off and 40−500 μL aliquots were removed for UPLC analysis. At least two test tubes were used for each solution condition and the data were averaged. Identical

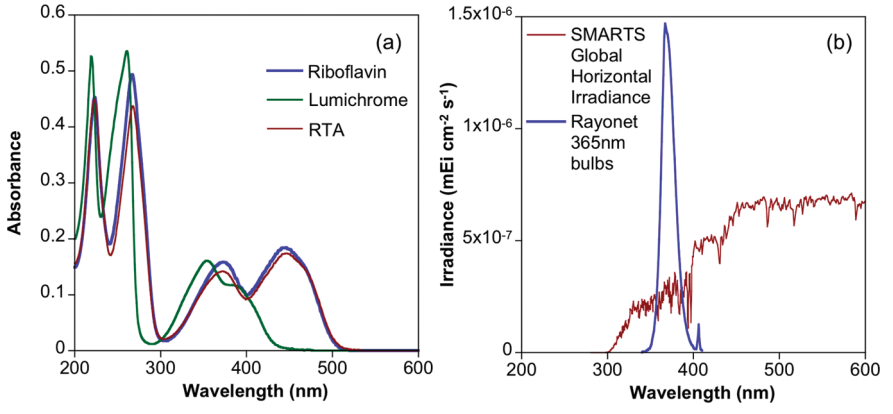


Figure 2. (a) Absorbance spectra of 16 μM riboflavin, lumichrome, and RTA at pH 7.4. (b) Global horizontal irradiance modeled with SMARTS for Zurich, Switzerland (47° 22′ 0″ N, 8° 33′ 0″ E) on July 1, 2010 and irradiance of the fixed-wavelength 365-nm bulbs used in the Rayonet.

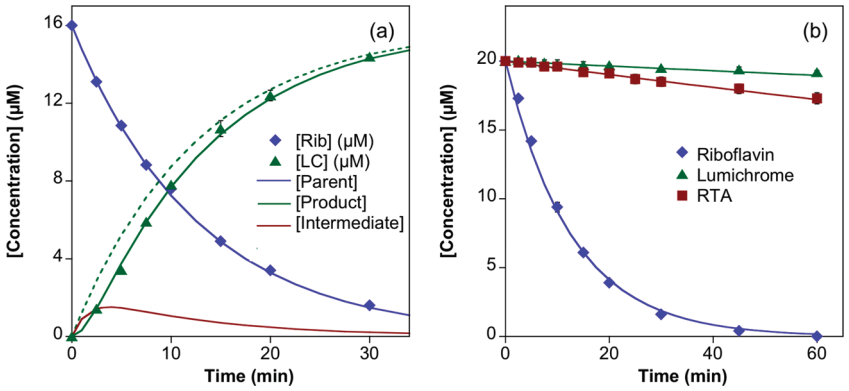


Figure 3. (a) Photodegradation of 16 μM riboflavin to produce lumichrome. Lines indicate model fits for formation of lumichrome directly (dashed; $k = 1.3 \times 10^{-3}$ M^{-1} s^{-1}) or via an intermediate (solid; $k_1 = 1.3 \times 10^{-3}$ M^{-1} s^{-1}; $k_2 = 1.0 \times 10^{-2}$ M^{-1} s^{-1}). (b) Direct photodegradation of 20 μM riboflavin, lumichrome, and RTA. Solid lines are fits of the data using exponential decay.

solutions were left in the dark during each experiment to serve as dark controls. Solutions containing 7.9 μM *p*-nitroanisole (pNA) and 9.14 mM pyridine were irradiated in separate test tubes as an actinometer[35] for each experiment. Initial degradation rate constants are shown in all plots and the error bars correspond to the standard deviation associated with the rate constant calculation.

Solutions containing 40 μM FFA and 0−20 μM flavin were irradiated under identical conditions to determine the steady-state concentration of $^1O_2$ for each flavin concentration. $[^1O_2]_{ss}$ was calculated by dividing the observed FFA degradation rate by the reaction rate between FFA and $^1O_2$ (8.3×10^7 M^{-1} s^{-1}).[36]

Several experiments with riboflavin were repeated in D_2O to provide further evidence of the contribution of $^1O_2$ to AA degradation and to verify the reaction rate between the amino acid and $^1O_2$. The rate constant of $^1O_2$ quenching by D_2O (reaction 7) is approximately an order of magnitude lower than that of H_2O and an increase in the AA degradation rate in the presence of D_2O is strong evidence that $^1O_2$ is involved.[10] The rate constant ($k_{AA}^{^1O_2}$) was calculated according to the following equation (derived in Supporting Information):

$$k_{AA}^{^1O_2} = \frac{k_{AA,obs}^{D_2O} - k_{AA,obs}^{H_2O}}{[^1O_2]_{ss}^{D_2O} - [^1O_2]_{ss}^{H_2O}} \quad (8)$$

where $k_{AA,obs}$ is the observed AA degradation rate constant and $[^1O_2]_{ss}$ is the $^1O_2$ steady-state concentration determined using FFA in H_2O or D_2O. Perinaphthenone, a well-characterized $^1O_2$-sensitizer, was used to verify the suitability of this approach under experimental conditions.

The UV−vis absorbance spectra of ~20 μM riboflavin, lumichrome, and RTA were measured on a Varian Cary 100 spectrophotometer at various pH values for pK_a determination. The absorbance at a single wavelength was plotted versus solution pH and the resultant curves were fit by least-squares minimization.

Analytical Methods. UPLC analysis was conducted on a Waters ACQUITY UPLC system equipped with a photodiode array detector (PDA) and a fluorescence detector (FLR). Details concerning quantification of riboflavin, lumichrome, RTA, FFA, and pNA by UV absorption, tyrosine and tryptophan by fluorescence, and methionine and histidine by AQC-derivatization followed by fluorescence are available in Supporting Information.

Absorbance spectra for all solutions were collected with a UV−vis spectrophotometer prior to photolysis for use in quantum yield calculations.

Calculations. The direct and $^1O_2$ quantum yields (Φ^{unk}) for each sensitizer were calculated relative to standard quantum yields (Φ^{std}):

$$\Phi^{unk} = \frac{k_{obs}^{unk}}{k_{obs}^{std}} \frac{k_{abs}^{std}}{k_{abs}^{unk}} \Phi^{std} \quad (9)$$

where k_{obs} is the observed degradation rate of the sensitizer for direct quantum yield calculations or the observed degradation rate of FFA for $^1O_2$ quantum yield calculations. The light absorbance rate constant (k_{abs}) is a function of the light intensity (I_λ), the solution decadic absorbance (α_λ), and a screening factor (S_λ):

$$k_{abs} = \sum_\lambda I_\lambda 2.303 \alpha_\lambda S_\lambda \quad (10)$$

integrated over the appropriate wavelength range. The pNA/pyridine actinometer was used as the standard for the direct quantum yield calculations[35] and riboflavin was used as the standard for the $^1O_2$ quantum yield calculations.[14]

The simple model of the atmospheric radiative transfer of sunshine (SMARTS)[37] was used to estimate sunlight irradiance for Zurich (47° latitude) to enable comparison between the 365-nm bulbs used in this study and typical solar irradiance.

RESULTS

Characterization and Photostability of Flavins. Riboflavin and RTA have nearly identical absorbance spectra, with peaks at 373 and 446 nm, while lumichrome absorbs very little visible light ($\lambda > 400$ nm) and has a peak at 353 nm at pH 7.4 (Figure 2). The absorbance spectra of the three flavins overlap well with the narrow-wavelength (λ_{max} = 365 nm, width at half-maximum = ± 9 nm) bulbs used in this study.

Substitution of the hydroxyl groups of the ribose chain in riboflavin with acetate groups has a minimal impact on the acid dissociation constant (pK_a) of the molecule (Figure S1). The measured pK_a of riboflavin, 10.23 ± 0.08, agreed with the literature value of 10.2.[38] RTA exhibited a similar pK_a of 10.10 ± 0.06. The measured pK_a values of lumichrome, 8.41 ± 0.04

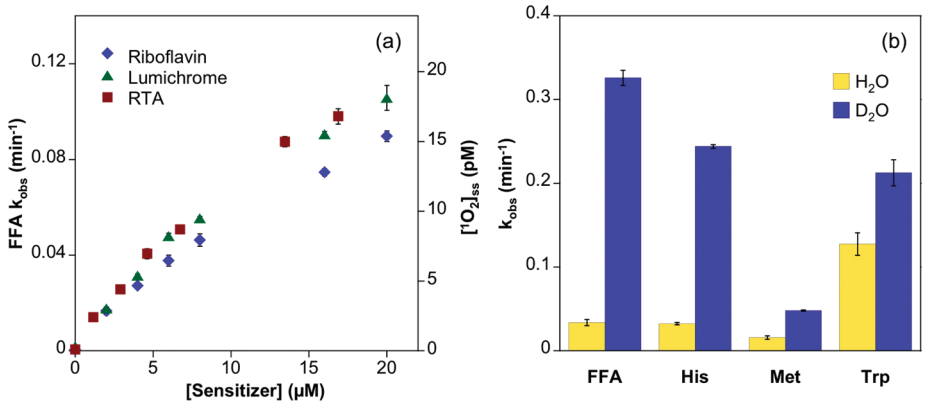


Figure 4. (a) Observed FFA degradation rates and $^1O_2$ steady-state concentrations in the presence of 0−20 μM riboflavin, lumichrome, and RTA. (b) Observed photodegradation rates of FFA, histidine, methionine, and tryptophan in the presence of 4 μM riboflavin in 5 mM phosphate buffer in H_2O (pH 7.4) or in D_2O (pH 7.8).

and 12.93 ± 0.04, were similar to the literature values of 8.28 and 12.9.[39]

Upon illumination, riboflavin undergoes rapid photodegradation to form lumichrome (Figure 3). Other potential products, such as lumiflavin, are more likely to form at higher pH values[22] and were not observed. Although the formation of lumichrome was stoichiometric after ~20 min of photolysis, modeling the conversion of riboflavin to lumichrome as a direct, one-step reaction overpredicted the lumichrome concentration at early time points. Assuming that riboflavin first forms an intermediate product that undergoes rapid transformation to form lumichrome resulted in a better fit to the data. The intermediate product of riboflavin was not identified, but its relatively rapid disappearance and predicted low concentration suggests that the species likely does not contribute significantly to target compound photodegradation.

The photoproduct lumichrome and the derivative RTA are substantially more photostable than riboflavin (Figure 3). The quantum yields, relative to the pNA/pyridine actinometer, are $(14.8 \pm 9.5) \times 10^{-4}$, $(0.14 \pm 0.08) \times 10^{-4}$, and $(0.68 \pm 0.39) \times 10^{-4}$ and the observed direct photodegradation half-lives are 7.9 ± 1.4, 784 ± 45, and 247 ± 67 min for riboflavin, lumichrome, and RTA, respectively. The photochemical behavior of lumichrome warrants consideration because it rapidly becomes dominant if riboflavin is used as a sensitizer.

Singlet Oxygen Production. The photosensitized degradation of FFA, and therefore $[^1O_2]_{ss}$, increased linearly with increasing concentrations of riboflavin, lumichrome, and RTA (Figure 4). The $^1O_2$ quantum yields of lumichrome and RTA were 0.63 ± 0.01 and 0.66 ± 0.03, averaged over all sensitizer concentrations used. The measured RTA yield agreed well with the literature value of 0.65 in H_2O,[40] whereas the lumichrome quantum yield fell between the values reported in D_2O (0.36)[41] and methanol (0.85).[42]

Amino Acid Photodegradation. Of the four amino acids included in this study, only tryptophan was susceptible to direct photodegradation in phosphate-buffered water ($t_{1/2}$ = 80 min; Table S2). This is due to the overlap of its absorption spectrum and the lamp irradiance (Figures 2, S2) and is consistent with previous observations.[43] All tryptophan degradation plots show indirect photodegradation rates only (i.e., $k_{obs} - k_{direct}$).

Identical experiments with riboflavin and perinaphthenone in H_2O and D_2O were performed to verify the reaction rates between $^1O_2$ and selected AAs ($k_{AA}^{^1O_2}$) under experimental conditions (Figure 4). In the case of histidine and methionine, the measured rate constants of $(6.0 \pm 0.1) \times 10^7$ and $(0.9 \pm 0.1) \times 10^7$ M^{-1} s^{-1} agreed with the literature values (Table S3). Previously reported rate constants for $^1O_2$ and tryptophan vary by an order of magnitude (e.g., 2−18 × 10^7 M^{-1} s^{-1}).[43,44] Nearly identical rate constants of $(2.2 \pm 0.4) \times 10^7$ and $(2.3 \pm 0.8) \times 10^7$ M^{-1} s^{-1} for the reaction of tryptophan with $^1O_2$ were found when riboflavin and perinaphthenone were used as sensitizers, respectively, in agreement with the lower reported rate constants.

The indirect photodegradation rates of histidine, methionine, tyrosine, and tryptophan in the presence of riboflavin, lumichrome, and RTA increased linearly with increasing $[^1O_2]_{ss}$ (Figure 5). The measured $[^1O_2]_{ss}$ was multiplied by $k_{AA}^{^1O_2}$ to predict the amino acid degradation rate attributable to sensitizer-generated $^1O_2$. AA degradation rates that fall on the predicted rate line indicate that $^1O_2$ is solely responsible for the observed indirect photodegradation, whiles rates that fall above the line indicate that Type I processes (Scheme 1) also contribute. The contribution of $^1O_2$ to indirect photodegradation was dependent on both the AA and the sensitizer.

Reaction with $^1O_2$ was the dominant mechanism of histidine photodegradation. Whereas 100.2 ± 1.3% of histidine degradation in the presence of lumichrome was due to reaction with $^1O_2$, only 72.2 ± 3.0% and 64.7 ± 6.7% of histidine degradation was attributable to reaction with riboflavin- and RTA-generated $^1O_2$ (Figure 5). Similarly, the increase in histidine degradation rate by riboflavin in D_2O relative to the rate observed in H_2O indicated that 78% of the histidine degradation was due to $^1O_2$ (Figure 4).

The contribution of $^1O_2$ to methionine degradation also was sensitizer dependent. Reaction with $^1O_2$ accounted for 32.8 ± 3.5%, 69.0 ± 2.2%, and 18.7 ± 1.8% of methionine photodegradation in the presence of riboflavin, lumichrome, and RTA, respectively. According to D_2O experiments, $^1O_2$ was responsible for 31% of methionine degradation by riboflavin.

The indirect photodegradation rates of tyrosine and tryptophan were much faster than the rates predicted by reaction with $^1O_2$ alone. Only 5.4 ± 0.4%, 10.2 ± 1.0%, and 6.0 ± 0.9% of tyrosine degradation and 7.1 ± 1.2%, 12.4 ± 1.3%, and 7.9 ± 0.6% of tryptophan degradation by photosensitized riboflavin, lumichrome, and RTA was attributable to reaction with $^1O_2$ (Figure 5). These results, in combination with the modest increase in tryptophan degradation in D_2O (Figure 4), indicate

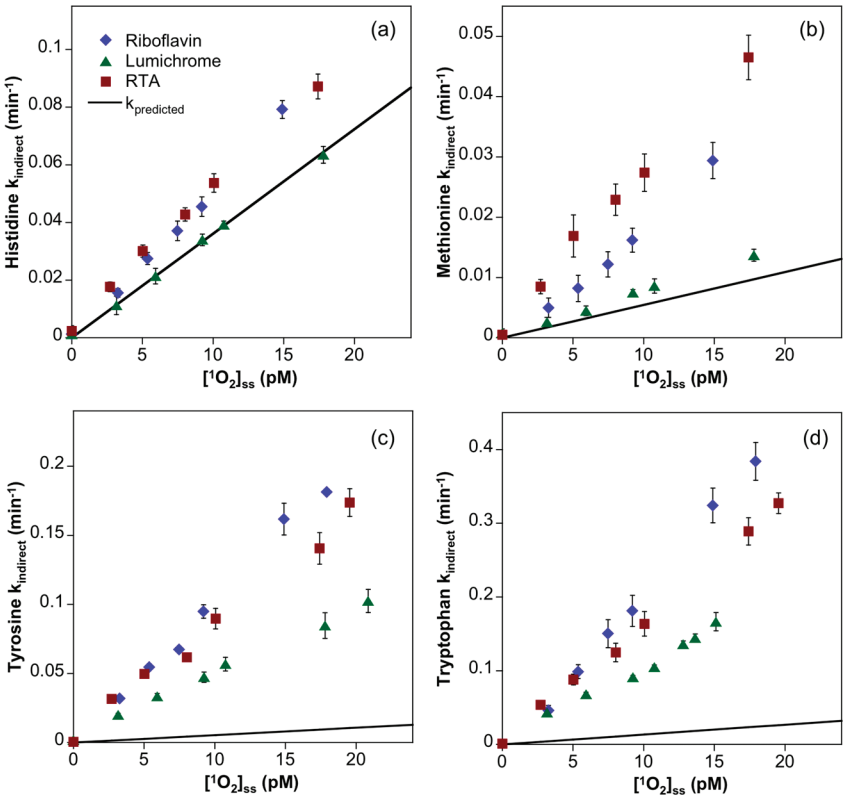


Figure 5. Indirect photodegradation rates of 40 μM (a) histidine, (b) methionine, (c) tyrosine, and (d) tryptophan in the presence of 0−20 μM riboflavin, lumichrome, and RTA plotted versus the steady-state $^1O_2$ concentration under the same conditions. The solid line indicates the predicted amino acid photodegradation rate due to reaction with $^1O_2$.

that nearly all of the tyrosine and tryptophan photodegradation was due to Type I reactions.

DISCUSSION

Comparison of Sensitizers. Although riboflavin is a well-characterized and biologically relevant sensitizer,[13,32,33,45] its relatively rapid degradation in the presence of light limits its use in practical applications. Lumichrome, the primary photodegradation product of riboflavin at circumneutral pH values, is nearly 100 times more photostable (Figure 3) and is a more effective $^1O_2$ sensitizer than riboflavin (Figure 4). Although the absorbance spectra of riboflavin and lumichrome overlap well with the narrow-wavelength bulbs used in this study, riboflavin absorbs considerably more light in the full sunlight spectrum (Figure 2). For example, the light absorbance rate constant (k_{abs}; reaction 10) of riboflavin is 1.1 times higher than that of lumichrome when calculated with the 365-nm bulb spectrum, but is 2.5 times higher when the SMARTS sunlight spectrum is used. This implies that a higher lumichrome concentration would be required to achieve the same relative increase in $[^1O_2]_{ss}$ observed here (e.g., 20.9 and 17.9 pM for 20 μM lumichrome and riboflavin, respectively).

The absorbance spectra and acid dissociation constant of RTA and riboflavin are very similar (Figures 2, S1). Although RTA and riboflavin absorb light at nearly the same rate, the half-life of direct RTA photodegradation is ~30 times longer than that of riboflavin (Figure 3). The increased photostability of RTA is attributable to inhibition of intramolecular photoreduction by the ribose side chain.[13,15,25,46] Furthermore, the $^1O_2$ quantum yield of RTA (Φ = 0.66) is higher than that of riboflavin (Φ = 0.48)[14] and a higher $[^1O_2]_{ss}$ would be observed in an equimolar solution of RTA relative to riboflavin during sunlight photolysis. The improved photostability and higher $^1O_2$ production efficiency of RTA suggest that the compound may be a better choice for use in sunlight-mediated disinfection processes.

Amino Acid Degradation Mechanisms. Singlet oxygen plays a role in the flavin-sensitized photodegradation of histidine, methionine, tyrosine, and tryptophan. Although the amino acids react with $^1O_2$ at similar rates (Table S3), the amount of AA degradation attributable to reaction with $^1O_2$ varied from <10% for tyrosine and tryptophan to 100% for histidine. As described below, previous studies with riboflavin and its conjugated form, flavin mononucleotide (FMN; riboflavin-5′-phosphate), provide further insight into the indirect photodegradation mechanisms of the AAs. We are unaware of previous studies that investigated AA degradation in the presence of lumichrome or RTA.

The reaction of histidine with $^1O_2$ in O_2-containing photosensitized systems is well-known and is generally considered to be the primary histidine degradation pathway.[43,47,48] Histidine is frequently used in photochemical studies as a specific $^1O_2$ quencher.[9] Accordingly, previous studies with riboflavin and FMN attributed histidine degradation solely to Type II processes (reaction 6).[32,47,49] While histidine photodegradation in the presence of lumichrome was completely attributable to reaction with $^1O_2$, degradation rates in riboflavin- and RTA-containing solutions were higher than those predicted based on a Type II reaction mechanism (Figure 5). These observations, combined with the lower-than-expected increase in histidine oxidation in

D_2O (Figure 4), demonstrate that triplet riboflavin and RTA can react with histidine via a Type I mechanism (reaction 4). In the absence of O_2, histidine reacts with ^{3}riboflavin and ^{3}FMN at reaction rates of $(1-6) \times 10^7\ M^{-1}\ s^{-1}$ at neutral pH[33,49,50] via H-atom abstraction.[50−52] It is interesting to note that this process contributes to the riboflavin- and RTA-sensitized degradation of histidine, despite the fact that O_2 is the dominant reaction partner for the triplet flavin in air-saturated solutions ($[O_2] \sim 250\ \mu M$; $k_{O_2}^{^3Rib} = 9.8 \times 10^8\ M^{-1}\ s^{-1}$).[33]

Photosensitized methionine degradation by riboflavin and FMN was previously attributed to reaction with $^1O_2$.[32] Furthermore, the reaction of methionine with triplet flavins proceeds via electron transfer at rates that are similar to those of histidine,[50,51,53] suggesting that direct reaction with the triplet species via a Type I mechanism is unlikely in the presence of O_2. However, estimation of methionine degradation based on $[^1O_2]_{ss}$ significantly underpredicted the indirect photodegradation rate, particularly in the case of riboflavin and RTA (Figure 5), and only a 3-fold increase in methionine degradation was observed in D_2O (Figure 4). Whereas 69% of the methionine degradation is due to $^1O_2$ in the presence of lumichrome, Type II processes are dominant when riboflavin or RTA are used as sensitizers. A similar mixed Type I/II mechanism was reported for the indirect photodegradation of methionine in the presence of dissolved organic matter, where 26−62% of degradation was attributable to oxidation by $^1O_2$.[43]

Tyrosine reacts with ^{3}riboflavin or ^{3}FMN at near-diffusion controlled rates ($(1-3) \times 10^9\ M^{-1}\ s^1$)[33,49−51] and can therefore outcompete O_2 for reaction with ^{3}riboflavin. This rapid reaction may be due in part to hydrogen bonding between riboflavin and the hydroxyl group of tyrosine.[13,54] Reaction with $^1O_2$ was responsible for <10% of the observed tyrosine degradation rate under experimental conditions (Figure 5), in agreement with previous studies that attributed photodegradation of tyrosine by riboflavin to Type I processes.[33,45] Despite density functional theory calculations that suggest the oxidation of tyrosine by triplet flavins proceeds by H-atom abstraction,[55] numerous laser flash photolysis studies provide evidence that electron transfer is more likely.[33,51,52,56]

Tryptophan also reacts with ^{3}riboflavin and ^{3}FMN at near-diffusion controlled rates,[33,49−51] suggesting that, as observed with tyrosine, Type I processes should be important. However, the photosensitized degradation of tryptophan in the presence of riboflavin or FMN has been attributed to both Type I[31−33,45,49,50] and Type II[31,45] reaction mechanisms. Under experimental conditions, <12% of tryptophan degradation was attributable to sensitizer-generated $^1O_2$ (Figures 4 and 5). Although oxidation by $^1O_2$ was slightly more important for tryptophan degradation relative to tyrosine degradation, the reaction pathways are quite similar. Numerous studies provide evidence for electron transfer between tryptophan and triplet riboflavin or FMN.[31,45,49,52,56]

Indirect degradation rates of histidine, methionine, tyrosine, and tryptophan were higher when riboflavin and RTA were used as sensitizers in comparison with lumichrome (Figure 5), suggesting that ^{3}riboflavin and ^{3}RTA are better oxidants than ^{3}lumichrome. One possible explanation for this observation is the difference in redox potentials of the sensitizers. The standard free energy for a light-induced, one-electron transfer reaction can be estimated as[49]

$$\Delta G^0 = -nF(E_{oxidant} - E_{reductant}) - \Delta E_{0,0} \qquad (11)$$

where the energy gap between ground and triplet-excited state levels ($\Delta E_{0,0}$) of riboflavin is 210 kJ mol^{-1}, corresponding to a ~570 nm phosphorescence peak.[49,57] Other isoalloxazines and alloxazines exhibit nearly identical phosphorescence peaks[57] and it is likely that $\Delta E_{0,0}$ for lumichrome is similar. Given that the reduction potentials of riboflavin and lumichrome ($E_{oxidant}$) are −0.3 V [58] and −0.5 V,[59] respectively, and taking tryptophan as the reductant ($E_{reductant}$ = 0.98 V),[49] ΔG^0 is −86.5 kJ/mol for ^{3}riboflavin and −67.2 kJ/mol for ^{3}lumichrome. The more negative free energy calculated for ^{3}riboflavin supports the general observation that ^{3}riboflavin is a better oxidant than ^{3}lumichrome. Although the redox potential of RTA is unknown, the riboflavin-derivative exhibited behavior similar to riboflavin and it is likely that ^{3}RTA behaves in the same manner as ^{3}riboflavin.

Implications for Photodisinfection Systems. The ability of riboflavin and its derivatives to rapidly photodegrade amino acids in homogeneous aqueous solutions provides the basis for applying these sensitizers in sunlight-based disinfection methods (e.g., SODIS). The four individual amino acids studied here, as well as cysteine, are the primary targets of photochemical oxidation in proteins[33,60] and are susceptible to oxidative damage in cells and viruses.[26,27] Furthermore, it is known that high concentrations of riboflavin are effective in enhancing photoinactivation of pathogens.[21] Further research is needed to understand the effect of riboflavin-based photosensitizers on proteins and pathogens on a mechanistic level and to determine if similar oxidative damage to enzymes observed during solar irradiation in the absence of riboflavin[29] contributes to the observed accelerated photoinactivation rates in the presence of the sensitizer.

Of the three riboflavin-based sensitizers included in this study, RTA has the most favorable photochemical properties for use in photoinactivation applications. Compared to riboflavin, RTA is more photostable, is a better $^1O_2$ sensitizer, and its triplet is an equally strong oxidant. RTA can be easily synthesized from riboflavin, which is widely available. An ideal application with RTA would involve immobilizing the sensitizer so that it could be easily separated from the disinfected water after exposure to sunlight, as done previously with Ru(II)-based photosensitizers.[61] Under these conditions, reaction with $^1O_2$ may become relatively more important due to limited interactions between the target molecules or organisms and the triplet flavin. Studies with immobilized flavin-derivatives to test this hypothesis are currently underway.

■ ASSOCIATED CONTENT

Supporting Information. Detailed methodology, Tables S1−S3, and Figures S1−S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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