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Highlight Article (Invited)

How Tryptophan Oxidation Arises by "Dark" Photoreactions from Chemiexcited Triplet Acetone

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ABSTRACT

Dioxetane intermediates readily decompose to chemiluminescent triplet carbonyls, giving rise to what has been paradoxically called photochemistry in the dark. In this issue of *Photochemistry and Photobiology*, Bechara et al. report on mechanistic advances in such a reaction. With the use of horseradish peroxidase for isobutyraldehyde-derived triplet acetone, light emission from acetone and singlet oxygen can be quenched. The experiments reveal that the reaction depends on oxygen and the amino acid. The analysis reveals that free tryptophan is a target of this form of "carbonyl stress," with the efficient formation of mono-, bi- and tricyclic compounds (*N*-formylkynurenine, indoline, 1λ^2-indole and 3*H*-indoles).

COMMENTARY

Ever since the demonstration of the chemiluminescence by triplet carbonyls (1-3), mechanistic details have been desired to further explore its potential relevance in biological processes. However, a challenge exists not only in terms of detection of the weakly emitted light, but also determination of biological damage and protection against the reactive species. Mechanistically, there are virtues in probing triplet carbonyl damage and factors that protect against it.

A mechanistic analysis using horseradish peroxidase (HRP) and H_2O_2 for isobutyraldehyde **1**-derived triplet acetone would improve the view (Fig. 1). The analysis shows that free amino acid quenching has importance to the concept of photochemistry in the dark by triplet-excited acetone. Amino acids are quenchers for carbonyl triplets, which is appreciated in the field (4-6). With the use of isobutyraldehyde and HRP in the presence of free Trp and oxygen, a series of products were produced. In this issue of *Photochemistry and Photobiology*, Bechara et al. (7) report mechanistic advances in such reactions: a triplet acetone from isobutyraldehyde and HRP emits light and undergoes quenching reactions that depend on the amino acid and O_2.

Reports of chemiluminescence reactions by excited triplet carbonyls have attracted much interest in the recent (8-14) and more distant past (15-18). Excited triplet carbonyls not only luminesce, they yield products from H-abstraction to oxygenation and fragmentation. Such reactions are of interest to biochemists for their damaging effects (19-21), however a challenge is that mechanistic analysis of chemiluminescent reactions is difficult in natural settings. Contributions from the Latin American photoscience community have made a high impact in the field, having emphasized dark photoreactions via chemiexcited species as not only important in chemical, but also biochemical systems (22-31).

Carbonyl structures arising from metabolic reactions can vary making their triplet excited states challenging to study—made even more difficult in biological settings. The various carbonyl structures can instead be studied by the use of proxy compounds such as isobutyraldehyde, to help deduce wider triplet carbonyl reactivity and untangle the dark photochemical origins of triplet carbonyls. Accessible by isobutyraldehyde is the direct detection of the weak chemiluminescence triplet acetone at 430 nm, along with quenching paths (5,32,33).

Quenching of triplet acetone luminescence by free amino acids (Ser, Thr, Lys, His, Cys and Trp) was examined with Stern-Volmer (K_{SV}) and quenching rate constants (k_q). The authors have not yet explored the effects of peptides, but showed quenching by His, Cys and Trp. Trp was key and was found as a dynamic quencher rather than a static quencher of triplet acetone. The reaction also included an O_2 dependence. Up to this point, products that arise in the isobutyraldehyde/HRP reaction with Trp and O_2 had not been identified.

To address this need, products generated by HRP incubated with isobutyraldehyde, Trp and O_2 were analyzed by HPLC-MS/MS. The mass spectrometry is useful in this type of reaction (34-36) and here the analysis led to the assignment of six products: a Schiff compound, *N*-formylkynurenine, and bicyclic and tricyclic alcohols, in which product distribution enabled a mechanistic analysis.

MECHANISM

The authors examined mechanistic steps tied in particular to product distribution, emphasizing how it relates to dark triplet formation for a photochemical reaction. Thus, the mechanism has distinct dark and light processes. Isobutyraldehyde **1** can condense to Trp to reach a Schiff compound. Isobutyraldehyde is oxidized, using HRP with H_2O_2 as the oxidizing agent. HRP catalyzes the oxidation of isobutyraldehyde to triplet acetone by several intermediates.

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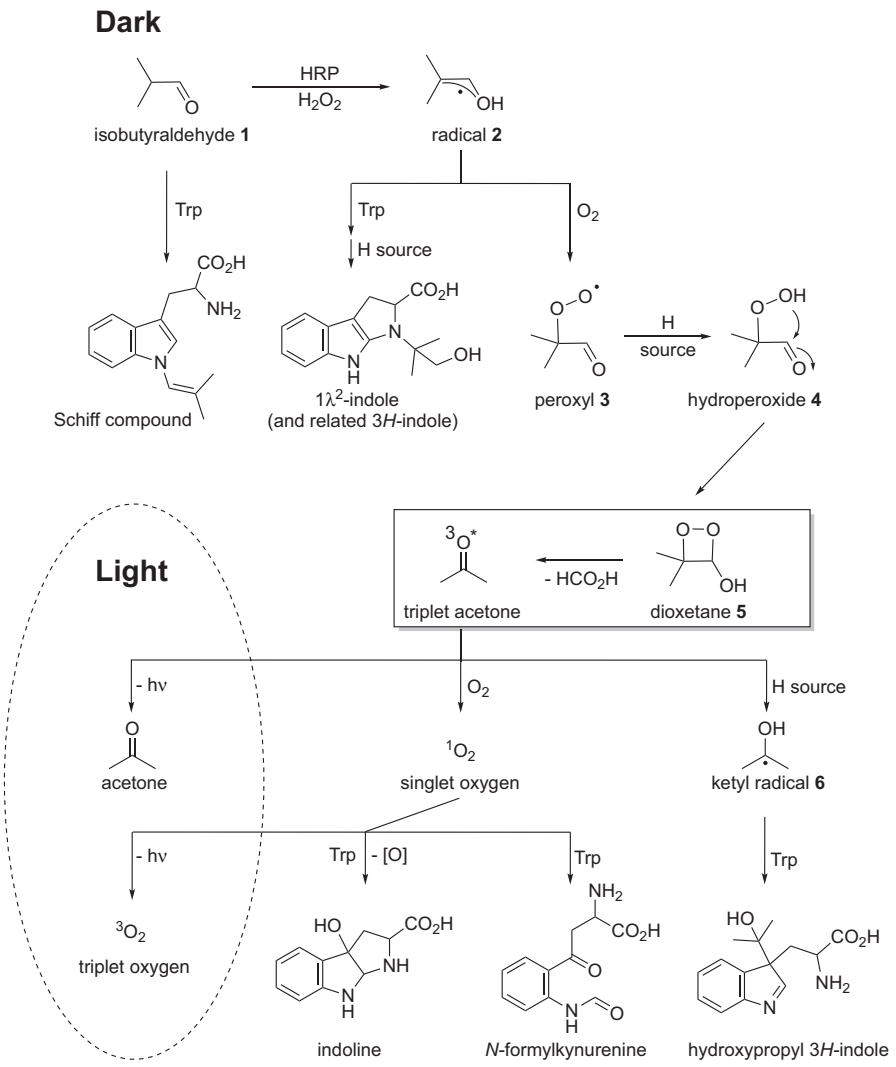

Figure 1. Isobutyraldehyde **1** is consumed by Trp in forming a Schiff compound. Isobutyraldehyde is also consumed by horseradish peroxidase (HRP) and H_2O_2 by H-abstraction, where the radical **2** undergoes molecular oxygenation to reach peroxyl radical **3**. A subsequent H atom abstraction leads to hydroperoxide **4**, enabling an intramolecular nucleophilic attack of the hydroperoxide to the adjacent C = O group leading to dioxetane **5**. Dioxetane **5** scission leads to triplet acetone and formic acid. The former can transfer energy to $^3O_2$ leading to singlet oxygen ($^1O_2$), and subsequent indoline and *N*-formylkynurenine products. While direct reactions of Trp with radicals **2** and **6** also form bi- and tricyclic compounds.

Mechanistically speaking, HRP abstracts an H atom from **1**, resulting in radical **2**, that in turn adds O_2 forming the peroxyl radical **3**. Importantly, **3** itself abstracts an H atom, setting up a nucleophilic attack of hydroperoxide **4** to the carbonyl group (C = O), which withdraws electron density yielding a dioxetane intermediate **5**. This nucleophilic formation of the dioxetane is analogous in the Kopecky dioxetane synthesis from bromo or iodohydroperoxides, common in organic chemistry (37,38), as well as other organic and biological dioxetane forming reactions (39). Dioxetane **5** is poised to release triplet acetone from oxygen–oxygen (O–O) and carbon–carbon (C–C) bond scission. Mechanistic facets of this scission have been of much interest, including dissymmetry in the process and the suggestion of the chemically initiated electron-exchange luminescence (CIEEL) provided in mechanistic organic photochemistry (40-42). Once released, triplet acetone can undergo a direct reaction (forming ketyl radical **6** that reacts with Trp to reach a 3*H*-indole compound) or transfer energy to triplet oxygen to produce $^1O_2$. Subsequent singlet oxygen reactions with Trp, which proceed by "ene" and [2 + 2] cycloaddition reactions, are implicated in the formation of an indoline compound and *N*-formylkynurenine.

Oxygen is a key reagent. One function of oxygen is to add to the isobutyraldehyde radical **2** and thereby enable triplet acetone formation. Oxygen's second function is as an energy transfer acceptor from triplet acetone in forming $^1O_2$. Both of oxygen's functions as well as triplet acetone contribute damaging effects on biomolecules, where the authors reveal Trp as a key target to this form of carbonyl stress. This carbonyl stress leads to reactive intermediates, including possible sugar-based free radicals and chain reaction processes. In fact, electrophoresis experiments with SDS-PAGE showed the damage to HRP by triplet acetone, as well as damage prevention by free Trp.

CONCLUSION

Bechara and colleagues' work underscores the importance of detailed mechanistic work (7). This can help inspire research on other triplet carbonyls. The isobutyraldehyde to triplet acetone

transformation is useful, and its success relates to transformations that natural carbonyls present. Future study can examine carbonyl stress from metabolized compounds, such as α-dialdehydes, β-ketoacids and aminoketones with variable distribution of biological triplets. The mechanistic insight into Trp protection should lead to better understanding of carbonyl stress. Emission or energy transfer paths will depend on facets such as the lifetime of triplet carbonyls, substituents and medium (43-46). The results raise questions for future study. One outstanding question is, does dark and light carbonyl stress relate to specific diseases in advanced glycation end-products (AGEs) and advanced lipid end-products (ALEs)? Future work exploring chemiluminescent triplet carbonyls, protein residues' influence, and when its protection is compromised, will provide further answers to questions about "photochemistry" in the dark.

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