

Quenching of horseradish peroxidase-generated triplet acetone by sorbate and ethyl sorbate

Thiago M. V. Gomes (PG),¹ Luiz D. Ramos (PG),² Cassius V. Stevani (PQ),² Etelvino J. H. Bechara (PQ)^{1,2*}

thiagodamata20@usp.br; ejhbechara@gmail.com

¹Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil; ²Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, Brazil

Palavras Chave: Photochemistry in the dark, Triplet acetone, Sorbate, HRP, Chemiluminescence, Quenching.

Highlights

1. Triplet carbonyls are produced by dioxetane and peroxy radical intermediates of biological processes.
2. Quenching of carbonyl triplets by conjugated dienes like sorbates is diffusion-controlled.

Abstract

Triplet excited carbonyls can initiate typical photochemical reactions even in the absence of light. This "photochemistry in the dark" paradoxical hypothesis was chemically anchored by synthesis and studies of 1,2-dioxetanes and 1,2-dioxetanones, which thermolysis yield triplet carbonyls, that exhibit ultraweak chemiluminescence. Due to their long lifetime and alcoxyl radical-like reactivity, it may start and propagate deleterious reactions on biological systems.¹ In this work, the quenching properties, with the classical Stern-Volmer treatment, of sorbate (2,4-hexadienoate) and ethyl sorbate (ethyl 2,4-hexadienoate) against horseradish peroxidase (HRP)/isobutanal (IBAL)-generated triplet acetone (Fig. 1) was investigated. The kinetic quenching constants (k_q) for sorbate, $3.0 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, and ethyl sorbate, $8.6 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, were obtained, when triplet acetone lifetime is $1.2 \mu\text{s}$.² These values are in agreement with a collisional suppression process, in which k_q expected is close to the reported diffusional coefficient in water ($\sim 5 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$).³ SDS-PAGE and CD show that enzymatic-generated triplet acetone may cause secondary and tertiary structural damages to the HRP. The use of sorbate and its alkyl ester during the reaction revealed the protection properties of the conjugated 1,2-dienes against IBAL-induced HRP structural damages.

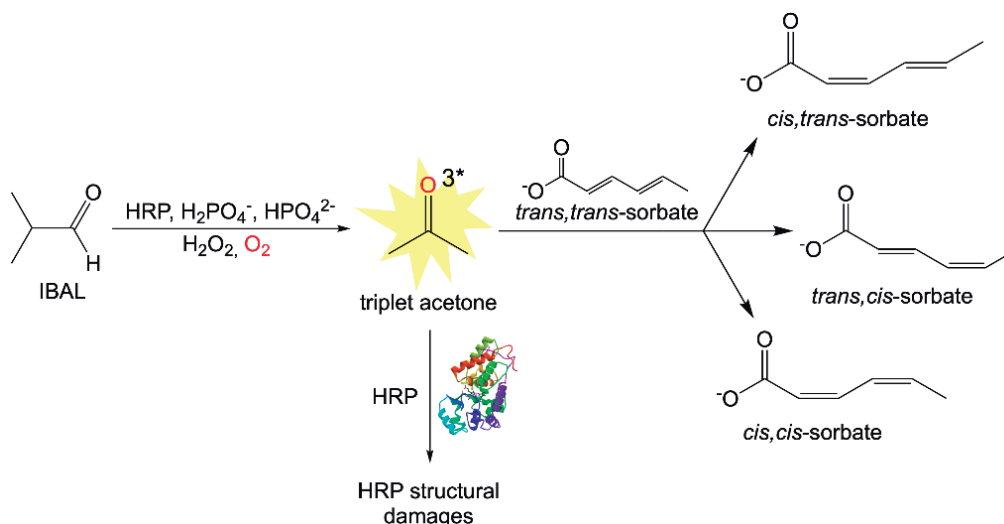


Fig. 1. Horseradish peroxidase (HRP)/isobutanal (IBAL)-generated triplet acetone mechanism, HRP structural damages and quenching of triplet acetone by sorbate, possibly generating *cis,trans* isomers.³

[1] BAADER, W. J. et al., Braz. Chem. Soc. 2015, 12, 2430-2447. [2] CATALANI, L. H. et al., Photochem. Photobiol. 1987, 45, 273-281. [3] VELOSA, A. C. et al., Chem. Res. Toxicol. 2007, 20 (8), 1162-1169.

Acknowledgments

The authors are grateful to FAPESP and CNPq for founding the research.