Scheme II

$$\begin{array}{c} \Big\uparrow \\ \Big\downarrow {\scriptstyle\text{fast}} \\ FeL_3^{2+*} + Q \overset{ET}{\rightleftarrows} FeL_3^{3+}\cdots Q^- \longrightarrow FeL_3^{3+} + Q^- \\ \Big\downarrow \\ FeL_3^{2+} + Q \end{array}$$
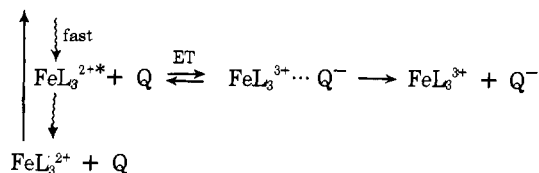
probability dependent on the ΔG of the electron-transfer reaction. This mechanism requires that the lifetime of the excited state which participates in the electron-transfer process be similar for the complexes with $E_{1/2} > 1.2$ V vs. Al. In any event, these lifetimes would be expected to be quite short (consistent with the absence of observable emission at room temperature).

Either of the above mechanisms can explain the data obtained, including the slope of approximately $-F/(2.3RT)$ in the region of $E_{1/2} > 1.2$ V vs. Al (broken straight line of Figure 2). Thus, for electron-transfer quenching of the excited state of the complex we can write

$$\Delta G_{ET} \text{ (eV)} = E_{1/2}(ML_3^{2+}/ML_3^{3+})$$
$$- E_{1/2}(Q^-/Q) - \Delta E_\infty$$

Taking the electronic excitation energy, ΔE_∞, to be 2.05 ± 0.1 eV and estimating the $E_{1/2}$ corresponding to $\Delta G_{ET} = 0$ as 1.15–1.20 V vs. Al, one finds $E_{1/2}(Q^-/Q) = -0.9 \pm 0.2$ V vs. Al. This value within the same uncertainty is obtained for ethylpyridinium using a linear correlation between $E_{1/2}$ for several compounds (11) in acetonitrile and in the molten salt medium, providing additional evidence for our identification of the pyridinium ion as the quencher in these systems.

Experiments involving irradiation of the complexes at the onset of the inverse charge-transfer band, which bear on the feasibility of the latter mechanism, are currently being elaborated.

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Helena Li Chum, D. Koran, R. A. Osteryoung*
*Department of Chemistry, Colorado State University*
Fort Collins, Colorado 80523

### Near-Ultraviolet Photooxidation of Tryptophan. Proof of Formation of Superoxide Ion

Sir:

Superoxide ion ($O_2^-\cdot$) plays a key role in a variety of diverse chemical processes, including a number which have biological significance.[1] Chemical[2] and biological[1,3] studies have implicated $O_2^-\cdot$ as a destructive agent in vivo, and recent speculation suggests an additional, beneficial, physiological role for this reactive species.[3,4] Thus, there is considerable interest in uncovering chemical processes which could give rise to $O_2^-\cdot$ in vivo. The results herein prove that $O_2^-\cdot$ (or $HO_2\cdot$)[5] is formed during the near-UV photooxidation of tryptophan (**1**) and indicate that this species has at least two fates, one of which is H_2O_2 formation.

Of the common amino acids, **1** is the most susceptible to photooxidation by near-UV (300–375 nm), owing to its conversion into N'-formylkynurenine (**2**), which is a particularly good near-UV (λ_{max} 320 nm) photosensitizer.[6] Elegant flash
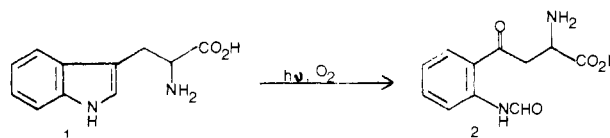
photolysis studies, in addition to revealing the role played by **2**, permitted the speculation that $O_2^-\cdot$ is formed during this process.[7] Recent work has shown that, in addition to **2** and other organic materials,[8] H_2O_2 is a major product formed upon near-UV photooxidation of **1**, even in the absence of added sensitizer,[9] and that it is H_2O_2 which is responsible for at least some of a number of biological activities associated with such photooxidation mixtures.[10] Related studies have revealed **2** to be an effective photosensitizer for nucleosides, and that H_2O_2 again is a major photoproduct.[7,11] It was therefore of interest to investigate the apparently general mechanism(s) which result in concomitant degradation of these biological materials and formation of H_2O_2, and in particular to investigate the possible role of $O_2^-\cdot$.

Superoxide dismutase (SOD)[12] can be used as a highly specific probe for the presence of $O_2^-\cdot$ (or $HO_2\cdot$) in chemical and biological reactions, owing to rapid ($k = 2 \times 10^9$ M^{-1} s^{-1}) reaction with this enzyme.[13]

$$2O_2^-\cdot + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$
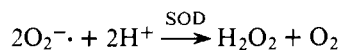
The complete absence of tryptophan residues in SOD lends it considerable resistance to near-UV and permitted its use in the present work to detect the photogeneration of $O_2^-\cdot$ (or $HO_2\cdot$) by the *enhancement* of H_2O_2 production. Figure 1 shows the amount[14] of H_2O_2 formed as a function of time during the photolysis[15] of an oxygenated, 0.03 M aqueous solution of **1** in the presence and absence of SOD (0.04 mg/mL[16]). Both at pH 6.0 and at pH 8.5 (0.01 M phosphate buffer), photolysis in the presence of SOD results in a marked increase in H_2O_2 formation, in spite of the fact that SOD converts into H_2O_2 only 50% of the $O_2^-\cdot$ with which it reacts. This not only provides definitive evidence for the photogeneration of $O_2^-\cdot$ (or
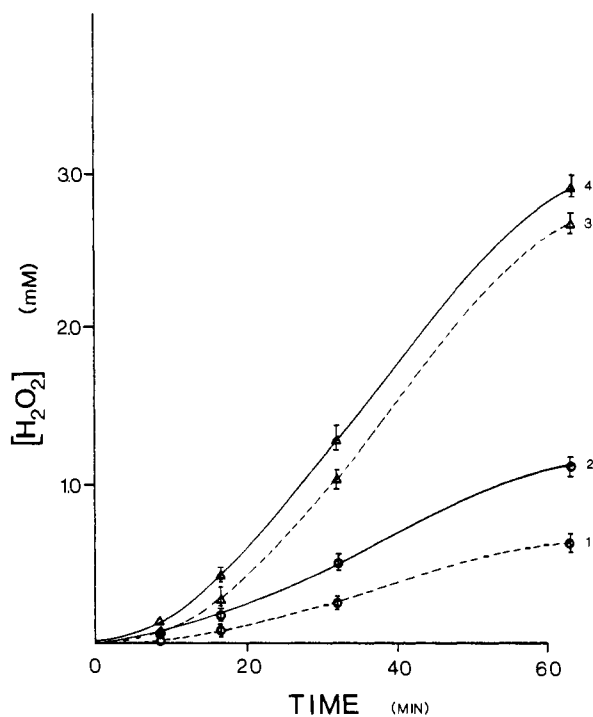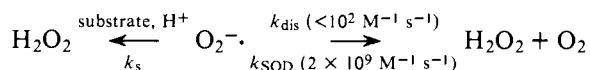
Figure 1. Formation of H_2O_2 during tryptophan (1) photooxidation in the presence and absence of SOD (0.04 mg/mL): curve 1, 1 alone, pH 6.0; curve 2, 1 alone, pH 8.5; curve 3, 1 + SOD, pH 6.0; curve 4, 1 + SOD, pH 8.5.

$HO_2\cdot$), but also indicates that only a portion of this species is transformed into H_2O_2 in the absence of SOD. These observations are consistent with the fates of $O_2^-\cdot$ shown, where k_s + k_{dis} account for at most 30% and 40% of $O_2^-\cdot$ reaction at pH values of 6.0 and 8.5, respectively.[17,18]

$$H_2O_2 \underset{k_s}{\overset{\text{substrate, } H^+}{\longleftarrow}} O_2^-\cdot \underset{k_{SOD} (2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1})}{\overset{k_{dis} (<10^2 \text{ M}^{-1} \text{ s}^{-1})}{\longrightarrow}} H_2O_2 + O_2$$

The formation of $O_2^-\cdot$ (or $HO_2\cdot$) presumably is mediated by 2,[7,19] a conclusion which is supported by the acceleration of the rate of H_2O_2 production in the early stages of the photolysis, followed by a decline in that rate as the reaction exhausts 1.[20] The extent of $^1O_2$ involvement in H_2O_2 formation can be estimated by the results of photolyses carried out in the presence of 0.05 M N_3^-,[21] which reduced the rate of H_2O_2 generation ~25 and 35% at pH values of 6.0 and 8.5, respectively, compared with the values obtained for 1 alone. Added N_3^- in photolysis mixtures containing SOD did not affect the amount of H_2O_2 produced.

The demonstration that $O_2^-\cdot$ is indeed formed upon near-UV photooxidation of 1 provides a basis for the synthesis of results acquired from experiments conducted separately concerning the chemical effects of $O_2^-\cdot$ and of near-UV on biological systems. Furthermore, the formation of $O_2^-\cdot$ and H_2O_2 together raises the possibility that this process can lead to the generation of the strongly oxidizing hydroxy radical.[2,22] These possibilities underscore the importance of further chemical and photochemical experiments to uncover the extent to which these results are applicable to in vivo processes stimulated by near-UV radiation, including the intriguing synergistic toxicity of near-UV and H_2O_2 to bacteria and bacteriophage.[23]

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J. P. McCormick,* Thomas Thomason

*Department of Chemistry, University of Missouri
Columbia, Missouri 65201*

Total Synthesis of (±)-Thienamycin

Sir:

Thienamycin (1, R = R' = R'' = H)[1] is a novel β-lactam antibiotic isolated from *Streptomyces cattleya*. Its unusually high potency against both gram-positive and gram-negative bacteria is quite surprising since the single 6-substituent is not only α but also lacks the traditional amide functionality. Of particular interest is its activity against *Pseudomonas* spp. and its resistance to bacterial β-lactamase.[2] Possibly the hydroxyl group can bind the same site normally bound by the 6β-amido group of the traditional β-lactam antibiotics when complexing with the bacterial cell wall enzymes, while the backbone of the 6α-substituent may mimic the 6α-methoxy group of the cephamycins to provide lactamase resistance. This unique and highly reactive compound offers a challenging synthetic problem, particularly the construction of the unusual ring