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RATE CONSTANTS FOR THE REACTION OF SINGLET OXYGEN WITH DERIVATIVES OF p-AMINOBENZOIC ACID

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SUMMARY

p-Aminobenzoic acid and its derivatives are often topically applied to prevent damage to the skin by actinic radiation. It has recently been suggested that reaction of these compounds with photochemically generated singlet oxygen may contribute to the protective effect. The total rate constants for interaction (reaction plus quenching) of several p-aminobenzoic acid derivatives with singlet oxygen have been determined in chloroform. The low rate constants for interaction, ethyl p-aminobenzoate, $8 \times 10^{4}\ M^{-1}s^{-1}$ ethyl p-N, N-dimethylaminobenzoate, $5.5 \times 10^{6}\ M^{-1}s^{-1}$, and p-N, N-dimethylaminobenzaldehyde, $1.1 \times 10^{6}\ M^{-1}s^{-1}$, indicate that interaction with singlet oxygen is not an important mode of protection for these compounds.

When topically applied, p-aminobenzoic acid and its derivatives provide protection against ultraviolet light-induced damage to the skin. Although these compounds are known to act as "sunscreens" because of their strong absorption in the ultraviolet region of the spectrum, it has recently been suggested that they may have an additional mode of action: reaction with actinically generated singlet oxygen, a highly reactive excited state of the oxygen molecule (1). p-Aminobenzoic acid, p-dimethylaminobenzoic acid, and ethyl p-dimethylaminobenzoate were destroyed when irradiated with hematoporphyrin sensitizer in non-degassed D_2O solution. The reactions were competitively inhibited by methionine, and their rates were decreased when H_2O was used in place of D_2O suggesting that singlet oxygen was responsible for destruction of the amines.

While the evidence is suggestive of the proposed mode of action, it is not conclusive since it has been shown that dye-sensitized amine oxidation can result from interaction of the amine with the sensitizer excited states as well as with singlet oxygen (2-6). A better measure of the efficiency with

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which p-aminobenzoic acid and its derivatives destroy singlet oxygen is the total rate constant for interaction between the amine and singlet oxygen. Rate constants for the reaction of singlet oxygen with a number of amines have been reported (2,3,7). Although no measurements have been reported for p-aminobenzoic acid and its derivatives, the values reported for a series substituted N,N-dimethylanilines (3) suggest that these values should be several orders of magnitude less than the diffusion rate. By the technique which has previously been used to measure the rate constants for the interaction of singlet oxygen with amines (7), olefins (8), sulfides (9), and nickel complexes (10), the rate constants for interaction of singlet oxygen with several derivatives of p-aminobenzoic acid have been measured.

MATERIALS AND METHODS

Rubrene, ethyl p-aminobenzoate, ethyl p-dimethylaminobenzoate, and p-dimethylaminobenzaldehyde were obtained from the Aldrich Chemical Company. Chloroform was obtained from Fischer Scientific.

Irradiations were carried out behind a black cloth to prevent exposure to UV light. Three ml samples of rubrene in chloroform, with and without added amine and all containing the same initial concentration of rubrene (0.8 mM, optical density at 546.1 nm ~4), were pipetted into 1-cm square Pyrex UV absorption cells. The samples were irradiated simultaneously on a merry-go-round (11) modified so that the turntable contained six 1-cm square holes. The 546.1 nm line of a Hanovia 679A36 450 watt medium pressure mercury vapor lamp was isolated by a combination of Corning C.S. 1-60, 3-68, and 4-72 filters. Six samples, two without amine and two each of two different amine concentrations, were irradiated at the same time. The samples were open to the air during irradiation to permit oxygen to diffuse in during reaction. Initial and final rubrene concentrations were measured spectrophotometrically at 440 nm on a Cary 14 spectrophotometer.

Interaction rate constants were calculated from the following equation (12):

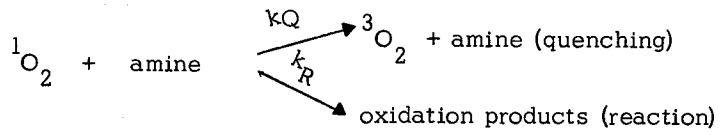
$$k = \frac{k_R ([R]_f^A - [R]_f^O) + k_d \ln ([R]_f^A / [R]_f^O)}{[A] \ln ([R]/[R]_f^A)}$$

when k_R is the rate constant for the reaction of $^1O_2$ with rubrene, k_d the rate constant for $^1O_2$ decay in chloroform, [R] the initial rubrene concentration, $[R]_f^A$ and $[R]_f^O$ the final rubrene concentrations in the solutions with and with-

out added amine, respectively, and [A] the amine concentration. Previously determined values for k_R ($5.3 \times 10^7 M^{-1}sec^{-1}$) and k_d ($1.7 \times 10^4 sec^{-1}$) were used (7). This method has been described in detail elsewhere (7).

RESULTS AND DISCUSSION

In the presence of an amine, singlet oxygen ($^1O_2$) may either be quenched to ground state oxygen ($^3O_2$) or react with the amine to yield oxidation products. As has been previously discussed (7), the technique used in



this study measures the total rate constant for removal of singlet oxygen by added amine, not how it removes it. The relative importance of the processes cannot be determined by this procedure. The measured rate constant is the sum of k_Q and k_R and represents the total rate constant for interaction of the amine with singlet oxygen. Since both processes destroy singlet oxygen, it is this overall rate constant which indicates if reaction of the amine with singlet oxygen might play a role in the protective effect. A low value for this overall rate constant would indicate that little if any of the protective effect arises from singlet oxygen destruction; a high value would indicate that singlet oxygen destruction is a possible additional mode of action.

The total rate constants for interaction with singlet oxygen were measured for three derivatives of p-aminobenzoic acid: ethyl p-dimethylaminobenzoate, p-dimethylaminobenzaldehyde, and ethyl p-aminobenzoate (Table 1). Although it was necessary to carry out these determinations in chloroform because rubrene is not soluble in methanol or water, the rate constant measured for p-dimethylaminobenzaldehyde is the same as the reported rate constant in methanol (3).

The overall rate constants for the two analogs of p-aminobenzoic acid, ethyl p-dimethylaminobenzoate, $5.5 \times 10^6 M^{-1}s^{-1}$, and ethyl p-aminobenzoate, $8 \times 10^4 M^{-1}s^{-1}$, are orders of magnitude below the rate for diffusion controlled reactions in chloroform, $\sim 1.1 \times 10^{10} M^{-1}s^{-1}$. In contrast, diamagnetic nickel

Table 1. Total rate constant for interaction of amines with singlet oxygen in chloroform.

Amine	Rate Constant ($M^{-1}sec^{-1}$)
Ethyl p-N,N-dimethylaminobenzoate	$(5.5 \pm 1.1) \times 10^6$
p-N,N-Dimethylaminobenzaldehyde	$(1.1 \pm 0.2) \times 10^6$ [a]
Ethyl p-aminobenzoate	$(8 \pm 2) \times 10^4$

[a] Literature: $(1.2 \pm 0.6) \times 10^6$ in methanol (Reference 2).

complexes, photostabilizers whose mode of action may include quenching of singlet oxygen, have quenching rates within a factor of ten of the diffusion rate (10). β-Carotene, which may function as a singlet oxygen quencher in biological systems, quenches at the diffusion controlled rate (13). This means that every collision of singlet oxygen with β-carotene destroys singlet oxygen while only about seven collisions out of a million of singlet oxygen with ethyl p-aminobenzoate destroy singlet oxygen.

While no mechanism for singlet oxygen mediated skin damage was proposed (1), it is known that singlet oxygen reacts with double bonds, such as those found in unsaturated fatty acids, and with certain of the amino acids in proteins. The rate constants for reaction of these substrates with singlet oxygen, disubstituted olefins, $\sim 8 \times 10^4 M^{-1}s^{-1}$ (14), histidine, $\sim 1 \times 10^8 M^{-1}s^{-1}$ (15), typtophane, $\sim 3 \times 10^7 M^{-1}s^{-1}$ (15), and methionine, $\sim 3 \times 10^7 M^{-1}s^{-1}$ (9,15,16), are about the same as or orders of magnitude larger than those measured for the p-aminobenzoic acid derivatives. It is unlikely that p-aminobenzoic acid could compete for singlet oxygen with these much more reactive substrates.

Since p- aminobenzoic acid and its N,N-dimethyl derivative are not soluble in chloroform, the measurements were carried out on their ethyl esters. While it is possible that the rate constants for the acids are considerably greater than those for the esters, the rate constants for singlet oxygen quenching for a series of substituted N,N-dimethylanilines have been reported to follow the Hammett equation (2,3). Since the substituent constant for the

ethyl carboxy group (0.45) is the same as that for the carboxy group (0.45) (17), it is unlikely that there is any large rate difference between the acids and their ethyl esters.

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