Quantitative Determination of Singlet Oxygen Generated by Excited State Aromatic Amino Acids, Proteins, and Immunoglobulins

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Singlet oxygen, \( ^1\text{O}_2 \), is a highly reactive electronically excited state of oxygen invoked in many physiological and pathological processes.\(^1\) While its generation in biological systems is mainly traced back to photosensitization by sunlight-absorbing cofactors and dark enzymatic pathways, \( ^1\text{O}_2 \) can also form by photosensitization with aromatic amino acids such as tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe), which are abundant light absorbers in the UV-B range (290 – 320 nm).\(^2\) Notably, though the role of \( ^1\text{O}_2 \) in the formation of \( \text{H}_2\text{O}_2 \) by in vitro UV irradiation of aromatic amino acids in immunoglobulins was reported by Wentworth et al.,\(^3\) the generation of \( ^1\text{O}_2 \) by aromatic amino acids and biological macromolecules has not been analyzed quantitatively.\(^4\) In this manuscript we report the quantum yields of \( ^1\text{O}_2 \) generation upon excitation with aromatic amino acids such as tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) in their zwitterionic forms, as methyl esters, and within a few test proteins and immunoglobulins.

The sensitized generation of \( ^1\text{O}_2 \) starts by excitation of the amino acid by absorption of UV light, followed by intersystem crossing to the triplet state (eqs 1 and 2), and is completed by energy transfer to oxygen in its triplet ground state (eq 3). The quantum yields of \( ^1\text{O}_2 \) can be obtained in air-saturated solutions by measuring the quantum yield of its near IR emission (eq 4 and Figure 1).

\[
\text{Tyr} + \hbar\nu \rightarrow ^3\text{Tyr} \\
^3\text{Tyr} \rightarrow ^3\text{Tyr} \\
^3\text{Tyr} + ^1\text{O}_2 \rightarrow ^3\text{Tyr} + ^1\text{O}_2 \\
^1\text{O}_2 + ^1\text{O}_2 + \hbar\nu(1270 \text{ nm})
\]

The amino acids Phe, Trp, and Tyr, the N-acetylated amino acids NAc-Phe, NAc-Trp, and NAc-Tyr, the proteins bovine serum albumin (BSA) and ovalbumin (OVA), and the immunoglobulins bovine-IgG, human-IgG, and sheep-IgG in \( \text{D}_2\text{O} \) or MeCN. Samples were irradiated with a 266 nm pulse from a frequency quadrupled continuum Nd:YAG laser (8 ns, 3 mJ). The near-IR luminescence from \( ^1\text{O}_2 \) was collected at right angles to excitation after focusing onto the variable slit of a SPEX 1681 monochromator and detected using a Hamamatsu PMT sensitive in the near-IR region.

The near-IR luminescence spectrum of singlet oxygen photosensitized by phenylalanine in an air-saturated \( \text{D}_2\text{O} \) solution is shown in Figure 1. There is a strong peak at about 1270 nm, characteristic of singlet oxygen phosphorescence. A time-resolved decay trace of the observed phosphorescence has a lifetime (\( \tau_x \)) of about 48 \( \mu \text{s} \), consistent with reported values of \( ^1\text{O}_2 \) in \( \text{D}_2\text{O} \). Solutions of tryptophan, tyrosine, bovine serum albumin (BSA), ovalbumin (OVA), the immunoglobulins bovine-IgG, human-IgG, and sheep-IgG in \( \text{D}_2\text{O} \), and the N-acetyl amino acids in MeCN were irradiated under similar conditions to emit spectra consistent with singlet oxygen luminescence. Argon-purged solutions of each compound showed no near-IR luminescence.

The quantum yields of oxygen (\( \Phi_\Delta \)) were determined using methylene blue in \( \text{D}_2\text{O} \) (\( \Phi_\Delta = 0.52 \)) or MeCN (\( \Phi_\Delta = 0.52 \)) as a reference.\(^5\) Tripletic kinetic analysis of decay traces for Tyr and Trp gave lifetimes for singlet oxygen of 35 – 40 \( \mu \text{s} \) in \( \text{D}_2\text{O} \), which are about 20% shorter than those observed with Phe. This may be attributed to quenching, in agreement with the high reactivity of singlet oxygen with these two amino acids. To ensure that singlet oxygen emission measured was generated by energy transfer from free amino acids and proteins in their native states, and not from photooxidation products, a fresh solution of each compound was used for acquisition after each laser shot.

Figure 1. Near-infrared luminescence of singlet oxygen sensitized by UV irradiation of phenylalanine in \( \text{D}_2\text{O} \). The emission spectrum shows a peak at about 1270 nm. Inset: Decay of singlet oxygen phosphorescence as a function of time (in \( \mu \text{s} \)), sensitized by phenylalanine. The black line is a curve fit of the decay.

The quantum yields of singlet oxygen emission are summarized in Table 1. The \( ^1\text{O}_2 \) quantum yields (\( \Phi_\Delta \)) for phenylalanine, tyrosine, and tryptophan (\( \Phi_\Delta^{\text{Trp}} = 0.065, \Phi_\Delta^{\text{Tyr}} = 0.138, \Phi_\Delta^{\text{Phe}} = 0.062 \)) are only a fraction of the corresponding quantum yields of triplet formation (\( \Phi_\Delta^{\text{Trp}} = 0.18, \Phi_\Delta^{\text{Tyr}} = 0.50, \Phi_\Delta^{\text{Phe}} = 0.40 \)). Given that the decrease in \( \Phi_\Delta \) with respect to \( \Phi_\tau \) is greater than the decrease in \( ^1\text{O}_2 \) lifetimes, we discount a significant amount of quenching by the original sensitizer.\(^6\) In fact, aromatic amino acids are known to undergo triplet state electron transfer, electron ejection, and photodissociation, all of which may contribute to the difference.

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Ovalbumin possesses only a small fraction (ca. 18%) of aromatic residues inferred by a close analysis of the structures of OVA and BSA. and the diffusivity of oxygen into the protein environment can be reduced by preventing it from accessing amino acids buried deep within the protein. In contrast, structural data from Human Serum Albumin, which is homologous to BSA, suggests a larger fraction (ca. 40%) of aromatic residues located on the surface, which can easily account for the observed quantum yield.

In conclusion, we have quantified the singlet oxygen quantum yields generated by excited-state aromatic amino acids tryptophan, tyrosine, and phenylalanine by time-resolved phosphorescence measurements. In addition, we have measured the singlet oxygen quantum yield from N-acetylated amino acids and from selected proteins and immunoglobulins. The three-dimensional conformation found in proteins and immunoglobulins results in decreased quantum yields. A crude analysis of the fraction of surface and internal residues in BSA and OVA suggests that molecular oxygen can diffuse through the polypeptide matrix and can be sensitized by residues buried within the folds of the protein structure. In agreement with previous tryptophan phosphorescence studies, singlet oxygen generation is hindered and limited by the increased viscosity of the protein matrix.

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Supporting Information Available: Experimental details and a detailed analysis of the number and location of aromatic residues in OVA and HSA. This material is available free of charge via the Internet at http://pubs.acs.org.

References


(12) For details, please see the Supporting Information.

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