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The role of singlet oxygen and oxygen concentration in photodynamic inactivation of bacteria

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Abstract:

New antibacterial strategies are required in view of the increasing resistance of bacteria to antibiotics. One promising technique involves the photodynamic inactivation of bacteria. Upon exposure to light, a photosensitizer in bacteria can generate singlet oxygen, which oxidizes proteins or lipids, leading to bacteria death. To elucidate the oxidative processes that occur during killing of bacteria, *Staphylococcus aureus* was incubated with a standard photosensitizer, and the generation and decay of singlet oxygen was detected directly by its luminescence at 1,270 nm. At low bacterial concentrations, the time-resolved luminescence of singlet oxygen showed a decay time of $6 \pm 2 \mu\text{s}$, which is an intermediate time for singlet oxygen decay in phospholipids of membranes ($14 \pm 2 \mu\text{s}$) and in the surrounding water ($3.5 \pm 0.5 \mu\text{s}$). Obviously, at low bacterial concentrations, singlet oxygen had sufficient access to water outside of *S. aureus* by diffusion. Thus, singlet oxygen seems to be generated in the outer cell wall areas or in adjacent cytoplasmic membranes of *S. aureus*. In addition, the detection of singlet oxygen luminescence can be used as a sensor of intracellular oxygen concentration. When singlet oxygen luminescence was measured at higher bacterial concentrations, the decay time increased significantly, up to $\approx 40 \mu\text{s}$, because of oxygen depletion at these concentrations. This observation is an important indicator that oxygen supply is a crucial factor in the efficacy of photodynamic inactivation of bacteria, and will be of particular significance should this approach be used against multiresistant bacteria.

Key-words: Luminescence, Oxygen depletion, *Staphylococcus aureus*