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# A proposed function for spermine and spermidine: Protection of replicating DNA against damage by singlet oxygen

(singlet oxygen quenching/polyamines)

AHSAN U. KHAN†, YING-HUA MEI‡, AND THÉRÈSE WILSON‡

†Department of Pathology, Harvard Medical School, Boston, MA 02115; and ‡Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

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ABSTRACT **Like all aliphatic amines, the polyamines spermine and spermidine are physical quenchers of singlet molecular oxygen ($^1O_2^*$). The rate constants of these processes were determined *in vitro* with photochemically generated $^1O_2^*$ and the hydrocarbon rubrene as substrate, in pyridine. At millimolar concentration, spermine and spermidine should quench $^1O_2^*$ *in vivo* and prevent it from damaging DNA. It is proposed that a biological function of polyamines is the protection of replicating DNA against oxidative damage.**

". . . spermidine and spermine are widely distributed in nature, but their function is not known with any certainty" (1). This sentence summarizes the status in 1991 of a problem that has been addressed in thousands of papers since Leeuwenhoek's discovery in 1677 of crystals of spermine phosphate in human semen (2), where spermine reaches the amazingly high concentration of 3.3 mg/g. It is known that polyamines (Table 1) slow down autoxidation processes (3–5) and thus inhibit oxidative damage caused by free radicals. We propose here that the primary function of polyamines is to protect DNA and RNA against singlet oxygen, $^1O_2^*$ ($^1\Delta_g$), a highly reactive and long-lived excited state of molecular oxygen. Tertiary and secondary amines are long known to be physical quenchers of singlet oxygen (6, 7). Chemical reaction is minimal, and the amines are therefore not destroyed in the quenching process. Spermidine and spermine (with one and two secondary amine groups, respectively; Table 1) are shown here to be no exception.

The recent paper of Balasundaram *et al.* (1) presents intriguing and unexplained results consistent with our proposal. These authors describe a mutant of *Saccharomyces cerevisiae* in which endogenous putrescine is present in normal levels, but the mutant is unable to synthesize spermine and spermidine and requires their exogenous addition for growth. However, this requirement holds *only* in aerobic conditions, not in an atmosphere of $N_2$/5% $CO_2$.

## Amine Quenching of Singlet Oxygen

The effectiveness of spermine and spermidine as *in vitro* quenchers of $^1O_2^*$ was determined by their ability to retard the self-sensitized photooxygenation of rubrene (5,6,11,12-tetraphenylnaphthacene), a bright-red hydrocarbon (R) that reacts rapidly with $^1O_2^*$ ($k_r = 4 \times 10^7\ M^{-1}\cdot s^{-1}$) (7) to form a colorless endoperoxide ($RO_2$). This reaction competes with quenching of $^1O_2^*$ by amine (A):

$$^1O_2^* + R \xrightarrow{k_r} RO_2$$

$$^1O_2^* + A \xrightarrow{k_q} A + {}^3O_2$$

Aerated pyridine solutions of rubrene (0.22 mM) were irradiated at 540 nm, and the concentration of rubrene was followed photometrically as a function of irradiation time, down to a concentration of rubrene of about one-half its initial concentration; this was repeated in the presence of different concentrations of amines in the millimolar range. Under these conditions, $k_o/k_A$, the ratio of the pseudo-first-order rate constant of decay of rubrene without amine to that with amine, can be treated in a Stern–Volmer fashion. The slopes of plots of $k_o/k_A$ vs. amine concentration is $k_q\tau$, where $\tau$ is the lifetime of $^1O_2^*$ in the absence of amine under the conditions of the experiment. These plots are linear up to ≈2 mM in the case of spermine and spermidine.

This procedure was used to determine $k_q\tau$ not only for spermine and spermidine but also for 1,4-diazabicyclo-[2.2.2]octane (DABCO), triethylamine, and diethylamine, three well-studied amines. The relative values are as follows: DABCO and $(C_2H_5)_3N$, 1; spermine, 0.5; spermidine, 0.4; $(C_2H_5)_2NH$, 0.2. The rate constants for $^1O_2^*$ quenching by spermine and spermidine, calculated on the basis of an average literature value (7) of $k_q = 2.5 \times 10^7\ M^{-1}\cdot s^{-1}$ for DABCO, are listed in Table 2. The order of $k_q$ was expected, since it reflects the known order of $k_q$ values of aliphatic amines (7): tertiary (1) > secondary (0.2) > primary (0.005). Amines quench $^1O_2^*$ by a charge-transfer process (6, 8); therefore, the lower the ionization potential, the better the quencher. Similar results were obtained in toluene solution with rose bengal as the sensitizer and irradiation at 560 nm.

## Biological Effects of Polyamines

In addition to the recent work with the yeast mutants, earlier studies with *Escherichia coli* mutants deficient in aliphatic polyamines clearly show the importance of these amines for growth (9, 10). In eukaryotes, an indication of their potentially crucial role is the fact that ornithine decarboxylase, the enzyme that catalyzes the first step of polyamine biosynthesis, is synthesized in a burst at the end of the $G_1$ phase, just before the S (synthesis) phase, and then is rapidly degraded (10). In mammalian cells, it has the fastest protein turnover rate of all eukaryotic enzymes.

There is no firm data on the cellular localization of polyamines *in vivo*, although the timing of the enzymatic synthesis of the polyamines and the high levels of ornithine decarboxylase certainly suggest a role for polyamines at the time of DNA synthesis. *E. coli* mutant studies indicate that polyamines affect the rate of movement of the DNA replicating fork (11). *In vitro* work shows a close association of polyamines with DNA, neutralizing at least in part its negative charges and stabilizing it (12, 13). Similarly, there is evidence for binding of polyamines to RNA (14) and an indication that polyamines increase the fidelity of translation

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Abbreviation: DABCO, 1,4-diazabicyclo[2.2.2]octane.

Table 1. Structures of some polyamines

| Amine | Structure |
|---|---|
| Putrescine | $NH_2$-$(CH_2)_4$-$NH_2$ |
| Spermidine | $NH_2$-$(CH_2)_3$-NH-$(CH_2)_4$-$NH_2$ |
| Spermine | $NH_2$-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-$NH_2$ |

(10). All this is consistent with the proposal that polyamines protect DNA and RNA against attack by $^1O_2^*$.

Many possible sources of $^1O_2^*$ in living cells have been identified, among them peroxidases (15, 16), dismutation (17) of superoxide ion $O_2^-$, and electron transfer from $O_2^-$ to metals (18, 19). As a result, singlet oxygen like polyamines is ubiquitous. The preferred site for $^1O_2^*$ attack on nucleic acids is the guanine residue. *In vitro*, the rate constant of the reaction with guanine has been estimated (20) at $5.3 \times 10^6$ $M^{-1}\cdot s^{-1}$ and that of $^1O_2^*$ with DNA at $5.1 \times 10^5$ $M^{-1}\cdot s^{-1}$. These rate constants are both smaller than that for quenching of $^1O_2^*$ by either spermine or spermidine. Therefore, these amines should afford protection to DNA, provided that they are strategically located near the site of $^1O_2^*$ attack, as is expected. Oxidation of guanine in DNA causes loss of transforming activity and mutagenesis, as well as some single-strand breaks (21–23).

In *E. coli*, the concentration of spermidine is reported to be 4.7 $\mu$mol/g of wet weight (24). On the assumption of $10^{12}$ cells per g of wet weight and a cell volume of 2 $\mu m^3$, one calculates a spermidine concentration, averaged over the whole cell, of $\approx$2 mM, well within the necessary range for DNA protection. Single-stranded DNA regions with exposed bases are the most likely targets of $^1O_2^*$ attack; if some of the spermidine were synthesized near the replication forks, the local concentration of amine could be much higher. Although putrescine (with only primary amine groups) is expected to be a weaker quencher, its presence may be sufficient to protect DNA in nonreplicating cells of the yeast mutant (1).

## Conclusions

The harmful effects of $^1O_2^*$ are, of course, not limited to nucleic acids. Its reactivity with amino acids as well as with lipids, leading to damage to cell membranes, is also well documented (25). In addition, it should be emphasized that $^1O_2^*$ is evidently not the only possible agent of oxidative damage. $O_2^-$, $H_2O_2$, and the extremely reactive free radical ·OH must all be considered (26). As mentioned earlier, several studies have shown that polyamines may also intervene in some of these reactions. For example, they retard free-radical autoxidation processes (3–5) and seem to moderate the toxicity of paraquat, a source of superoxide ion (27).

We believe that a case can be made for a role of polyamines in the protection of DNA against oxidative attack, as documented in a companion paper (28), and that this may be their primary function. One might speculate that, downstream, the presence of polyamines could then allow protein synthesis, once the integrity of DNA and RNA was ensured.

The present ideas could have implications for research in fertility.

Table 2. Rate constants, $k_q$, for $^1O_2^*$ quenching by amines

| Amine | $k_q$,† $M^{-1}\cdot s^{-1} \times 10^{-7}$ |
|---|---|
| $(C_2H_5)_3N$ | 2.5 |
| DABCO | 2.5 |
| Spermine | 1.2 |
| Spermidine | 1.0 |
| $(C_2H_5)_2NH$ | 0.5 |

†Calculated on the basis of an average literature value (7) of $2.5 \times 10^7$ $M^{-1}\cdot s^{-1}$ for DABCO.

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