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Photosensitized DNA cleavage promoted by amino acids†

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A novel class of DNA cleavage agents are reported that derive activity from amino acids tethered to a photoactive intercalator.

A variety of compounds are known that promote DNA strand scission upon photoactivation.[1] These agents either directly attack the DNA backbone or bases or indirectly facilitate strand cleavage through the photosensitization of singlet oxygen or the generation of other diffusible and reactive species.

We have characterized a new class of peptide–intercalator conjugates exhibiting a novel type of DNA photocleavage activity. The strand-scission chemistry requires the presence of specific amino acids. Previous efforts to develop intercalator-based probes utilizing the diverse chemistry of amino acids for novel DNA-binding reagents have yielded compounds with nuclease activity.[2] However, in most of the systems described to date, the intercalating moiety functions solely to deliver peptides with low intrinsic binding affinity to DNA and does not contribute to chemical reactivity. Here, we describe a family of conjugates that derive DNA cleavage activity from a reaction that requires both a photoexcited intercalator and appended amino acids.

Fig. 1 illustrates the series of compounds synthesized and tested for DNA photocleavage activity. The conjugates feature thiazole orange (TO), a fluorescent DNA intercalator,[3,4] conjugated to synthetic dipeptides through a linker attached to the quinoline nitrogen of the heterocycle. These compounds were prepared using standard solid-phase peptide synthesis and a carboxy-functionalized TO derivative.[5] The first TO–dipeptide conjugates that were investigated and compared with the parent compound featured either glycine (TO–GK), tyrosine (TO–YK), or tryptophan (TO–WK) intervening between a terminal lysine and TO. These compounds display DNA-binding affinities and fluorescence quantum yields that are comparable to the parent compound.[6]

Upon irradiation with visible light, the TO–WK conjugate efficiently cleaved supercoiled plasmid DNA (Fig. 2A), while TO–GK or underivatized TO did not produce significant levels of cleavage. Irradiation of DNA in the presence of a high concentration of a WK dipeptide or TO did not result in strand scission (Fig. 2B). These experiments indicated that the reaction observed required both TO and W. The involvement of both the intercalator and amino acid was confirmed by monitoring photocleavage in samples where TO and a W-containing peptide was introduced to plasmid DNA *in trans*.[7] DNA photocleavage was detected even in the absence of a covalent linkage between TO and W, indicating that only the presence of these two reactants and light was required for the chemistry to occur.

A TO–YK conjugate also produced DNA cleavage upon photoexcitation, although with lower efficiency than TO–WK (Fig. 2B). The observation of activity for TO–WK and TO–YK that was significantly higher than for TO–GK indicates that the aromatic amino acids form reactive species in the presence of the TO excited state that are not accessible with the aliphatic amino acid.

To obtain information about the origin of the amino acid dependent DNA cleavage activity, a series of experiments was conducted to test for the involvement of diffusible species generated during photoexcitation of TO (Fig. 3A). Photocleavage of plasmid DNA by TO–WK was monitored in the presence of superoxide dismutase (SOD), catalase, and mannitol to test for the involvement of superoxide or hydroxyl radicals. The addition of these agents did not significantly affect the cleavage efficiency.[8]

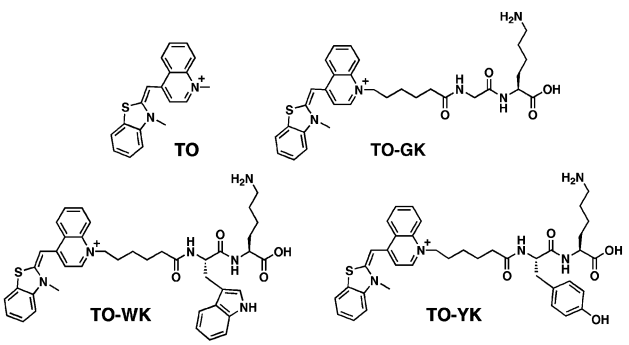

Fig. 1 Structures of TO and TO–dipeptide conjugates (see ESI for synthesis and characterization†).

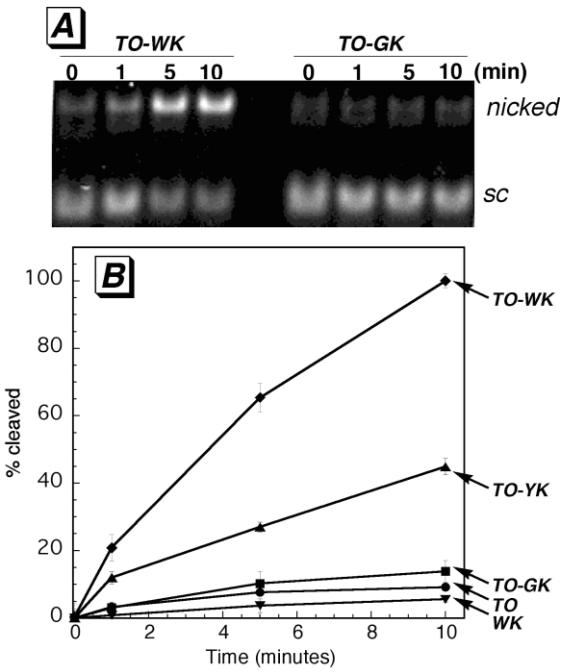

Fig. 2 A) Photocleavage of pUC18 plasmid DNA by TO dipeptide derivatives analyzed by agarose gel electrophoresis. Solutions contained 20 μM TO or TO–peptide conjugate, 75 μM (bp) pUC18, and 25 mM sodium phosphate (pH 7). B) Time dependence of photocleavage activity for TO, TO–dipeptides and a WK dipeptide.

† Electronic Supplementary Information (ESI) available: procedures for TO–peptide syntheses and experimental details for photochemical experiments. See http://www.rsc.org/suppdata/cc/b3/b306008j/

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To determine whether photogenerated singlet oxygen contributed to the DNA cleavage reaction, the effect of D_2O, NaN_3, and argon was investigated (Fig. 3A). The introduction of D_2O, a solvent that increases the lifetime of singlet oxygen,[9] increased the cleavage efficiency by over 50%. NaN_3, a singlet oxygen scavenger,[10] decreased the cleavage efficiency by $>65\%$. In addition, saturation of samples with argon before irradiation decreased the cleavage efficiency by $>90\%$. These results strongly suggested that singlet oxygen was involved in the DNA cleavage reaction.

Several features of the TO–peptide reactivity indicated that the cleavage mechanism was not a result of a direct reaction between $^1O_2$ and DNA. Singlet oxygen reacts with DNA, but typically generates base damage that requires alkaline or heat workup for strand scission.[11] The cleavage that is observed with the TO–peptide conjugates appears to involve direct strand scission, as no workup is required. Moreover, since the fluorophore responsible for generating $^1O_2$ is identical among the active and inactive TO–peptide conjugates, additional chemistry subsequent to the generation of $^1O_2$ must occur to impart DNA photocleavage activity to TO–WK and TO–YK but not TO–GK.

A subset of naturally-occurring amino acids is known to react with singlet oxygen to form peroxides.[12] Trp and Tyr efficiently react with $^1O_2$, with quenching rate constants (k_{tot}) of 3.2×10^7 and 0.5×10^7 $M^{-1}s^{-1}$, respectively.[13] Gly exhibits very low reactivity upon exposure to $^1O_2$, with $k_{tot} < 0.1 \times 10^7$ $M^{-1}s^{-1}$. The trend in the rate constants coincides with the DNA cleavage activity of the TO–peptide conjugates.

As shown in Fig. 3A, trolox, a peroxyl-radical scavenger,[14] significantly decreased the efficiency of DNA photocleavage by TO–WK (Fig. 3A). To test directly whether peroxides were formed upon irradiation of the TO–peptide conjugates, a modified FOX assay was employed.[15] This analysis showed significant levels of peroxide formation upon irradiation of TO–WK that increased when D_2O was introduced into the samples. These results are consistent with the production of amino-acid based peroxides formed by a reaction with $^1O_2$ generated by photoexcited TO. A previous report of DNA cleavage by thermally-generated peroxides provides a precedent for strand scission by this class of chemical species.[16]

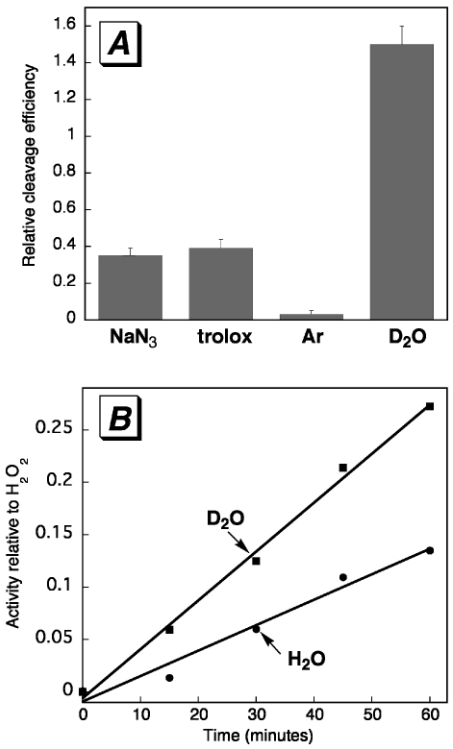

Fig. 3 A) Effect of different agents on photocleavage of DNA by TO–WK; 5 minute irradiations were perfomed as described to quantitate changes in efficiency relative to TO–WK in buffered H_2O under ambient conditions. B) Time dependence of Trp-based peroxide formation upon irradiation of TO–WK in 70% glycerol–30% X_2O measured using a modified FOX assay.

The DNA-binding peptide–intercalator conjugates described here exhibit DNA cleavage activity that appears to result from the reaction of $^1O_2$ with amino acids. Damage to protein side chains is proposed to be a potential source of the deleterious effects of $^1O_2$.[17] The discovery of a model system that permits the photogeneration of $^1O_2$ in proximity to reactive residues will facilitate studies of this damage pathway. Morever, TO–peptide conjugates will provide useful tools for analysis of the chemical reactions of amino acid-based peroxides with DNA.

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6 The K_d values corresponding to TO, TO–GK, TO–WK, and TO–YK bound to calf thymus (CT) DNA were 1.8 ± 0.1, 2.1 ± 0.1, 2.4 ± 0.3, and 2.9 ± 0.2 μM, respectively. The quantum yields of TO, TO–GK, TO–WK, and TO–YK bound to CT DNA were 0.11,[4] 0.20 ± 0.02, 0.16 ± 0.02, 0.22 ± 0.01. See ESI† for procedures and conditions used to obtain these values.

7 Solutions containing 20 μM TO, 200 μM KWK, and 75 μM bp pUC18 DNA exhibited cleavage yields that were ~50% relative to the covalent TO–WK conjugate. No cleavage was observed with TO + DNA or KWK + DNA after irradiation.

8 In the presence of 100 mM D-mannitol, 5 ng ul^{-1} SOD, 5 ng ul^{-1} catalase, or 5 ng ul^{-1} SOD and 5 ng ul^{-1} catalase cleavage yields did not vary more than 15%.

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