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INTERACTION OF RUTHENIUM COMPLEXES WITH NUCLEIC ACIDS. DNA DAMAGE VIA PHOTOSENSITIZED RADICAL PRODUCTION

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The binding of tris(2,2'-bipyridyl)- and tris(1,10-phenanthroline)ruthenium(II) ($Ru(bpy)_3^{2+}$ and $Ru(phen)_3^{2+}$, respectively) to double-stranded (ds) DNA or other nucleic acids has been characterized using absorption and luminescence spectroscopy and excited state lifetime kinetics.[1-3] Effectively all ruthenium complex ions (sensitizers) bind electrostatically to the nucleic acids at a [Nucleotide]/[Sensitizer] (N/S) ratio greater than 3.[2] For dsDNA a further contribution to binding comes from the partial intercalation of only one polypyridyl ligand between two DNA base-pairs. The other two ligands and the metal centre remain external to the DNA duplex in the major groove, preventing full intercalation of the inserted ligand. Most sensitizer cations are bound in this manner at N/S ratios greater than 10.[1,4] Partial intercalation depends on the size of the inserted ligand so that $Ru(phen)_3^{2+}$, for example, binds more strongly than $Ru(bpy)_3^{2+}$. The electrostatic component to binding is important since sensitizers can be released from the nucleic acid in the presence of relatively low concentrations of salt cations such as Na^+ or Mg^{2+} which compete for electrostatic binding sites.[4] The concentration of salt cations required for this release depends markedly on their valence, their size, on the sensitizer, and on the N/S ratio.

$Ru(bpy)_3^{2+}$ bound to nucleic acids such as polyuridylic acid (poly(U)) in the presence of $K_2S_2O_8$ can be used to sensitize the production of the reactive species[5] $SO_4^{\cdot-}$ and $Ru(bpy)_3^{3+}$ in close proximity to the strand.[3] These lead to single-strand break (ssb) formation as in the following reactions:

$$Ru(II) + S_2O_8^{2-} \xrightarrow{h\nu} Ru(III) + SO_4^{\cdot-} + SO_4^{2-} \quad (1)$$

$$poly(U) + SO_4^{\cdot-} + H_2O \rightarrow poly(U^{\cdot}OH) + SO_4^{2-} + H^+ \quad (2)$$

$$poly(U) + Ru(III) + H_2O \rightarrow poly(U^{\cdot}OH) + Ru(II) + H^+ \quad (3)$$

$$poly(U^{\cdot}OH) \rightarrow ssb\ formation \quad (4)$$

Here Ru(II) and Ru(III) denote $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{3+}$, respectively, and poly($U^{\cdot}OH$) refers to a base OH-adduct radical which is probably formed by hydrolysis of a base radical cation, in turn formed by attack of $SO_4^{\cdot-}$ or Ru(III) on a base. H-abstraction from a sugar by a base OH radical leads to heterolytic decay of the sugar radical and strand breakage.[6]

The formation of ssb in poly(U) was measured by monitoring the release of counterions resulting from strand breakage,[7] using transient conductivity, and the disappearance of Ru(III) was measured by monitoring spectral changes, following either flash photolysis or rapid mixing (Figure 1). The rates of the slower conductivity increase (due to ssb) and of Ru(III) disappearance are virtually identical at N/S = 10

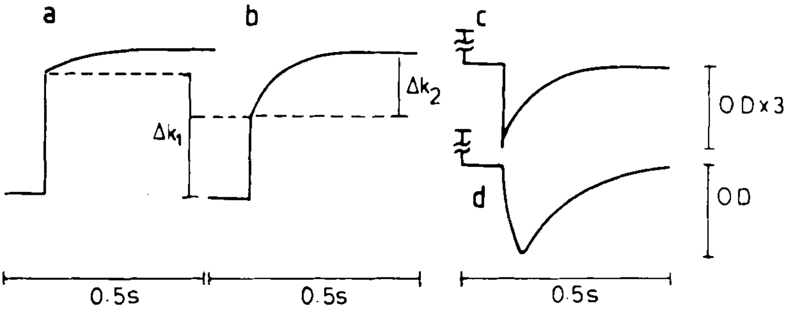

FIGURE 1 Transient conductivity increase (a) without and (b) with poly(U) and (c) transient absorption changes at 452 nm for the Ru(bpy)$_3^{2+}$/$S_2O_8^{2-}$/poly(U) system (0.05 / 1 / 0.5 mM) following the laser pulse (λ_{exc} = 353 nm); (d) transient absorption changes at 452 nm following rapid mixing of Ru(III) ($\simeq$ 0.025 mM) with poly(U) (0.5 mM).

(Table 1). This points to a reaction of Ru(III) either with bases or base radicals as the rate limiting step.

The Ru(bpy)$_3^{2+}$/$S_2O_8^{2-}$/nucleic acid system has also been used to study cleavage in ds plasmid DNA (pBR322) by observing the transition from form I to form II (ssb formation) and to form III (double-strand break) and its biological deactivation (bd) when transformed into *E. coli* bacteria (Table 2).[8] Φ_{ssb} and Φ_{bd} increase linearly with $S_2O_8^{2-}$ concentration, are approximately 2.5 times smaller in aerated than in argon saturated solutions, and are drastically reduced on addition of mono- and divalent salts. At N/S = 18, about 33 SO_4^{-} radical anions are required per one lethal event. Φ_{ssb} and Φ_{bd} are three times smaller when Ru(bpy)$_3^{2+}$ is replaced by Ru(phen)$_3^{2+}$.

TABLE 1
Transient conductivity and absorption data for the Ru(bpy)$_3^{2+}$/$S_2O_8^{2-}$/poly(U) system[7]

N/S	$\Delta\kappa_1^a$	$\Delta\kappa_2^a$	$k_{\Delta k}^b$	$k_{LF(s^{-1})}^c$	k_{RM}^c
0	1.0	0.3	< 1		
3	0.9	1.3	4.5	6.7	6.7
10	0.6	0.6	14.3	14.2	11.7

(a) $\Delta\kappa_1$ and $\Delta\kappa_2$ are the respective amplitudes of the fast and slow conductivity increases (Figure 1) normalized against ΔK_1 at N/S=σ (b) the rate of slow conductivity increase; (c) the rates of Ru(III) disappearance after laser flash (LF) and rapid mixing (RM). [Ru(bpy)$_3^{2+}$] = 50 μM and [$K_2S_2O_8$] = 1 mM.

TABLE 2
Quantum yields for ssb formation, Φ_{ssb}, and biological deactivation, Φ_{bd}, of plasmid DNA[8]

N/S	Condition		Φ_{ssb}	Φ_{bd}
3	Argon		0.0052	0.0040
3	Air		0.0020	0.0014
6	Argon		0.0064	0.0052
9	Argon		0.0060	0.0048
18	Argon		0.0084	0.0076
18	Air		0.0036	0.0032
3	Argon	$NaClO_4$ (0.02 M)		0.0007
3	Argon	$MgCl_2$ (0.02 M)		0.0005

[Ru(bpy)$_3^{2+}$] = 7 μM, [$K_2S_2O_8$] = 0.5 mM, [DNA] = 20–120 μM.

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