Enhanced DNA Binding and Photocleavage Abilities of β-Cyclodextrin Appended Ru(II) Complex through Supramolecular Strategy

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ABSTRACT: Photosensitizers with high photocleavage ability are urgently needed to improve photodynamic therapy efficacy. Herein, a supramolecular complex was constructed through host–guest self-assembly using hexa-β-CD-appended ruthenium polypyridyl (6CD-Ru) and adamantane-modified anthracene (ADA-AN) in water. The targeted DNA-intercalation of peripheral anthracenes can remarkably enhance photocleavage ability and antitumor activity of the complex irradiated with visible light.

Photodynamic therapy (PDT), a light-activated chemotherapeutic treatment, has emerged as a more effective and safer approach toward cancer therapy. This treatment utilizes the excited state photosensitizer (PS) to generate cytotoxic reactive oxygen species (ROS) including singlet oxygen \( ^1\text{O}_2 \) through energy transfer to the \( ^3\text{MLCT} \) state and large \( ^1\text{O}_2 \) yields, these compounds have been intensively applied as cytotoxic agents in PDT pathways. Winkel and Yu Liu have found that anthracene could enhance the PDT reactivity of Ru(II) complexes. However, owing to their limited water solubility, these complexes usually possess no more than two polycyclic aromatic, which hinder the further advance for PDT.

Supramolecular chemistry provides a facile and rapid procedure to construct complicated molecular assemblies through noncovalent interactions. Of all macrocyclic host molecules, cyclodextrins (CDs) have attracted great attention for their natural availability, benign water solubility, biological compatibility, and low toxicity. A number of host–guest supramolecular systems based on CDs have been reported for applications in biomedical science. Very recently, Mao’s group have constructed tumor-targeted metallo-anticancer agents using β-CD modified ruthenium-complex and adamantane-functionalized peptide through inclusion interaction.

With the goal of targeted binding, then photocleavage toward DNA, herein, we presented a supramolecular strategy to construct a complex using hexa β-CD appended ruthenium polypyridyl (6CD-Ru) with adamantane-modified anthracene (ADA-AN) and investigated its abilities toward DNA binding and photocleavage (see Scheme 1). The constructed complex possesses inherent advantages as (1) the good water solubility through the appended CD moieties and the formation of the host–guest complexes between adamantane and CD, and (2) the six anthracene groups jointly enhance the DNA binding.
strength, then facilitate the photocleavage ability of the (Ru) core when irradiated with visible light.

6CD-Ru and ADA-AN were obtained according to our previous reports.20,21 Through the strong host–guest interaction between β-CD and adamantane group, \( K_S = 8 \times 10^4 \text{ M}^{-1} \),21 we constructed a water-soluble supramolecular complex by mixing 6CD-Ru and 6 equiv ADA-AN in H2O. The fluorescence titration data were analyzed to estimate the binding constant \( (K_b) \) of the complex toward ctDNA. As shown in Figure 1, the fluorescence intensity of anthracene decreases obviously with increasing concentration of ctDNA, indicating the intercalative binding of the anthryl group into the DNA helix.22 The \( K_b \) value was obtained as 2.5 \( \times 10^5 \text{ M}^{-1} \) according to the Stern–Volmer equation.23 By monitoring the absorption in UV–vis titration experiment (Figure S1), the \( K_b \) value was estimated to be 8.6 \( \times 10^5 \text{ M}^{-1} \) (Figure S2), which is in reasonable agreement with the result from fluorescence titration within the experimental error, lending credibility to these measurements. \( K_b \) value of the complex is one order greater than monomer ADA-AN22 and other anthracene derivatives reported in the literature.15,22 This can be attributed to the six anthracene units connected on one core (Ru), which could enhance the binding ability through multiple binding sites toward DNA. The complex is hence expected to enhance photocleavage ability.

More convincing evidence for the interaction mode of the complex with ctDNA comes from the displacement experiments using ethidium bromide (EB), a fluorescence dye that can intercalate with DNA duplex. As shown in Figure S3, with the addition of the complex to the EB/ctDNA solution, the EB fluorescence was obviously quenched, possibly because the intercalated EB was excluded out by the anthryl group in the complex.24 This phenomenon confirms the intercalation binding mode of the complex toward DNA.

Circular dichroic (CD) spectral techniques can reveal how the conformation of the DNA chain is affected by the bound complex. As shown in Figure 2, the CD spectrum of free ctDNA is composed of two major peaks, one negative peak at 247 nm attributed to helicity and one positive peak at 280 nm ascribed to base stacking. These characteristic spectra are consistent with double helical DNA in a right-handed B form.21 The changes of these signals were observed in the presence of the complex. As shown in Figure 2, the positive band split into two, one at 258 nm with greater intensity and one at 274 nm with decreased intensity. The greater intensity could be ascribed to a partial B to A-DNA conformational transition, whereas the decreased intensity is due to base destacking as anthracene intercalation. Similar spectral changes were reported when intercalation occurred.25,26 The more winding helix of A-DNA is reflected in the enhanced intensity of the negative band at 247 nm. These significant changes in the conformation of ctDNA indicate strong DNA-intercalation ability of the complex.

Agarose-gel electrophoresis was used to monitor the conversion of pBR322 DNA with different molar concentrations of the complex. Control runs (Figure 3, Lane 1) suggested that cleavage of pBR322 DNA in the absence of complex was not significant, where the plasmid was mainly in the supercoiled form (form I) with only a small amount of nicked impurity (form II). After keeping the mixtures under irradiation at 450 nm for 1 h, the complex showed concentration-dependent cleavage abilities (Lanes 2–5). As displayed in Figure 3, supercoiled DNA could be completely photocleaved in the presence of 2.7 \( \mu \text{M} [\text{Ru}] \) ([ADA-AN] =...
communication as 1O2 scavengers, DMSO and NaI as hydroxyl radicals (shown in Figure 4, obvious inhibitions were observed in the dark for 1 h in each case. Lanes 6–10, solutions with concentrations corresponding to 1–5 but kept in the dark for 1 h in each case.

16.2 μM). The gradual loss of form I with no increase in form II in lanes 4–5 is due to the presence of DNA fragments after photocleavage, which is smaller than form III DNA and difficult to detect in the agarose-gel electrophoresis.27,28

In the dark control experiments, no obvious DNA cleavage was observed, whether or not it was treated with the complex (lanes 6–10). In the parallel control experiments, no clear DNA cleavage was observed even at 27 μM of monomer 6CD-Ru (Figure S4), and complete photocleavage was not observed until 112 μM of monomer ADA-AN upon irradiation (Figure S5). Compared with monomer compound 6CD-Ru and ADA-Ru (Figure S4), and complete photocleavage was not observed of magnitude toward supercoiled DNA. The large di

In attempts to unravel the probable mechanisms of the complex toward DNA photocleavage, control experiments were conducted through addition of various scavengers of the reactive oxygen. Sodium azide (NaN3) and L-histidine could act as 1O2 scavengers, DMSO and NaI as hydroxyl radicals (·OH) scavengers, superoxide dismutase (SOD) as superoxide anion radical (O2⁻), and catalase as H2O2 scavengers, respectively. As shown in Figure 4, obvious inhibitions were observed in the presence of NaN3, NaI, and to a lesser extent by l-histidine, DMSO scavengers. These findings suggest that 1O2 and ·OH together act as ROS in light-induced DNA cleavage. Systems exhibiting multiple reactivities are desirable for PDT agents in order to improve PDT efficacy.29

To further confirm the formation of ROS in the complex system, EPR spin-trapping experiments were carried out in the presence of DMPO or TEMP. Upon irradiation at 450 nm, the characteristic EPR spectra for the DMPO-OH adducts30 with a 1:2:2:1 quadruple signal with \( a_N = a_H = 14.9 \) G were observed in Figure 5a. In addition, a 1:1:1 triplet characteristic signal of TEMPO (with \( a_N = 16.0 \) G) was observed in Figure 5b, attributed to the adduct of 1O2 and TEMP.31 These resulting paramagnetic products confirm the production of 1O2 and ·OH in the system.

To investigate the specific cellular targeting of the complex, the cellular uptake was confirmed by colocalization assays. The confocal microscopy images in Figure 6 reveal that the complex overlapped well with the commercial nuclear dye acridine orange (AO) in human lung carcinoma A549 cells. This result demonstrates that the complex can effectively penetrate the nuclear membrane and are mainly accumulated within the nuclear.

A549 cell was employed as the model cell to evaluate the in vitro cytotoxicity of the complex. The dark and light cytotoxicity profiles are given in Figure 7. After incubation with the complex in the dark for 24 h, the dose-dependent antitumor activity was clearly observed. A possible reason may be that the specific intercalation of anthracene units on the complex toward the DNA, as B to A-DNA conformational changes (Figure 2) could prevent DNA duplication and transcription to mRNA.26,32 After irradiation at 450 ± 5 nm for 10 min, the complex displayed better anticancer activity, as the relative cell mortality rate could be dramatically enhanced over that without irradiation. The phototoxic complex could annihilate 95% of the cancer cell population at concentration 8 μM after PDT treatment. The half maximal inhibitory concentration (IC50) after PDT treatment was determined to be as low as 2.8 μM (Figure S6), which is considerably lower compared to photofrin,33 cisplatin,34 and other ruthenium base photosensitizers, indicating greatly enhanced cellular toxicity of the complex.

The production of intracellular ROS by the complex under irradiation was probed using ROS probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA). Upon DCFH-DA being oxidized...
to 2',7'-dichlorofluorescin (DCF) by ROS, it could be converted from a nonfluorescent to highly fluorescent state. Flow cytometric analysis (Figure S7) reveals a concentration-dependent fluorescent increase of DCF in A549 cells treated with the complex, which demonstrates that the enhanced cell death in Figure 7 induced by the complex under irradiation is mediated by ROS. Based on the above results, we speculated that both the intercalated binding toward DNA and the production of ROS under irradiation induce antitumor activity of the complex.

In summary, benefiting from the highly noncovalent binding, a promising DNA photocleavage supramolecular complex was successfully fabricated using 6CD-Ru and ADA-AN in aqueous solution. This complex can be accumulated in the nuclei of cancer cells. The presence of six anthracene intercalative groups in one assembly increased the affinity of the complex for DNA; thus, targeted delivery of ROS to the DNA was achieved. Consequently the supramolecular assembly exhibited excellent photoinduced DNA cleavage activity and efficient anticancer activity. This strategy presents a new opportunity for the construction of highly efficient photosensitizer in photodynamic therapy.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.8b00191.

Instrumentation, methods, and characterization (PDF)

REFERENCES

Bioconjugate Chemistry


