DNA topoisomerase I changes the mode of interaction between camptothecin drugs and DNA as probed by UV-resonance Raman spectroscopy

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Abstract Pronounced differences of interactions of camptothecin (CPT) and its derivative 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT11), inhibitors of DNA topoisomerase I, with oligonucleotides were found using UV resonance Raman spectroscopy. 30-mer oligonucleotides were derived from the sequences of the topoisomerase I-induced and CPT-enhanced cleavage sites in SV40 DNA. CPT induces well-defined alterations of the oligo structure, whereas CPT11 interacts with oligonucleotides more weakly and in another manner than CPT. Formation of cleavable ternary complexes between CPT11, topoisomerase I and oligonucleotides causes CPT11 to interact with oligonucleotides in the same fashion as was found for its parent compound CPT, and enhances this interaction as compared to CPT-oligonucleotide complexes. The data present evidence of molecular interactions of CPT11 with both other partners (topoisomerase I and oligonucleotide) of the ternary cleavable complex at the oligonucleotide-enzyme interface.

Key words: Camptothecin and analog; Topoisomerase I; Cleavable ternary complex; Topoisomerase I inhibitor; Anti-tumor action; Resonance Raman spectroscopy

1. Introduction

Camptothecin (CPT) isolated from the Chinese tree *Camptotheca acuminata* (Fig. 1) and several recently synthesized CPT derivatives are potent antitumor agents [1]. Cytotoxicity of camptothecins was shown to be related to their inhibition of DNA topoisomerase I (topo1) by stabilizing DNA-drug-enzyme ternary, so called cleavable, complexes and preventing in this way the ligation step of the enzyme action [2–5]. Mechanism of formation and structure of these cleavable complexes are currently under intense investigation [6]. CPT is not a DNA intercalating drug, but it is suggested that its quasi-planar multiring system binds by stacking with the guanine at the 5′-terminus of the DNA breaks produced by topo1 [7]. Among other factors the presence of hydrolyzable α-hydroxy-δ-lactone ring is considered to be an important factor of CPT functional activity [5,8], whereas the carboxylate form of CPT (Fig. 1) is biologically inactive and potentially toxic [9]. Up to now the studies were concentrated on the parent compound CPT, and practically nothing is known about the features of interactions of CPT derivatives in the course of formation of ternary complexes. Here we report the first attempt to elucidate some of these features for one of the CPT derivatives, CPT11 (Fig. 1), which is the most advanced in clinical research [1]. Our studies were performed using 30-mer oligonucleotides (Fig. 1) derived from the sequences of the topo1-induced (oligo(−)) and CPT-enhanced (oligo(+)) cleavage sites in SV40 DNA [10]. Cleavage of both oligonucleotides by topo1 was shown to occur with similar intensity and at the same position as in SV40 DNA [7]. An enhanced cleavage of oligo(+) and weaker cleavage of oligo(−) by topo1 were observed in the presence of CPT and found to be in qualitative agreement with the cleavage pattern induced by the drug in SV40 DNA [7,10]. Though a unique cleavage pattern was reported for CPT and its analogs [5], the data presented in this paper give evidence that substitutions (at least for CPT11) affect strongly the mode of the drug interaction with oligonucleotides. The results obtained provide proof for the interaction of CPT11 with both topo1 and oligonucleotide at the oligonucleotide-enzyme interface of the ternary cleavable complex.

2. Materials and methods

Oligonucleotides (Fig. 1) were purchased from Eurogentec (Belgium). Calf thymus topo1 (25 units/μg) and CPT11 were supplied by Rhone-Poulenc Rorer, S.A. CPT was purchased from Sigma. All other chemicals were of analytical reagent grade and all solvents were of HPLC grade.

One unit of topo1 was defined as described earlier [11]. To obtain duplex oligonucleotides the annealing procedure was performed as described elsewhere [7]. Complexes and reaction mixtures of CPT or CPT11 with oligonucleotides, CPT11 with topo1 and oligonucleotides as well as oligonucleotide with topo1 were prepared by mixing the components in 40 μl of the reaction buffer (20 mM Tris-HCl (pH 7.0), 60 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, and 0.5 mM dithiothreitol) at concentrations of 10 μM of the enzyme, 20 μM of the

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Abbreviations: topo1, DNA topoisomerase I; CPT, camptothecin; CPT11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; UV RR, ultraviolet resonance Raman; oligo(+), oligomer d(5′-CAAAGTCAGGTTGATGAGCATATTTTACTC-3′)$_2$; oligo(−), oligomer d(5′-CAAAGTCAGGTTGATTAGCATATTTTACTC-3′)$_2$

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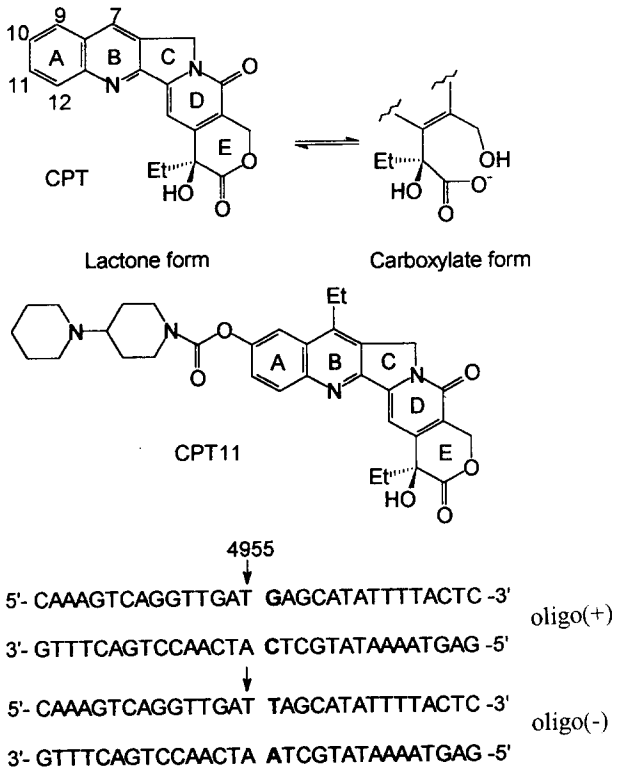

Fig. 1. Chemical structures of CPT and CPT11. Base sequences of the studied oligonucleotides. Position of topo1-induced DNA cleavage in SV40 DNA and altered nucleotide bases are marked.

oligonucleotide and 20 or 40 μM of the drug. After mixing the complexes were incubated at 20°C for 15 min, and then placed in the cell for UV RR measurement. Preparing a complex a stock solution of 15 mM CPT or CPT11 in DMSO was pre-diluted in the buffer at 4°C and immediately mixed with the other components in the necessary proportion. At the conditions used (pH 7.0, $t = 20$°C) kinetics of hydrolysis of the lactone ring of CPT or CPT11 was very slow (rate constant $< 0.48\ h^{-1}$ [12,13]), and the drug was in lactone form during the spectrum recording. Carboxylate form of CPT11 was prepared by pre-diluting the drug in the buffer (pH 7.0, $t = 20$°C) and storing it for 12 h before addition to the reaction mixture.

The excitation of Raman spectra at 257.27 nm (5 mW) was performed with a Spectra Physics Ar^+ cw laser (model 2025-3) equipped with a home-made intracavity second harmonic generation system. UV RR spectra were accumulated with resolution ca. 8 cm^{-1} using the spectrograph module of a multichannel XY-UV spectrometer (Dilor, France). The sample solution (40 μl) was continuously stirred in a small quartz cell (3×3 mm) with a Hellma magnetic stirrer (model CUV-O-STIR 333) supplied with a small (2 mm) stir bar to avoid photodegradation during measurement. Comparing three consecutive records (an average of 16 accumulations at 10–15 s integration time) the probable time-dependent and irradiation-induced spectral changes were evaluated. Three independent experiments were performed to test the reproducibility of the results. The 3400 cm^{-1} band of water was used as internal standard of intensity to normalize and compare the spectra as well as for simulation of the spectra of complexes by combination of the spectra of individual components.

3. Results and discussion

3.1. General description of the UV RR spectra of oligonucleotides, topo1 and drugs

Comparing relative intensities of the UV RR spectra of oligonucleotides, topo1, CPT11 and CPT (Fig. 2, I), the contribution of each component to the spectra of binary and ternary complexes was evaluated. The dominance of the oligonucleotide signals was observed due to the strong resonance of the excitation wavelength with electronic transitions of the nucleotide bases. Vibrational modes of deoxyribose and phosphate backbone being out of resonance did not contribute to the UV RR spectra of the oligonucleotides. The very similar

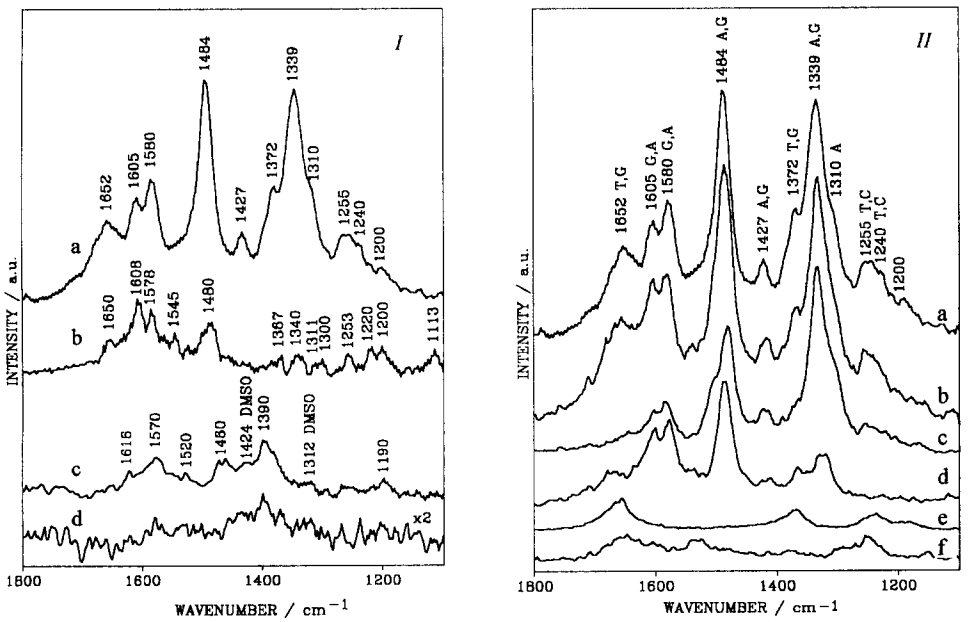

Fig. 2. I: UV RR spectra of oligo(+) (a), topo1 (b), CPT11 (c), and CPT (d). The spectra of oligo(−) and oligo(+) are very similar. The signal of water has been subtracted. Fluorescence background was subtracted from the spectra of the enzyme and drugs. The same scale of intensity was used for all spectra except for the CPT one to show the relative intensity of the spectra. [oligo(+)] = 20 μM, [CPT11] = 20 μM, [CPT] = 20 μM, [topo1] = 10 μM. II: UV RR spectrum of oligo(+) (a). Simulated spectrum of oligonucleotide (b) obtained as the sum of UV RR spectra of dAMP (c), dGMP (d), dTMP (e), dCMP (f). The same scale of intensity was used to show the relative contribution of each type of nucleotide in the overall intensity of the oligonucleotide spectrum.

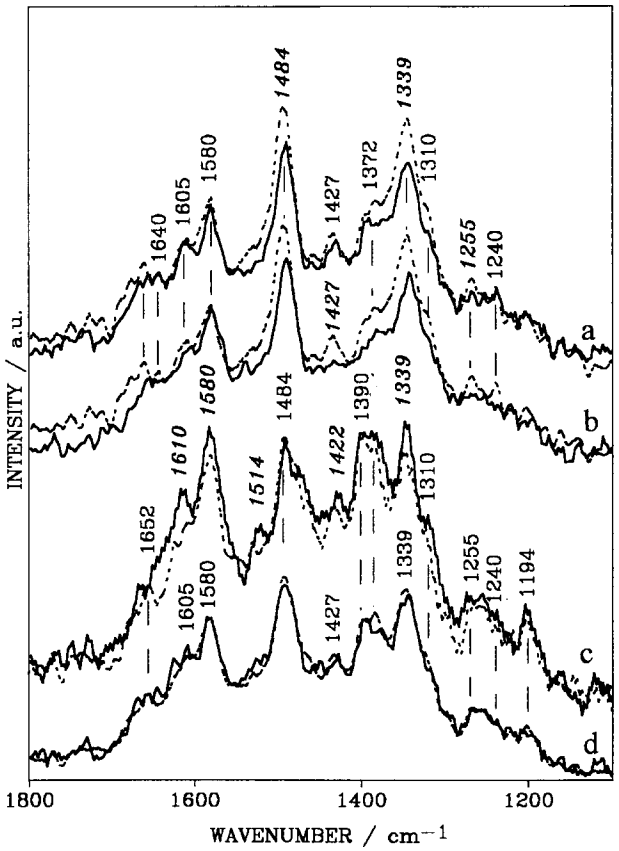

Fig. 3. Solid lines: UV RR spectra of CPT-oligo(−) (a), CPT-oligo(+) (b), CPT11-oligo(−) (c) and CPT11-oligo(+) (d) reaction mixtures. Dashed lines: simulated spectra of the corresponding mixtures obtained as the sum of normalized UV RR spectra of pure components. Italics are used to mark the bands affected by complex formation. The signals of water and fluorescence background have been subtracted. For spectra (a), (b) and (d): [CPT] = 20 μM, [oligo(+)] = 20 μM, [oligo(−)] = 20 μM, [CPT11] = 20 μM. For spectrum (c): [CPT11] = 40 μM, [oligo(−)] = 20 μM. See text for details.

UV RR spectra were recorded for both duplex oligonucleotides because their sequences differed only in two nucleotide bases. Simulating the spectrum of oligonucleotide by superposition of the deoxynucleotide spectra (Fig. 2, II), the vibrations of adenine, guanine, thymine and cytosine bases were concluded to contribute to the oligonucleotide spectrum ca. 45%, 35%, 11% and 9% of integral intensity, respectively. The contribution of cytosine was very weak at any excitation in the region of 240–300 nm due to its small Raman cross-sections as compared with other nucleotides [14]. The intensities of the thymine bands were found to be reduced markedly due to a stacking induced hypochromism within double-stranded helixes [15,16]. A tentative assignment of the oligonucleotide bands is shown in Fig. 2, II.

The UV RR spectrum of topo1 (Fig. 2, I,b) consists of the bands of aromatic amino acid residues and amide bands of the polypeptide backbone [17]. CPT and CPT11 exhibit a strong electronic transition at 254 nm, thus enabling detection of their spectra at low concentrations when the 257 nm excitation is used (Fig. 2 I,c,d). The spectrum of CPT is weaker than the CPT11 one. The UV RR spectra of lactone and carboxylate forms of the drugs were found to be similar leading us to the conclusion that vibrational modes of α-hydroxy-δ-lactone ring were weakly coupled with the 254 nm electronic transition of the drugs.

3.2. The UV RR spectra of the oligonucleotides in the presence of the drugs

On adding CPT to oligonucleotides the UV RR spectra of both oligo(−) and oligo(+) were found to be strongly perturbed (Fig. 3a,b) clearly indicating the occurrence of interactions between the drug and the oligonucleotides. A decrease in the intensity of the 1484 cm^{-1} (A, G) and 1339 cm^{-1} (A, G), and to a lesser extent the 1652 cm^{-1} (T, G) and 1255 cm^{-1} (T, C) bands was observed both in the spectrum of CPT-oligo(−) and in that of CPT-oligo(+). These similar spectral changes assume the same type of interaction between the drug and both oligonucleotides being independent of the existence of a CPT-dependent cleavage site. The only feature that can be tentatively ascribed to some specific interactions of the drug with oligo(+) induced by the altered (but only in the two nucleotide bases) oligonucleotide sequence is a lack of the 1427 cm^{-1} (A, G) band as compared to the spectrum of CPT-oligo(−).

In contrast to CPT, the CPT11-induced changes of the oligonucleotide signals were found to be weak and were detected only in the spectrum of CPT11-oligo(−) (Fig. 3c). An increased drug/oligo molar ratio was used to observe these changes clearly. The changes included a moderate increase in the intensity of the 1580 cm^{-1} (G, A), 1514 cm^{-1} and 1339 cm^{-1} (A, G) bands as well as both intensity increase and frequency shifts of the 1610 cm^{-1} (G, A) and 1422 cm^{-1} (A, G) bands. These spectral features differed obviously from those observed in the CPT-oligonucleotide spectra (Fig. 3a,b). The spectrum of CPT11-oligo(+) (Fig. 3d) was found to coincide with the superposition of the free CPT11 and oligo(+) spectra (even at the increased drug/oligo molar ratio), indicating an absence of detectable interaction between the drug and oligo(+). These observations suggest a pronounced alteration of the ability of this derivative to interact with DNA as compared to CPT itself. It is reasonable to conclude that substitutions at the 10 and 7 positions of the drug molecule (Fig. 1) make weaker or even exclude the interactions between the conjugated ring system (A–E rings) of the drug and oligonucleotides. The effect of substitutions seems to depend on the structure of the oligonucleotide as it follows from the differences of CPT11 action on oligo(−) and oligo(+).

3.3. The UV RR spectra of the ternary complexes

A strong decrease in the 1339 cm^{-1} (A, G) and 1484 cm^{-1} (A, G) band intensity was observed in the spectra of the CPT11-topo1-oligo(−) (Fig. 4a) and CPT11-topo1-oligo(+) (Fig. 4b) ternary complexes. These spectral features were not observed in the spectra of the complexes formed between the oligonucleotides and CPT11 (Fig. 3c,d) nor were they induced by addition of topo1 to oligo(+) (Fig. 4d) or by mixing the carboxylate form of CPT11 with oligo(+) and topo1 (Fig. 4c). Therefore, their appearance is due to concerted action of the drug and topo1 on the oligonucleotides. It should be noted that similar but less pronounced features were observed in the spectra of CPT with oligonucleotides (Fig. 3a,b). It suggests that topo1 stimulates the mode of interaction between CPT11 and oligonucleotides which is a characteristic of the parent compound CPT, and enhances this interaction. These observations confirm the suggestion

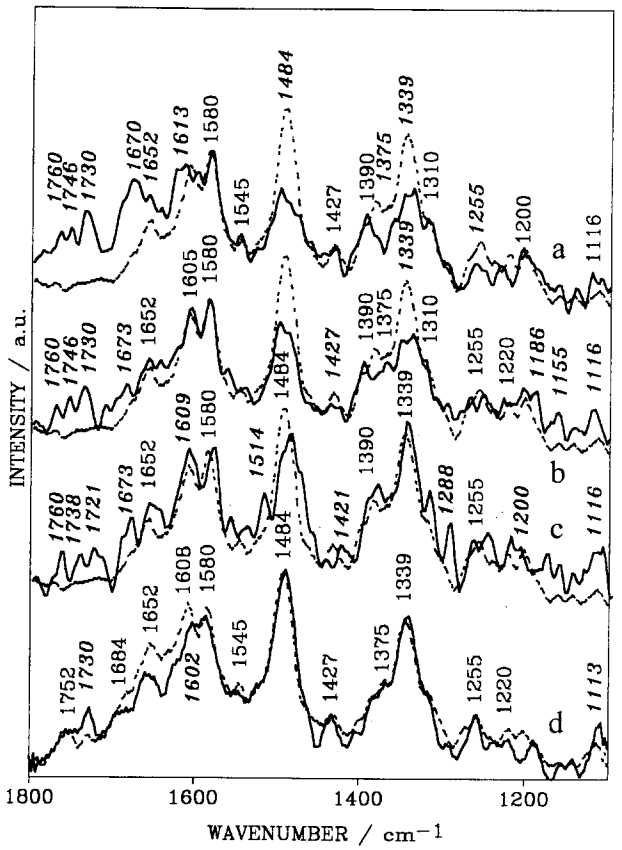

Fig. 4. Solid lines: UV RR spectra of topo1-CPT11-oligo(−) (a), topo1-CPT11-oligo(+) (b), topo1-CPT11-oligo(+) (carboxylate form) (c) and the topo1-oligo(+) (d) complexes. Dashed lines: simulated spectra of the corresponding complexes obtained as the sum of the UV RR spectra of pure components. Italics are used to mark the bands affected by the complex formation. The signals of water and fluorescence background have been subtracted. [CPT11] = 20 μM, [oligo(+)] = 20 μM, [oligo(−)] = 20 μM, [topo1] = 10 μM.

that camptothecins interact with an asymmetrical receptor site of the enzyme or enzyme-DNA complex [5]. The binding of CPT11 to this site was detected in our spectra in a form of specific re-orientation of the molecule which led the conjugated ring system of the drug to be accessible to the interaction with the oligonucleotides. The appearance of the interaction features of the CPT derivative in dependence on whether oligonucleotide alone or together with topo1 is added to the drug is consistent with recent findings [11,18], where some biological effects induced by different modes of binding with DNA, topoisomerases and in ternary complexes were observed for the derivatives of intoplicine.

A moderate decrease in the 1484 cm^{-1} band intensity of the spectrum of the carboxylate CPT11-topo1-oligo(+) (Fig. 4c) shows that even a carboxylate form of CPT11 interacts with the oligonucleotide in the presence of topo1. At the same time some specific interactions, in particular manifesting themselves by the decreasing of the 1339 cm^{-1} band (Fig. 4a,b), disappear after the opening of the lactone ring. Since the adenine bases contribute mainly to the 1339 cm^{-1} band of the oligonucleotide, whereas the 1484 cm^{-1} band is equally attributed to both guanine and adenine bases (Fig. 2, II), a predominant interaction of the carboxylate form of CPT11 with guanines of oligo(+) can be suggested. In contrast, the lactone form of CPT11 seems to interact with both guanine and adenine bases within the CPT11-topo1-oligo(+) complex (Fig. 4b).

Considering the features of the interactions within the ternary complexes, an intensity increase of the 1670 cm^{-1} band and a decrease of the 1375 and 1250 cm^{-1} bands of oligo(−) as compared with oligo(+) should be noted. The 1670 cm^{-1} feature should be assigned to the thymine band, which appeared due to the splitting of the 1652 cm^{-1} band into 1670/1652 cm^{-1} doublet [19]. Taken together, this 1670/1652 cm^{-1} doublet and these 1375, 1250 cm^{-1} bands correspond to the most intensive bands in the spectrum of dTMP (Fig. 2, II,e). Therefore, thymine bases are more affected in oligo(−) than in oligo(+) upon ternary complex formation. This correlates with the fact that the cleavage site of oligo(−) is enriched by thymine bases as compared to that of oligo(+). It can be supposed that alteration of the oligonucleotide sequence modifies the structure of duplex oligonucleotides and changes the accessibility of the thymine bases for the interaction with CPT11 and/or topo1 in the ternary complexes.

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