Interactions of lactone, carboxylate and self-aggregated forms of camptothecin with human and bovine serum albumins

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Abstract Pronounced differences in the interactions of monomeric (lactone and carboxylate) and the J-type self-aggregated form of camptothecin (CPT), an inhibitor of DNA topoisomerase I (topo I), with human (HSA) and bovine (BSA) serum albumins were observed by using circular dichroism (CD) spectroscopy. HSA binding changes the geometry of the covalent structure of CPT due to hydrophobic contacts of the chromophore within the protein interior. The carbonyl group of the ring D of CPT (Fig. 1A) interacts with the positively charged amino acid residues of HSA. Interaction with HSA induces disaggregation of the J-type self-aggregates of CPT. On the other hand, neither heat-denatured HSA nor native BSA participated in binding of the lactone or carboxylate or self-aggregate forms of CPT. Analysis of HSA and BSA homology within the IIA and IIIA principle ligand-binding structural domains suggests that the binding site for the CPT chromophore is located in subdomain IIA. Hydrophobic contacts with Leu-203, Phe-211, and Ala-215 and electrostatic interactions with Lys-199 and/or Arg-222 of HSA may play a key role in formation of the drug-HSA complex.

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Key words: Topoisomerase inhibitor; Circular dichroism; Camptothecin; Human serum albumin

1. Introduction

20(S)-Camptothecin (CPT), an alkaloid isolated from Camptotheca acuminata (Fig. 1A), and several of its recently synthesised derivatives have aroused considerable interest due to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1]. The CPT family of drugs exhibits its cytotoxicity to their ability to halt the growth of a wide range of human tumours [1].

In this study, we have employed the circular dichroism (CD) approach in order to characterise the interactions of lactone, carboxylate, and self-aggregated forms of CPT with HSA and BSA. In contrast to fluorescence analysis, CD spectroscopy enables the detection of changes in the ground-state molecular conformation. Differences in CPT-HSA and CPT-BSA interactions and analysis of the homology of the binding domains of two proteins allowed localisation of the binding site of CPT within HSA. J-aggregates of CPT were found to interconvert into the monomers in the presence of HSA but not when BSA was present. Interactions of CPT monomers

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Abbreviations: topo I, DNA topoisomerase I; 20(5)-CPT or CPT, 20(S)-camptothecin; BSA, bovine serum albumin; CD, circular dichroism; PBS, potassium buffered saline

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with HSA were supposed to include both hydrophobic contacts (with the Leu-203, Phe-211, and Ala-215 residues of the IIA binding domain, Fig. 1B) changing the geometry of the CPT structure and an interaction of the CPT carbonyl group of ring D and the carboxylate group of hydrolysed ring E (Fig. 1A) with charged (Lys-199 and/or Arg-222, Fig. 1B) HSA amino acid residues.

2. Materials and methods

20(S)-CPT, crystallised HSA and BSA were purchased from Sigma. 9NH₂-CPT and the 20(R)-stereoisomer of CPT were supplied by the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. Drug stock solutions were made in DMSO (ACS spectrophotometric grade, Aldrich) at concentrations of 10, 1 or 0.1 mM, and aliquots were stored in the dark at —70°C. Further dilutions with HSA were supposed to include both hydrophobic contacts (with the Leu-203, Phe-211, and Ala-215 residues of the IIA binding domain, Fig. 1B) changing the geometry of the CPT structure and an interaction of the CPT carbonyl group of ring D and the carboxylate group of hydrolysed ring E (Fig. 1A) with charged (Lys-199 and/or Arg-222, Fig. 1B) HSA amino acid residues.

3. Results and discussion

3.1. General characteristics of CPT chromophore

CPT includes two chromophores conjugated through ring C—rings AB (quinoline) and ring D with hydrolyzable ring E (Fig. 1A). Ring E of CPT does not exert a strong influence on the electronic properties of the whole chromophore: upon hydrolysis, the UV-Vis spectra of the lactone and carboxylate forms of CPT are quite similar. Molecular modelling analysis shows that the molecule in solution is almost planar and exhibits low ellipticity values in the corresponding CD spectra. All other chemicals were of analytical-reagent grade and all solvents were of HPLC grade.

CD spectra were measured using Jobin Yvon, Mark III and Jasco spectro-photometers. Protein and CPT concentrations were estimated from their molar absorption coefficients in PBS: ε₃₉₀₀ = 39 800 M⁻¹ cm⁻¹ and ε₃₈₀₀ = 19 900 M⁻¹ cm⁻¹, respectively. UV-Vis spectra were recorded with a Philips PU 8720 UV-Vis scanning spectrophotometer. Experimental conditions are indicated in the figure legends. Measurements were performed using quartz cells of 1, 0.1, 0.01, and 0.001 cm path length. The CD spectra were scaled to molecular ellipticity (α).

3.2. CD spectra of CPT monomers and kinetics of lactone hydrolysis followed by CD spectroscopy

According to Clar’s classification [11] all long-wavelength bands in the UV-Vis spectra of CPT are typical for quinoline (rings AB, Fig. 1A) but are shifted to the red as a result of conjugation of rings AB and D(E). For this type of conjugated and slightly twisted π-electron system, there are 9 main electronic transitions detectable in the UV-Vis and CD spectra. They include 3 transitions within the long-wavelength band ILa (approx. 380, 365, and 354 nm) and 6 others within the short-wavelength region [12]. In the case of the ILa band, the 380 nm band exhibits the lowest rotational activity in the CD spectra whereas the other two show the highest values, however, the signs of all the CD bands are the same (Fig. 2A). The rotational activity (CD signals) for each transition reflects the extent to which the transition contributes to the phenomenon of optical activity. As a rule, all CD bands corresponding to the same group of transitions (e.g. the bands at approx. 380, 365, and 354 nm corresponding to the same ILa group) should have the same sign upon alterations induced by effects changing the molecular symmetry. Hydrolysis of the lactone ring changes the symmetry of the CPT molecule and induces inversion of the signs of the bands at 380, 365, and 354 nm (Fig. 2A). After hydrolysis the relative contribution of each of these bands to the optical activity is indeed changed as compared with the lactone form, but simultaneous inversion of the sign for all three bands clearly confirms their attribution to the same π-π* transitions within the ILa band.

The CD spectrum of the lactone form of CPT in the region 300–340 nm shows another two bands with behaviour different from that of the bands of the ILa group mentioned above. These bands (approx. 305 and 330 nm) do not change sign on going from the lactone to the carboxylate form of CPT (Fig. 2A). The band at 305 nm is always positive and even remains unchanged in intensity upon hydrolysis, the band at 330 nm becoming smaller and totally disappearing on progressing from the lactone to the carboxylate form. The band at approx. 305 nm is probably attributable to the second (1La) electronic transition of the π-electron system of CPT chromophore [12]. Being very similar to the corresponding band of quinolines [13], this transition is essentially localised on rings AB which are less conjugated with the electron system of ring DE.

In contrast to the band at 305 nm, the band at 330 nm totally disappears after hydrolysis-induced relaxation of the structure of the 20(S)-CPT chromophore (Fig. 2A). This band, which is positive for the lactone form of 20(R)-CPT, also disappears upon hydrolysis of this isomer (data not shown). As noted above, reduced strength in the coherent structure of CPT leads to a symmetric configuration of the carbonyl group of ring D relative to the plane of chromophore. In UV-Vis and CD spectra, the n-π* transition of the carbonyl group in aromatic ketones (structures corresponding to conjugated ring D of CPT) exhibits very similar bands at approx. 330 nm [12]. We assign the band at approx. 330 nm for CPT to the n-π* transition of the carbonyl group of ring D. Hydrolysis-induced relaxation of the 20(S)-CPT (20(R)-CPT) structure leading to the lower- (upper-) plane transfer...
Fig. 1. (A) Structures of lactone and carboxylate forms of 20(5)-CPT and its synthetic stereoisomer 20(R)-CPT. (B) Amino acid sequences of BSA and HSA in the regions of II A and III A principle ligand-binding structural domains. Amino acid residues forming 3D structural ligand-binding domains [17] are in bold-face. Amino acid residues forming 3D structural ligand-binding domains in HSA and replaced by others in BSA are in bold-face and encircled. Deletions in the primary structures correspond to the regions of total homology of the HSA and BSA. Panel B is modified from [16].

3.3. Interaction of monomers of CPT with the HSA

HSA binding induces three effects on the CD spectra of the CPT chromophore: (i) a pronounced effect of induced optical activity: the lactone form exhibits an approx. 3.5-fold increase and the carboxylate form an approx. 5-fold increase in molar ellipticity as compared with free CPT in solution; (ii) a long-wavelength shift of the bands within the lLa electronic transition; and (iii) disappearance of the approx. 330 nm band for the lactone CPT-HSA complex and induction of this band for the carboxylate CPT-HSA complex (compare Fig. 2A,B).

The theory of optical activity predicts that a chromophore of a low-molecular-weight ligand may become optically active or increase its internal optical activity as a result of two types of interactions: individual interactions of each chromophore with a macromolecule-asymmetric matrix (monomeric effect) or co-operative interactions between the ligands fixed on the matrix in close vicinity to each other (oligomeric effect) [14]. A characteristic feature of the oligomeric effect is its dependence on the ligand/matrix molar ratio. The CD spectra of CPT-HSA complexes recorded at 1/1 and 1/5 molar ratios (data not shown) were found to be identical both in the levels of induced optical activities and in the relative ratios of the CD bands. Therefore, the chromophore-chromophore interaction was concluded to be small and the effect of the induced optical activity of the CPT chromophores should be determined by the ‘monomeric’ CPT interactions with the HSA asymmetric matrix. Induced optical activity of the monomers in the region of the electronic transition of the molecule is determined by three factors: (i) asymmetrization electron rotation around the fixed nucleus; (ii) replacement of the equilibrium positions of the nucleus along the asymmetric normal coordinates in the ground state; and (iii) the different character-
characteristics of asymmetrization of the molecule in the ground and excited states [15]. The first two effects induce CD with a form similar to that of the UV-Vis spectrum, i.e. without changes in the signs of the CD bands within the region of optically active electronic transitions. On the other hand, asymmetrization of the molecule in the ground and excited states induces splitting of the CD bands with changes in their signs, hence the forms of the CD and UV-Vis spectra become very different [14].

In our case, the CD bands of CPT-HSA complexes do not change sign within the 1La electronic transition but do exhibit a long-wavelength shift as compared with free CPT in aqueous buffer and exhibit the same position and profile as for CPT in non-polar DMSO solution (Fig. 2B). Hence, the effects of induced optical activity of CPT and changes in profile of the CD bands within the 1La transition in the presence of HSA is caused by replacement of the equilibrium positions of the nucleus along the asymmetric normal coordinates in the ground state (changes in the geometry of the molecule) due to the hydrophobic interactions.

Asymmetrization of electron rotation around the fixed nucleus should induce perturbations within the UV-Vis spectra accompanied by corresponding changes of the CD bands. This is the case for CPT derivatives with the charged groups introduced within or in close vicinity to the π-electron system of the chromophore [7,8]. On the other hand, changes in the geometry of the molecule do not induce pronounced changes in the UV-Vis spectra, whereas the corresponding CD spectra may be quite different. For example, hydrolysis of the lactone ring of free CPT does not induce strong changes in the UV-Vis spectra (i.e. no strong redistribution of electron density within the chromophore) but leads to dramatic differences in the CD spectra (Fig. 2A, changes in the signs of CD bands). The UV-Vis spectra of CPT in the complex with HSA show slight modifications comparable with those on hydrolysis of the lactone ring.

Interaction of the lactone form of CPT with HSA results in the disappearance of the optical activity of the 330 nm n-π* carbonyl electronic transition (Fig. 2B). It should be noted that if hydrolysis of free lactone leads to the loss of optical activity of the n-π* transition (Fig. 2A), then the interaction of the carboxylate form of CPT with HSA restores its optical activity (intense shoulder at approx. 330 nm, Fig. 2B). This reflects an interaction of the carbonyl of ring D of the carboxylate form of CPT within the HSA moiety. Interaction of the carboxyl group of hydrolysed ring E with the charged groups of HSA may also contribute to the changes in CPT carbonyl symmetry.

Hence, the monomeric effects of induced optical activity of CPT in the presence of HSA are caused by replacement of the equilibrium positions of the nucleus in the ground state, i.e. by deformations of the covalent structure of the molecule due to the hydrophobic contacts of CPT with HSA, and modifications within the 330 nm band may reflect interactions of the carbonyl group of ring D of the lactone form of CPT and interactions of the carbonyl group of ring D and the carboxyl group of hydrolysed ring E of the carboxylate form of CPT with the positively charged amino acid residues of the protein.

3.4. Interactions of J-aggregates of CPT with HSA

We have recently shown that some derivatives of the CPT family in aqueous buffer solution may form J-type aggregates [7,8]. These aggregates appear under certain dilution conditions for stock DMSO solutions of 20(S)-CPT, 10,11-methyleneoxy-CPT and 7-ethyl-10-hydroxy-CPT and are characterised by very large CD signals with molar ellipticity values in range of 20–40 M⁻¹ cm⁻¹. The aggregates were found to be stereospecific, being undetectable for the 20(R)-stereoisomer of CPT and were formed by the stacking interaction between the quinoline rings of the CPT chromophores with the inverse
position of the nitrogen atoms. The aggregates were found to penetrate within the cells with much higher efficiency and to exhibit much greater cytotoxic effects as compared with the monomeric form of the drugs [7,8]. In this paper, we have shown that addition of HSA to the aqueous buffer solution of self-aggregated CPT induces disaggregation of the drug which is detectable as a decrease in the corresponding CD signals (Fig. 3A). It is difficult to say whether, in the presence of HSA, the rate of hydrolysis of the lactone ring within the self-aggregated species is the same as for the monomers: it is not possible to detect the CD signals of the monomers on the background of the approx. 100-fold stronger bands of the aggregates. However, it is clear that the interaction of CPT chromophores with HSA is much stronger than the interchromophores interaction within the aggregates.

3.5. Interactions of CPT with denatured HSA and with native BSA

Interactions of lactone, carboxylate or self-aggregated forms of CPT with HSA were found to be strongly affected by alterations of the native protein structure. Heat-denatured HSA used at the same concentrations as the native protein does not exert any effects on the CD spectra of free CPTs. Therefore, the effects described above were found to be specific.

The CD spectra of CPT monomers do not exhibit effects of induced optical activity or changes in profile of the bands which are characteristic of the free monomeric CPT chromophores in aqueous solution (Fig. 3C); also, the CD spectra of CPT J-aggregates do not exhibit effects of disaggregation (Fig. 3B) in the presence of BSA.

HSA and BSA are highly homologous proteins [16], especially within the structural domains responsible for the ligand-protein interactions (Fig. 1B, the so-called IIA and IIIA domains [17]). Moreover, these ligand-binding domains in HSA and BSA are characterised by extremely high structural homology [18,19]. Hence, the disappearance of specific CPT/protein interactions in the presence of BSA compared with HSA provides us with the possibility of identifying individual amino acid residues playing a key role in CPT/HSA interactions. Replacement of these residues by others in BSA as compared with HSA (Fig. 1B) leads to the absence of CPT binding by BSA (Fig. 3B,C).

It should be noted that crystallographic analysis of CPT-HSA complexes is in progress [9] and the possibility of predicting the exact amino acid residues involved in the drug-protein interactions may have a fundamental interest.

The binding cavity in the IIIA domain of albumins is the most active and accommodating on HSA and BSA. Many ligands were found to bind preferentially at this location, for example, digitoxin, ibuprofen and tryptophan [17]. Aspirin and iodinated aspirin analogues show nearly equal distribution between binding sites located in IIA and IIIA. Warfarin occupies a single site in IIA (see [17] and references herein). Residues Trp-214, Lys-199 and Tyr-411 have been implicated in the binding process, and each is located strategically in the IIA or IIIA hydrophobic pockets [20,21].


Three key hydrophobic residues of the IIA domain of HSA do not have their counterparts in BSA. Leu-203, Phe-211, and Ala-215 of HSA are replaced by Ile-203, Leu-211 and Ser-215 in BSA (Fig. 1B). Moreover, ‘a focal point’ of domain IIA structural organisation, Lys-199 of HSA, is replaced by Arg-199 when progressing from HSA to BSA. Lys-199 together with Arg-222 was shown to play a key role in the process of carboxylate binding by HSA [17]. Both these residues are replaced (by Arg-199 and Lys-222) on progressing from HSA to BSA (Fig. 1B).

The residues forming the IIA domain are totally (with only one minor exception: Val-456 of HSA is changed by Ile-456 in BSA) homologous with those forming the corresponding domain of BSA (Fig. 1B). It is difficult to imagine that the
replacement of one hydrophobic residue by another (and also hydrophobic) may eliminate the interactions between the CPT and the BSA.

Finally, domain IIA of HSA is supposed to be a principle site of CPT binding. The residues Leu-203, Phe-211, and Ala-215 of HSA may undergo direct hydrophobic interactions with the CPT chromophore whereas the positively charged groups of Lys-199 and Arg-222 may stabilise the complex by direct interaction with the carbonyl group of ring D of the lactone form and with the carbonyl group of ring D and the carboxyl of hydrolysed ring E of the carboxylate form of CPT.

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References