Scientific paper

Effect of SDS Micelles on Actinomycin D – DNA Complexes

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Abstract

DNA thermal denaturation was evaluated as a measure of the effect of antitumor drug actinomycin D on the stability of the double helix and also the effect of SDS micelles on actinomycin D – DNA complexes. The results indicated that the melting temperature of DNA was dependent on drug concentration, increasing with actinomycin D concentration. High thermal stabilization (about 10 °C) of the DNA helix after the association with actinomycin D clearly demonstrates the intercalative binding mode. The presence of SDS micelles leads to the release of intercalated actinomcyin D molecules from DNA double helix and their further relocation in surfactant micelles. These results highlighted that the drug release can be controlled in time and by varying the concentration and nature of surfactant.

Keywords: Actinomycin D, DNA, micelles, thermal denaturation

1. Introduction

Actinomycin D (ActD) is a chromopeptide anticancer drug used for the treatment of pediatric rhabdomyosarcoma and Wilms' tumor as a component of VAC (vincristine, actinomycin D, cyclophosphamide) therapy.[1] Structurally, actinomycin D contains a 2-aminophenoxazin-3-one chromophore and two identical cyclic pentapeptide lactones (Fig. 1). Actinomycin D exerts the anticancer activity by tight binding to DNA which determines the inhibition of transcription elongation by blocking RNA polymerase.[2,3] The interaction of actinomycin D with DNA in terms of binding mode and sequence specificity was extensively investigated using different experimental techniques.[4–8] The actinomycin D-DNA complex is made by intercalation of planar phenoxazone ring between 5'GpC3' sequence and strong hydrogen bonds are formed in the minor groove between the guanine 2-amino groups and the carbonyl oxygen atoms of the L-threonine residues of the pentapeptides chains.[4,5] Additional stabilization of this complex are acquired from hydrophobic interactions between groups on the pentapeptides and sugar residues and from other specific hydrogen bonding and atom–atom intermolecular interactions.[6,9] Actinomycin D also binds to some DNA sequences that do not contain G-C sites[10,11] and to single stranded DNA.[12,13]

Stopped-flow kinetic studies indicate that the binding of actinomycin D to DNA is characterized by five rate constants with three slow processes.[14,15] As these slow processes are absent in the binding of actinomine (a peptide-lacking analog of actinomycin D) to DNA, the slow
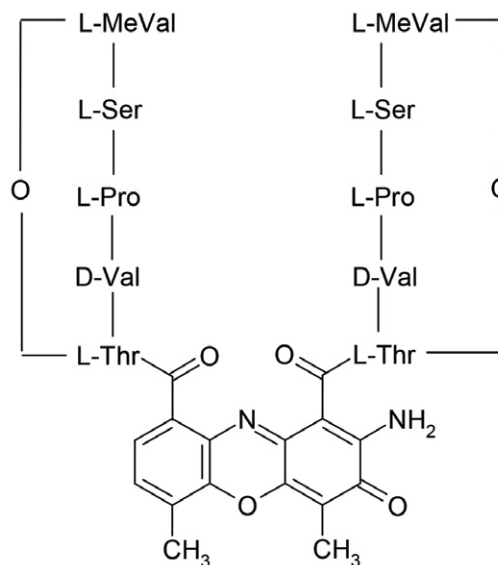

Figure 1. Molecular structure of actinomycin D (ActD).

kinetics of interaction of actinomycin D with DNA was explained by conformational changes in the peptide chains of actinomycin D during the accommodation of molecules into double helix of DNA.[14] Also, SDS induced actinomycin D dissociation from DNA presents multiexponential decay, with the slowest rate of 1500 seconds.[16]

In the present work, the thermal denaturation profile of DNA is used to evaluate the effect of anticancer drug actinomycin D on the stability of the DNA double helix. These results together with our previous research can provide information about the nature and the strength of interaction between actinomycin D and DNA. Moreover, the effect of SDS micelles on actinomycin D – DNA complexes is investigated taking into account that micelle-induced sequestration can be applied as a simple method for the removal of drug molecules from DNA.[17]

2. Materials and Methods

2. 1. Materials

Actinomycin D (ActD), deoxyribonucleic acid (DNA) sodium salt from calf thymus and sodium dodecyl sulphate (SDS) were purchased from Sigma-Aldrich. The concentration of the prepared DNA stock solutions was determined by measuring the absorbance at 260 nm using the molar absorbance coefficient, $\varepsilon = 6600$ M^{-1} cm^{-1} (in nucleotide concentration). All the sample solutions were prepared in 0.1 M phosphate buffer, pH 7.4. All chemicals used were of analytical grade or higher and were used without further purification. The concentration of actinomycin D was determined spectrophotometrically using the molar absorption coefficient at 440 nm, $\varepsilon = 24400$ M^{-1} cm^{-1}.

2. 2. Thermal Denaturation Experiments

Thermal denaturation experiments were performed in stoppered quartz cuvettes on a Jasco V-550 UV-VIS spectrophotometer equipped with a Jasco ETC-505T cell-temperature controller. The temperature of DNA solutions alone and in the presence of actinomycin D at ratios (R) of [ActD]/[DNA]: 0.05, 0.1, 0.2, 0.5 and 1.00 was increased gradually from 25 to 103 °C at a speed of 1 °C/min and the absorbance at 260 nm was read automatically. The melting temperature (T_m) of DNA in the absence and the presence of actinomycin D was determined using the second derivative method in the frame of Melting Temperature Calculation Program provided by the spectrophotometer. All measurements of T_m were repeated three times and the data presented are the average values.

The hyperchromicity (%H) of DNA was calculated in the absence and presence of the different concentrations of actinomycin D at 260 nm using the equation (1):[18]

$$\%H = \frac{A_U - A_L}{A_L} \times 100 \qquad (1)$$

where A_U and A_L are the absorbance of the upper baseline and the absorbance of the lower baseline respectively.

3. Results and Discussion

The DNA helix is a fairly stable structure due to hydrogen bonds between base-pairs and base stacking interactions. Thermal DNA denaturation or DNA melting is the process of the dissociation of double helix of DNA into two single strands by the breaking of hydrogen bonds between the bases when temperature is raised. The thermal behavior of DNA in the presence of different ligand molecules can give information about the nature and the strength of the interaction of ligands with DNA. In general, the intercalation binding stabilize the DNA double helix leading to a significant rise in T_m by about 5–8 °C, while in the case of non-intercalative interaction (groove binding or electrostatic binding at the DNA surface), a small change in the T_m is observed.[19,20]

The experimental melting curves of DNA upon addition of actinomycin D within the concentration range 0 < R < 1, where R = [ActD]/[DNA] ratios are presented in Fig. 2. The parameters of the helix-to-coil transition (the melting temperature (T_m), the melting shift (ΔT_m – the difference between the melting temperatures of actinomycin D – DNA complexes and DNA alone) and the hyperchromicity (%H)) are reported in Table 1 and Fig. 2. In the present work, the melting temperature of DNA is about 86.51 °C under our experimental conditions. The addition of actinomycin D to DNA solution results in an increase in the T_m of DNA with the increasing R ratio. The high positive ΔT_m values (Fig.3) indicate the stabilization of double helix of DNA by intercalation mode of binding of actinomycin D drug. The value of $\Delta T_m \sim 10$ °C is in the range of the values corresponding to other intercalative anticancer drugs, such as mitoxantrone, doxorubicin, daunomy-
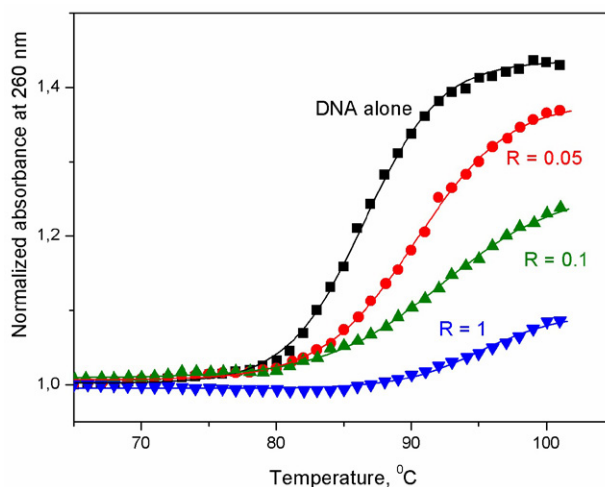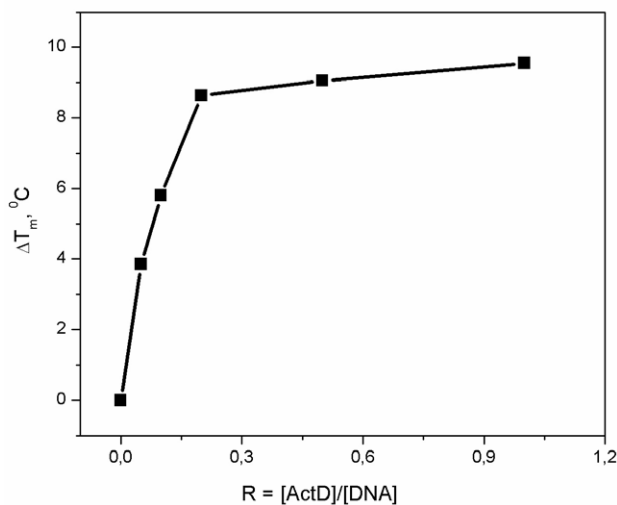

Figure 2. Thermal melting profile of DNA alone and in the presence of actinomycin D at different molar ratio (R = [ActD]/[DNA]).

cin.[21,22] In addition, it can also see that the maximum value of ΔT_m is reached at R = 1. However, the magnitude of ΔT_m in the range of R = 0.2 – 1 does not differ significantly.

Figure 3. The melting shift ΔT_m at different molar ratios, R = [ActD]/[DNA].

Table 1. DNA melting temperature (T_m) and the hyperchromicity (%H) at different molar ratios (R = [ActD]/[DNA]).

R = [ActD]/[DNA]	T_m (°C)[a]	%H
DNA alone	86.5±0.3	53.3
0.05	90.4±0.4	42.4
0.1	92.3±0.2	24.3
0.2	95.1±0.2	18.6
0.5	95.6±0.1	13.0
1	96.1±0.3	7.87

[a] All values are the average ± standard deviation of three experiments.

Also, in Fig. 2 a decrease of the hyperchromicity (%H) is observed. In the absence of the drug, the hyperchromicity of DNA calculated using the equation (1) is about 53.3% (Table 1). As the concentration of actinomycin D increases (R ratio increases), the hyperchromicity of DNA significantly decreases. These results suggest that the dissociation of double helix in the DNA solutions containing actinomycin D is obstructed to take place completely.

The reduction of hyperchromicity and high increase of T_m clearly indicate that the interaction of actinomycin D with DNA determine a high stabilization of double helix of DNA. These results are in agreement with the intercalation mode of binding of actinomycin D molecule between the base pairs of DNA. Also, specific hydrogen bonding and other atom-atom intermolecular interactions contribute to the stabilization of the actinomycin D-DNA complexes.[9]

Further, the influence of SDS concentration on (ActD-DNA) complexes was investigated. The use of surfactant micelles to sequestrate the drug molecules dissociated from DNA is a well-established method to study the kinetics of dissociation of drug – DNA complexes.[15,23] Also, the interaction of drug molecules with surfactant micelles is important to understand the nature of drug-biomembrane interactions but can also help in the case of drug overdoses to remove the excess of the drug and the removal of mutagens.[24,25] Previous studies (absorption, thermal denaturation and circular dichroism) indicate that the native B-form of DNA is not altered by the presence of SDS micelles.[21] Also, DNA hold its native B-form even in the presence of the highest SDS concentration (6.98 × 10⁻³ M) used in the present study, as is indicated by the melting curve (Fig. 4) and the value of melting temperature (85.87 °C) which is very close to the value of melting temperature of DNA alone.

The critical micellar concentration (CMC) of pure SDS in 0.1 M phosphate buffer (pH 7.4) was previously determined from conductivity measurements and it is 9.28 × 10⁻⁴ M.[26] This value is smaller than the CMC of SDS in pure water (8.08 × 10⁻³ M) and it is an agreement with literature data which indicate that the CMC value decreases in phosphate buffer as the concentration of electrolyte increases.[27] Submicellar (6.09 × 10⁻⁴ M) and micellar (2.24 × 10⁻³ M, 4.38 × 10⁻³ M, 6.98 × 10⁻³ M) SDS concentrations were used to assess the influence of SDS surfactant on ActD – DNA complexes.

The presence of submicellar SDS concentration does not change significantly the melting temperature and the hyperchromicity of actinomycin D – DNA complexes (Fig. 4). Instead, the presence of increasing micellar SDS concentrations leads to the continuous decrease of melting temperature of actinomycin D-DNA complexes up to almost the melting temperature value of DNA alone. This decrease of T_m after SDS micelles addition signifies that actinomycin D – DNA complex is disrupted and actinomycin D molecules are relocated from DNA into SDS micelles. In other words, the deintercalation of actinomycin D molecules from DNA takes place in the presence of the micelles and not in the presence of monomeric surfactant molecules.

Also, in the presence of increasing micellar SDS concentrations, the hyperchromicity enhances but it does not reach the value obtained for DNA in the absence of drug. Similar with actinomycin D, the intercalative binding of mitoxantrone to DNA induces the increase of melting temperature with about 10 °C.[21] The addition of SDS micelles conducts to the exclusion of intercalated actinomycin D and mitoxantrone from DNA helix, the melting temperature decreases and reaches the value corresponding to DNA alone. In the case of mitoxantrone – DNA complex, the initial value of DNA hyperchromicity is restored in the presence of SDS micelles,[21] as against actinomycin D – DNA complex when the hyperchromicity enhances in the presence of micelles but is not recovered completely. The most likely explanation for the lack of recovery of the

hyperchromicity could be the fact that some actinomycin D molecules are still associated with DNA. Also, this different behavior between the two drugs could be due to the more complex structure of actinomycin D and hence probably a more complicated mode of interaction with DNA.
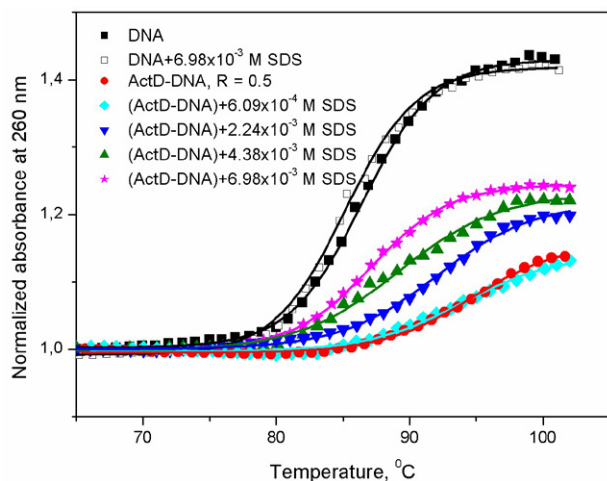

Figure 4. Thermal melting profile of DNA alone, in the presence of 6.98×10^{-3} M SDS, actinomycin D, and actinomycin D and different SDS concentrations.

Table 2. The DNA melting temperature (T_m), the melting shift (ΔT_m) and the hyperchromicity (%H) of actinomycin D-DNA complexes (R = 0.5) in the presence of different SDS concentrations.

System	T_m (°C)	ΔT_m (°C)	%H
(ActD-DNA)	95.56	9.05	12.96
(ActD-DNA) + 6.09×10^{-4} M SDS	94.92	8.41	12.37
(ActD-DNA) + 2.24×10^{-3} M SDS	92.07	5.56	20.43
(ActD-DNA) + 4.38×10^{-3} M SDS	89.12	2.61	21.72
(ActD-DNA) + 6.98×10^{-3} M SDS	87.12	0.61	23.68

The deintercalation of actinomycin D molecules from DNA helix is also observed in the absorption spectra when SDS micelles are added to intercalated (ActD-DNA) complex.

The absorption spectrum of actinomycin D is characterized by a broad absorption band around 440 nm. The formation of actinomycin D – DNA complex results in a hypochromic effect and a shift of the maximum towards longer wavelength. In our previous study, these changes in absorption spectra accompanying the titration of actinomycin D with DNA were used to calculate binding constant and the size of binding site.[28] The addition of micellar concentrations of SDS leads to an increase in the absorbance and the splitting of the absorption maximum in
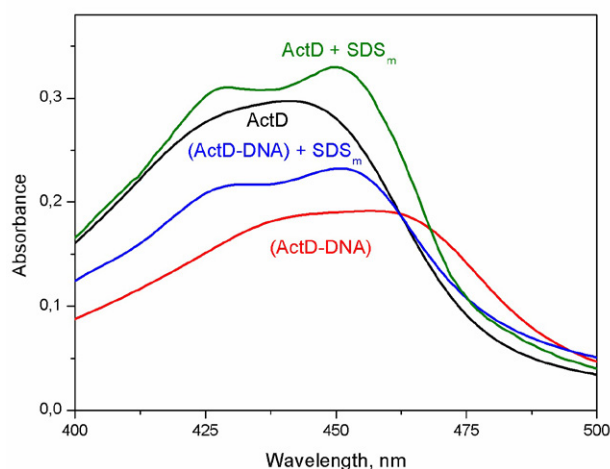
Figure 5. Absorption spectra of actinomycin D, (ActD-DNA) complex, and actinomycin D and (ActD-DNA) complex in the presence of micellar SDS concentration (SDS_m).

two peaks. The spectral modulation in Fig. 5 suggests that upon addition of SDS micelles, actinomycin D molecules experiences a different environment than that in DNA, the absorption spectrum being similar with the spectrum of actinomycin D in SDS micelles.[29] These results are in agreement with melting experiments and lead to the conclusion that the presence of SDS micelles induces the deintercalation of actinomcyin D molecules from DNA double helix and their further relocation in surfactant micelles.

4. Conclusions

The consequences of actinomycin D – DNA complex formation on the stability of the double helix of DNA as well as the influence of anionic surfactant SDS on drug-DNA complexes were evaluated by recording the DNA melting profiles. The melting temperature values indicate an increasing stabilization of DNA as the concentration of added actinomycin D increases. Also, the large values of melting shift (~10 °C) point out for the intercalation mode of binding of actinomycin D to DNA. Addition of SDS micelles to actinomycin D – DNA complexes leads to the deintercalation of drug molecules from DNA helix and their further relocation into surfactant micelles. Besides providing an insight into the stability of actinomycin D – DNA complexes, the present work also demonstrates the excretion of the drug molecules from the biomacromolecular assembly using surfactant micelles.

5. References

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Povzetek

Termično denaturacijo DNA smo ovrednotili kot merilo učinka protitumorske učinkovine aktinomicina D na stabilnost dvojne vijačnice in tudi učinka micel SDS na komplekse aktinomicin D – DNA. Rezultati so pokazali, da je bila temperatura tališča DNK odvisna od koncentracije zdravila, ki se povečuje s koncentracijo aktinomicina D. Visoka toplotna stabilizacija (približno 10 °C) vijačnice DNA po vezavi z aktinomicinom D jasno kaže interkalativni način vezave. Prisotnost micel SDS vodi do sproščanja interkaliranih molekul aktinomciina D iz dvojne vijačnice DNA in njihove nadaljnje premestitve v micele površinsko aktivne snovi. Ti rezultati so pokazali, da je sproščanje zdravila mogoče nadzorovati pravočasno in s spreminjanjem koncentracije in narave površinsko aktivne snovi.