Stabilization of Poly(dA)·[poly(dT)]$_2$ Triplex by Anthryl and Acridine Derivatives: Effect of Side Chains and Nitrogen Atom on the Polycyclic Aromatic Ring

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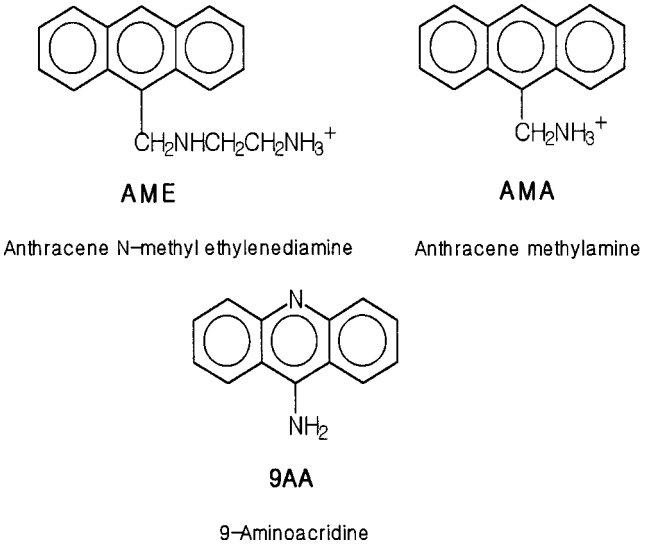
The effects of anthryl derivatives and 9-aminoacridine on the thermal melting profile of a poly(dA)·[poly(dT)]$_2$ triplex were compared. 9-Aminoacridine stabilizes the triplex far more effectively than anthryl derivatives. The absorption and CD and LD spectroscopic characteristics of anthryl derivatives are similar to those of 9-aminoacridine when complexed with the triplex; the N atom of acridine, which can act as a hydrogen bond acceptor, plays an important role in triplex stabilization.

Introduction

Sequence-specific recognition of duplex DNA *via* triplex formation has recently drawn attention due to its potential biological and therapeutic applications.[1,2] However, use of the triplex *in vivo* is restricted by the instability of the third strand in physiological conditions. Several approaches have been taken to enhance the stability of the third strand, including the use of photoactivated cross linking reagents,[3] attaching intercalators to the third strand oligonucleotides,[4] and the use of a triplex-selective ligand.[5] It appears that intercalators in general, including ethidium derivatives,[6,7] benzopyridoindole derivatives,[8,9] Ru(II)[(1,10-phenanthroline)$_2$L]$^{2+}$ complexes,[10] and acridine derivatives,[11] stabilize the third strand of a poly(dA)·[poly(dT)]$_2$ triplex. All these intercalators possess N atom in the cyclic moiety; therefore, N atoms may play some role in triplex stabilization. We compared the effect of anthryl derivatives on the thermal melting profile of triplex DNA with 9-aminoacridine to determine the effect of the N atom on triplex DNA stabilization. Anthryl derivatives with methylamine and methylethylenediamine side chains (referred to as AMA and AME in this article, Scheme 1) do not possess an N atom in the cyclic part.



Scheme 1

Materials and Methods

Triple helical poly(dA)·[poly(dT)]$_2$ was prepared by simmering a 2 : 1 molar ratio of poly(dT) and poly(dA)(purchased from Pharmacia), followed by overnight annealing at room temperature. We used a 5mM cacodylate buffer containing 5 mM NaCl and 100 μM $MgCl_2$ at pH 7.0.

The absorption and circular dichroism (CD) spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer and Jasco J715 spectropolarimeter, respectively. We used the Benesi-Hildebrand approach to obtain the equilibrium constant.[12,13] The linear dichroism (LD) was measured by a Jasco J500C spectropolarimeter and the reduced linear dichroism (LD^r) was calculated using the method described by Nordén *et al*.[14,15] The spectroscopic measurements were averaged over an appropriate number of scans when necessary. The method used to measure the thermal melting profile of the triple helical poly(dA)·[poly(dT)]$_2$ is described elsewhere.[10,11] The temperature was increased at a rate of 0.3 °C/min from 30-90 °C, with a reading taken every 2 minutes. The concentration of the triplex for the melting profile measurement was 14 μM base triplet.

Results

Spectroscopic properties of the anthryl derivatives-poly(dA)·[poly(dT)]$_2$ complex. Binding, especially intercalation, of a drug to DNA results in a hypochromism, broadening of the envelop, and red-shift. The absorption spectra of free AME in the presence and absence of poly(dA)·[poly(dT)]$_2$ are depicted in Figure 1a. The absorption spectrum of the triplex was subtracted for easy comparison. The poly(dA)·[poly(dT)]$_2$-AME complex exhibits about 30% of the hypochromism of DNA-free AME. An equilib-

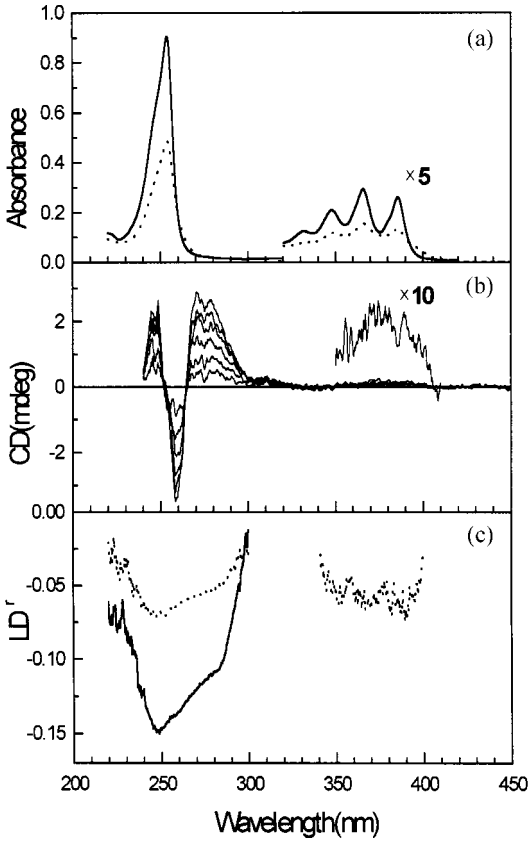


Figure 1. (a) Absorption spectrum of AME complexed with triple helical poly(dA)·[poly(dT)]$_2$ (dashed curve) and polynucleotide-free ligand (solid curve). Absorption spectrum of the triplex was subtracted for easy of comparison. [AME] = 20 μM, [triplex] = 50 μM base triplet (150 μM in base). (b) Induced CD spectrum of AME complexed with poly(dA)·[poly(dT)]$_2$ triplex. [triplex] = 50 μM base triplet. The mixing ration of 0.3, 0.25, 0.2, 0.15, 0.1, 0.05 (from top at 280 nm) (c) LD^r spectrum of AME complexed with triple helical poly(dA)·[poly(dT)]$_2$ (dashed curve) and drug-free triplex (solid curve). The concentrations are the same as in (a).

rium constant of 3.5×10^4 M^{-1} was estimated using a Benesi-Hildebrand analysis[13] with an increasing triplex concentration. The equilibrium constant of AMA was similar, indicating that the variations of the side chains did not affect the binding affinity.

Although neither anthryl derivative possesses any chiral center, a CD signal is expected to be induced by the interaction of the transition moment of the anthracene chromophore and the chirally arranged nucleo-base transition. The induced CD spectrum of AME is depicted in Figure 1b; that of AMA is not shown because it was almost identical. The DNA-shaped CD spectrum of AME in the DNA absorption region was invariant with a concentration change, and two isosbestic points around 251 nm and 264 nm were apparent, indicating that the conformation of AME is independent of the binding ratio. A positive band at 274 nm and a negative band at 258 nm appeared in the DNA absorption region. A weak positive band can be seen in the longer wavelength region (330 nm-400 nm), where AMEs lowest transition lies. The characteristics of the triplex-bound CD spectrum of AME are very similar to those of 9-aminoacridine,[11] indicating that the conformation of AME and AMA in the triplex are similar to that of 9-aminoacridine, since the induced CD spectrum of the DNA-bound drug is known to be very sensitive to its environment.[16,17]

The measured LD spectra of the poly(dA)·[poly(dT)]$_2$ triple helix and the poly(dA)·[poly(dT)]$_2$-AME complex were divided by the isotropic absorption spectrum to produce LD^r spectrum (Figure 1c), which is a powerful tool to determine the orientation of the drug in DNA double helix.[14,15] Forty percent of the added AME remained unbound since the equilibrium constant was low. Therefore, the absorption spectrum of the poly(dA)·[poly(dT)]$_2$-AME complex was adjusted for this factor prior to its use to determine the LD^r spectrum. The LD^r spectra of both the triplex poly(dA)·[poly(dT)]$_2$ and poly(dA)·[poly(dT)]$_2$-AME complexes had negative signals at all wavelengths, indicating that the drugs were, on average, more parallel than perpendicular to the nucleo-bases. The magnitude of the LD^r spectrum in the DNA absorption region significantly decreased upon AME binding. This result indicates that the orientability of the triplex was decreased due to the increase in the flexibility or bending. The possibility of a contribution from the positive LD signal of AME may be excluded, since the LD^r was reduced at both 260 nm and 280 nm. The LD^r magnitude in the AME absorption region (330 nm-400 nm) was approximately the same as that of the triplex, indicating that the planar part of the drug was oriented, on average, more perpendicular than parallel to the helix axis. In summary, the absorption and CD and LD^r properties of the poly(dA)·[poly(dT)]$_2$-AME complex were the same as those of a poly(dA)·[poly(dT)]$_2$-9-aminoacridine complex,[11] indicating that the conformation of AME is identical to that of 9-aminoacridine. The spectroscopic properties of the AMA complex were identical to those of the poly(dA)·[poly(dT)]$_2$-AME complex, and thus are not shown. In addition to the equilibrium not being affected by variations in the side chains, the conformation of the triplex bound drug was not affected in the case of anthryl derivatives.

Stabilization of a poly(dA)·[poly(dT)]$_2$ triplex by anthryl derivatives. Figure 2a shows an example of the effect of the AME on the thermal stability of triple helical poly(dA)·[poly(dT)]$_2$. The thermal melting profiles of the triplex poly(dA)·[poly(dT)]$_2$ in the presence and absence of AME or AMA are biphasic. The first melting temperature (T_{m1}) of the triplex, corresponding to the breaking of the Hoogsteen base-paired poly(dT) strand, was observed at 50 ^{o}C in the absence of drugs. The second melting, which corresponds to the dissociation of the Watson-Crick base pair, occurred at 68 ^{o}C. The T_{m1} for the third strand dissociation increased in the presence of both AMA and AME (Figure 2b). At a mixing ratio of 0.05 [drug]/[DNA base triplet], the T_{m1} value was observed at 57.6 ^{o}C and the T_{m2} at 69.2 ^{o}C, indicating that anthryl derivatives have a triplex stabilization effect. The deviation of the triplex melting temperature from the reported one (54.5 ^{o}C,[11]) may be attributed to differences in the salt condition and concentration of the triplex. When

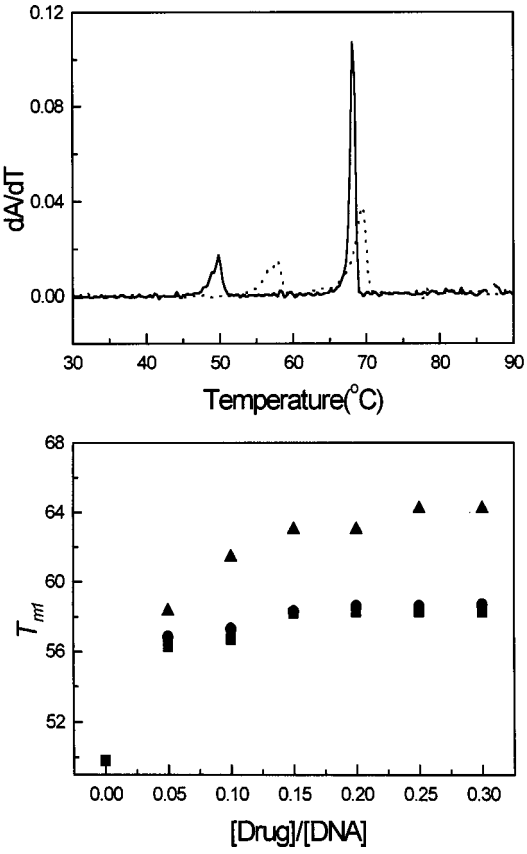


Figure 2. (a) An example of thermal melting profiles of poly(dA)·[poly(dT)]$_2$ triplex in the presence (dashed curve) and absence (solid curve) of AME. [triplex] = 14 μM triplet, [AME] = 0.7 μM. The change in T_{m1} with increasing concentration of AMA (squares), AME (circles) and 9-aminoacridine (triangles). [triplex] = 14 μM base triplet

the mixing ratio reached 0.1, AMA and AME exhibited the maximum stabilization effect (T_{m1} = 58 °C), and no further increase in T_{m1} was observed beyond this ratio. The triplex stabilization effects of AMA and AME were the same, indicating that the variations of the side chains did not affect the stabilization. In contrast, the T_{m1} increased to 63 °C when 9-aminoacridine was mixed in. The maximum was reached at a mixing ratio of about 0.2.

Discussion

Binding mode of anthryl derivatives in a poly(dA)·[poly(dT)]$_2$ triplex. Thorough fluorescence studies have demonstrated that both AMA and AME intercalate between the base pairs of double helical DNAs.[18,19] When they are complexed with a triplex with poly(dA)·[poly(dT)]$_2$, the spectroscopic properties of both anthryl derivatives can be summarized as a hypochromism in the absorption spectrum, a DNA-shaped CD spectrum in the short wavelength, and a weak positive band outside of the DNA absorption region. Both anthryl derivatives caused a significant reduction of the triplex orientation in the flow LD experiment, and the cyclic moiety was approximately parallel to the plane of the base triplet. All these properties are identical to a 9-aminoacridine-poly(dA)·[poly(dT)]$_2$ complex,[11] indicating that the binding modes of both AMA and AME to triplex poly(dA)·[poly(dT)]$_2$ are similar to that of 9-aminoacridine. Although we cannot specify the precise binding geometry, some important features of the binding modes can be deduced. First, the LD^r data lead us to conclude that the anthryl moiety is intercalated. Second, significant decreases in the LD^r magnitude in the DNA absorption region upon complexation with AMA and AME provided information about the binding. DNA generally becomes elongated and stiffened with a reduction of flexibility upon drug intercalation, resulting in increases in the LD and LD^r magnitudes of the DNA. However, in the above case, the orientability of the triplex decreased upon AMA or AME intercalation. When anthryl derivatives intercalate, DNA bending occur, probably because the impetus is not sufficient to part the bases of the third strand. The increase in flexibility of DNA due to DNA binding is also possible.

Stabilization of the poly(dA)·[poly(dT)]$_2$ triplex by intercalators. The third strand of the triplex is known to be stabilized by the formation of a Hoogsteen type hydrogen bond with the template duplex. The reasons for the enhanced stabilization by intercalated drugs may be the formation of a hydrogen bond between the intercalated drugs and the third strand or the expansion of the stacking interaction to the base of the third strand, in addition to the solvent and ion rearrangement. Since most known intercalators exhibit an additional stabilization effect,[6-11] rearrangement of the solvent structure probably plays an essential role. The additional stabilization effect of 9-aminoacridine is apparent when compared with anthryl derivatives and 9-aminoacridine, even though the conformation of these drugs are the same. Since 9-aminoacridine has an N atom at the cyclic part that can act as a hydrogen bond acceptor, formation of the hydrogen bond is conceivably an important factor in additional stabilization. Expansion of the stacking interaction between the intercalated drug and the base of the third strand is not likely to occur because, if this was the case, an increase in the LD magnitude in the DNA absorption region would be observed.

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