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Structure and drug interactions of parallel-stranded DNA studied by infrared spectroscopy and fluorescence

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ABSTRACT

The infrared spectra of three different 25-mer parallel-stranded DNAs (ps-DNA) have been studied. We have used ps-DNAs containing either exclusively dA·dT base pairs or substitution with four dG·dC base pairs and have them compared with their antiparallel-stranded (aps) reference duplexes in a conventional B-DNA conformation. Significant differences have been found in the region of the thymine C=O stretching vibrations. The parallel-stranded duplexes showed characteristic marker bands for the C2=O2 and C4=O4 carbonyl stretching vibrations of thymine at 1685 cm^{-1} and 1668 cm^{-1}, respectively, as compared to values of 1696 cm^{-1} and 1663 cm^{-1} for the antiparallel-stranded reference duplexes. The results confirm previous studies indicating that the secondary structure in parallel-stranded DNA is established by reversed Watson–Crick base pairing of dA·dT with hydrogen bonds between N6H...O2 and N1...HN3. The duplex structure of the ps-DNA is much more sensitive to dehydration than that of the aps-DNA. Interaction with three drugs known to bind in the minor groove of aps-DNA — netropsin, distamycin A and Hoechst 33258 — induces shifts of the C=O stretching vibrations of ps-DNA even at low ratio of drug per DNA base pair. These results suggest a conformational change of the ps-DNA to optimize the DNA-drug interaction. As demonstrated by excimer fluorescence of strands labeled with pyrene at the 5′-end, the drugs induce dissociation of the ps-DNA duplex with subsequent formation of imperfectly matched aps-DNA to allow the more favorable drug binding to aps-DNA. Similarly, attempts to form a triple helix of the type $d(T)_n \cdot d(A)_n \cdot d(T)_n$ with ps-DNA failed and resulted in the dissociation of the ps-DNA duplex and reformation of a triple helix based upon an aps-DNA duplex core $d(T)_{10} \cdot d(A)_{10}$.

INTRODUCTION

Parallel-stranded DNA (ps-DNA) is an unusual DNA conformation that has been demonstrated to exist in hairpins as well as in linear duplex structures (1,–3). Ps-DNA has been assigned a number of potential biological functions in gene expression, recombination, and the stabilization of genomic structure (1, 3–6). It was suggested that ps-DNA is formed by reverse Watson–Crick base pairing (7). The consequence of this base pairing scheme is an approximate equality of the major and the minor groove. Structural details are emerging from high-resolution NMR (8) but there are as yet no crystallographic data. Previous studies have demonstrated that parallel-stranded DNA differs from common antiparallel-stranded DNA (aps-DNA) with regard to chemical, physical and biochemical behavior (2, 3). Recently, it was shown that the Raman spectra of ps-DNA and aps-DNA are significantly different, and the reverse Watson–Crick base pairing was confirmed (9). In this study, we used infrared spectroscopy to study the structure of three different ps-DNAs in comparison with the corresponding aps-DNAs. We studied the interaction of ps-DNA with three non-intercalating drugs (netropsin, distamycin A and Hoechst 33258) all known to bind in the minor groove of B-DNA, preferably to AT clusters (10). Lastly, we investigated the ability of ps-DNA containing exclusively A·T base pairs to form a triple helix of the type $d(T)_n \cdot d(A)_n \cdot d(T)_n$. We used the excimer fluorescence of 5′-end labeled ps-DNA to assess the maintenance of the parallel-stranded duplex structure upon addition of drugs or $d(T)_n$.

MATERIALS AND METHODS

Materials

The following sequences were synthesized as described previously (11, 12, 13):

5′ AAAAAAAAAATAATTTTAAATATTT 3′	(D1)
5′ TTTTTTTTTTATTAAAATTTATAAA 3′	(D2)
5′ AAATATTTAAAATTATTTTTTTTTT 3′	(D3)
5′ TTTATAAATTTTAATAAAAAAAAAA 3′	(D4)
5′ AAAAAGAAAGTAGTTTTAAGTATTT 3′	(D5)
5′ TTTTTCTTTCATCAAAATTCATAAA 3′	(D6)
5′ AAATACTTAAAACTACTTTCTTTTT 3′	(D7)

The three ps duplexes D1·D2, D3·D4 and D5·D6 were formed by heating mixtures of equimolar solutions of the respective single

strands at 70°C for 30 min and cooling very slowly. The corresponding aps duplexes D1·D3, D2·D4, and D5·D7, respectively, were formed by the same procedure. A small amount of Mg^{2+} (2mM) was present to stabilize the duplex of ps-DNA. The final solution used for the infrared spectra contained 150 mM DNA (in base pairs) in 30 mM Mg^{2+} and 150 mM NaCl. The fluorescence label pyrene was attached to the 5′ terminus of D3 and D4 as described recently (3). The drugs netropsin, distamycin A and Hoechst 33258 were research samples or commercial samples from Serva. They were used without further purification. The oligonucleotide deoxydecathymidylate, $d(T)_{10}$, was a commercial sample from Pharmacia (lot 85192).

Methods

The infrared spectra were recorded at a resolution of 1 cm^{-1} using a Fourier-Transform infrared spectrophotometer Perkin Elmer Model 1760 equipped with a 7000 microcomputer. The solution spectra in H_2O and D_2O and the film spectra as a function of the relative humidity were taken as described previously (14). The final solutions contained 175 mM DNA, 30 mM $MgCl_2$ and 150 mM NaCl. The spectra were processed using SpectraCalc (Galactic Industries Corporation, USA). Usually, a smoothing by the Savitzky-Golay algorithms with 5, 7 or 11 points was applied, and the base line corrected for the solvent. The precision of the wavenumber is ±1 cm^{-1}. The excimer fluorescence of pyrene was observed at 7°C with a sample containing ps-DNA D3·D4 with both strands 5′-end labeled with pyrene using a SLM 8000 spectrofluorimeter as discussed previously (3). The DNA-drug complexes and the DNA triple helix were prepared by direct mixing of the DNA duplex with the drug or the decadeoxythymidylate, $d(T)_{10}$, in aqueous solution.

RESULTS

Comparison of the infrared spectra of ps-DNA and aps-DNA

Striking differences between the spectra of the three ps-DNAs, D1·D2, D3·D4 and D5·D6, and of the three aps-DNAs, D1·D3, D2·D4 and D5·D7, were found in the region of the C=O stretching vibration at 1650−1700 cm^{-1}. The spectra of the D_2O solutions of ps-D1·D2 and aps-D1·D3 clearly show a frequency shift of the vibration bands at 1663 cm^{-1} and 1696−1697 cm^{-1} of the reference aps-DNA D1·D3 to new positions of the ps-D1·D2 at 1668 cm^{-1} and 1685 cm^{-1}, respectively (Table 1, Fig. 1). These values hold for ps-D3·D4 as well as for aps-D2·D4, which also contains exclusively dA·dT base pairs. The C=O stretching vibrations of the corresponding pair ps-D5·D6 and aps-D5·D7 incorporating four dG·dC base pairs were slightly shifted (Table 1). No further differences in the infrared spectra of ps-DNA and aps-DNA were found in D_2O or in H_2O solution. As an example, the spectra of ps-D1·D2 and aps-D1·D3 are shown in H_2O solution (Fig. 2). The infrared spectra of the single strands of D3 and D4 were measured in D_2O solution at two different concentrations (approximately 200 mM and approximately 40 mM) for comparison (Table 1). In the diluted solutions, the two C=O stretching vibrations were at 1696 cm^{-1} and 1662−1663 cm^{-1}; these values correspond to those of aps-DNA. However, the infrared absorption band at 1641 cm^{-1} found in all ps-DNA and aps-DNA duplexes was absent or only very weak. We investigated the temperature dependence of the infrared spectra

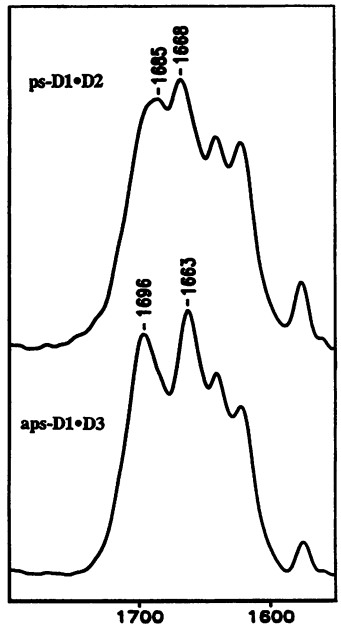

Figure 1. Infrared spectra of parallel-stranded DNA (ps-D1·D2) (top) and antiparallel-stranded DNA (aps-D1·D3) (bottom) in D_2O solution in the spectral region of 1800−1550 cm^{-1}.

Table 1. Wavenumber (in cm^{-1}) of infrared bands in the region of 1650 cm^{-1}−1700 cm^{-1} of parallel-stranded (ps) and antiparallel-stranded (aps) DNAs, as well as of the corresponding single strands, in D_2O solution.

Sample	Wavenumber (cm^{-1})
ps-D1·D2[a]	1685; 1668; 1641; 1622
aps-D1·D3[a]	1696; 1663; 1641; 1622
ps-D3·D4[a]	1685; 1668; 1642; 1622
aps-D2·D4[a]	1697; 1663; 1640; 1620
ps-D5·D6[a]	1690; 1669; 1643; 1625
aps-D5·D7[a]	1698; 1669; 1641; 1623
D3, concentrated[b]	1696; 1663; 1641; 1621
D3, dilute[c]	1696; 1663; 1621
D4, concentrated[b]	1696; 1663; 1637; 1624
D4, dilute[c]	1696; 1662; (1637); 1625

[a]175 mM. [b]approximately 200 mM. [c]approximately 40 mM.

of the ps-D3·D4 in D_2O between 7°C−52°C (Fig. 3). Above 35°C, the band at 1685 cm^{-1} shifted gradually to 1697 cm^{-1}, and the band at 1668 cm^{-1} to 1663 cm^{-1}. A weak band at 1685 cm^{-1} remained at 52°C indicating the existence of a small fraction of ps-DNA. The band at 1641 cm^{-1}, however, disappeared completely at this temperature.

Stability of ps-DNA against dehydration

Films of ps-D1·D2 and of aps-D1·D3 were studied as a function of the relative humidity, RH. In the spectra of both DNA films, significant changes in the shape of the band at 1054 (±1) cm^{-1} occurred with the decrease of RH (Fig. 4). This band decreased in intensity with progressing dehydration. However, the changes in the spectra of ps-DNA occurred at much higher values of RH, i.e. at a much lower degree of dehydration. For example, the infrared spectrum in the region of 1000 cm^{-1}−1100 cm^{-1} of aps-DNA at 47% RH was similar to that of ps-DNA at 76% RH (Fig. 4). In ps-DNA, the band at 1054 cm^{-1} was gradually

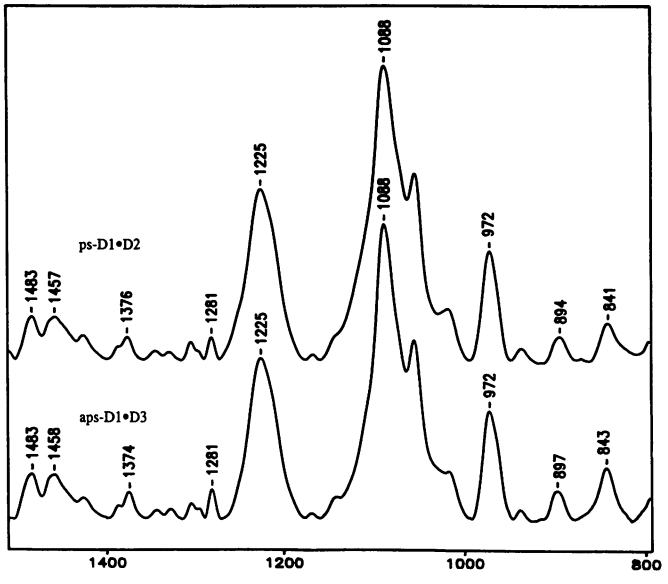

Figure 2. Infrared spectra of ps-D1·D2 (top) and aps-D1·D3 (bottom) in H_2O solution in the region of 1500–800 cm^{-1}.

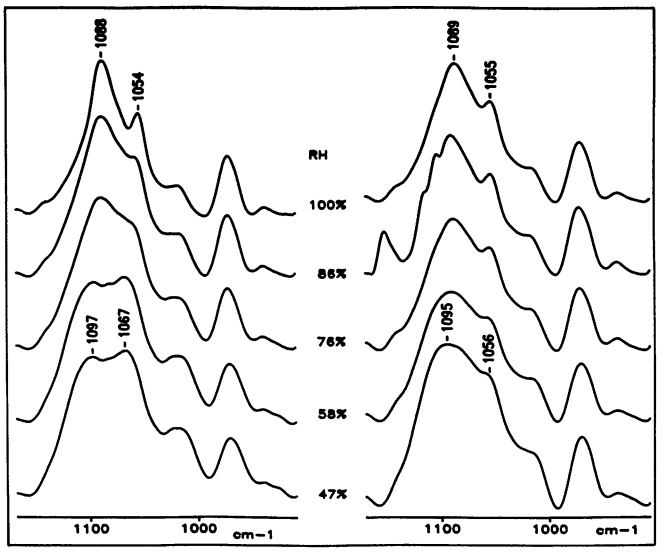

Figure 4. Infrared spectra of films of ps-D1·D2 (left) and aps-D1·D3 (right) exposed to different relative humidity (RH) as indicated.

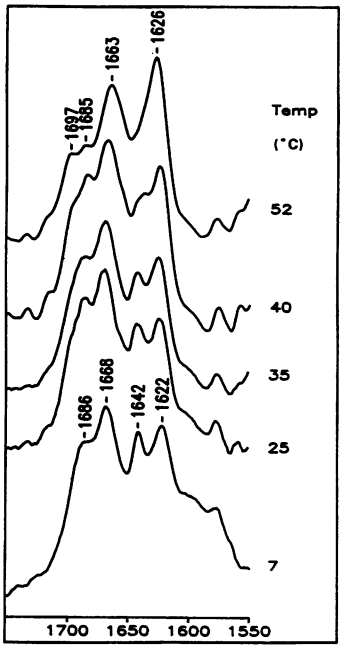

Figure 3. Infrared spectra of the ps-D3·D4 in D_2O solution as a function of temperature between 7°C and 52°C.

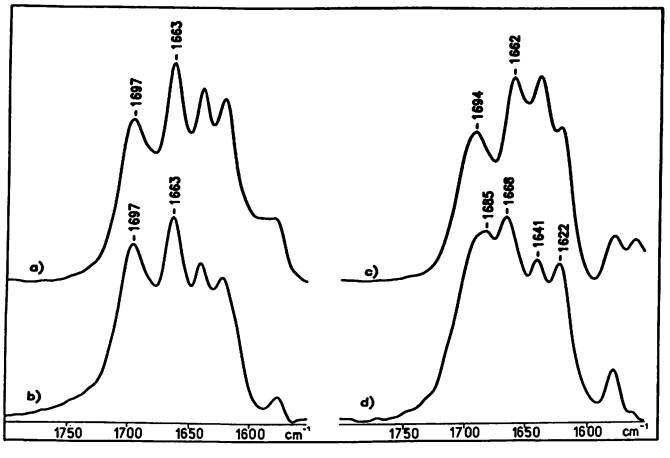

Figure 5. Infrared spectra (1800–1550 cm^{-1}) of complexes of ps-D1·D2 with the drugs (**a**) netropsin, (**b**) Hoechst 33258, (**c**) distamycin A; (**d**) drug-free ps-DNA D1·D2 for comparison. D_2O solution, input ratio of the drugs was 0.2 drug/base pair.

replaced by a new band at 1067 cm^{-1}. (Fig. 4, left hand spectra). The origin of the band at 1142 cm^{-1} in the 86% RH spectrum of aps-DNA is probably due to contamination.

Interaction with non-intercalating minor-groove binding drugs

The three drugs netropsin, distamycin A and Hoechst 33258 are known to bind into the minor groove of B-DNA, preferring A·T base pairs (10). We observed significant changes in the infrared spectra both of aps-D1·D3 and the ps-D1·D2. However, shifts in the region of the C=O stretching vibrations were found only in the spectra of ps-DNA D1·D2 after mixing with drugs. All three drugs induced shifts of both bands at 1685 cm^{-1} and 1668 cm^{-1} of ps-D1·D2. The band at 1685 cm^{-1} was shifted to higher wavenumbers, but the band at 1668 cm^{-1} to lower wavenumbers. Figure 5 shows the infrared spectra of ps-D1·D2 in the absence of drugs and in the complex with drugs at an input ratio of 0.2 drug/base pair, equivalent to 1 drug per 5 base pairs. The drug induced shifts of the two thymine carbonyl stretching vibrations already at very low drug/base pair ratios, e.g. at 0.02 drug/base pair or one drug molecule per 50 DNA base pairs. This unexpected feature is illustrated by a plot of the C2=O2 stretching vibration wavenumber of the ps-DNA-netropsin

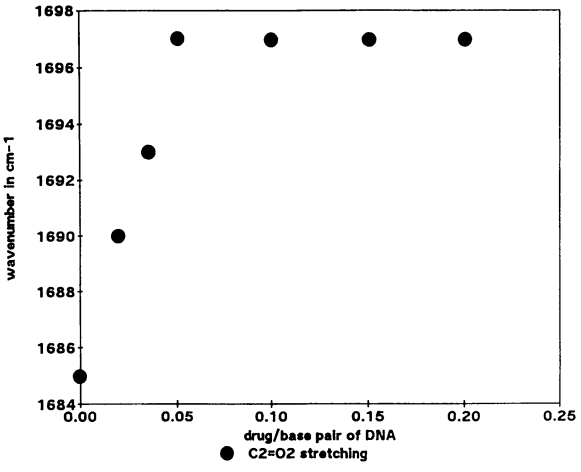

Figure 6. Wavenumber shift of the C2=O2 vibration of ps-DNA in the netropsin complex as a function of the drug/base pair input ratio.

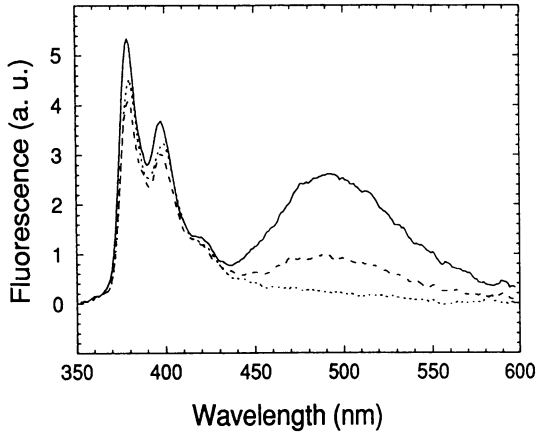

Figure 7. Fluorescence of ps-D3·D4 5′-end labeled with pyrene. The monomer fluorescence is at 378 nm, and the excimer fluorescence due to short-range pyrene-pyrene contacts at 490 nm. (——) ps-DNA without drug; (- - -) addition of netropsin to ps-DNA, r=0.072 drug/base pair or 1.8 drug/duplex; (···) remeasured after 16 hours.

complex as a function of the drug/base pair input ratio (Fig. 6). A plateau was reached at r=0.05 (approximately one drug per duplex) with a shift of the two C=O stretching bands to 1697 cm^{-1} and 1663 cm^{-1}, respectively, in the case of netropsin and Hoechst 33258, and to 1694 cm^{-1} and 1662 cm^{-1} with distamycin A. The drug-induced spectral changes in the spectra of ps-DNA and aps-DNA at r >0.05 were very similar in the carbonyl stretching region (Fig. 5) as well as in the region of 1500−1300 cm^{-1} (not shown). Addition of the minor-groove binding drug pentamidine (15) to ps-D1·D2 produced the same changes in the carbonyl stretching region as described for the drugs netropsin and Hoechst 33258 (data not shown).

Excimer fluorescence of 5′-end labeled ps-DNA and drug interactions

5′-end pyrene labeled ps-D3·D4 shows significant excimer fluorescence at 490 nm, in addition to the monomer fluorescence

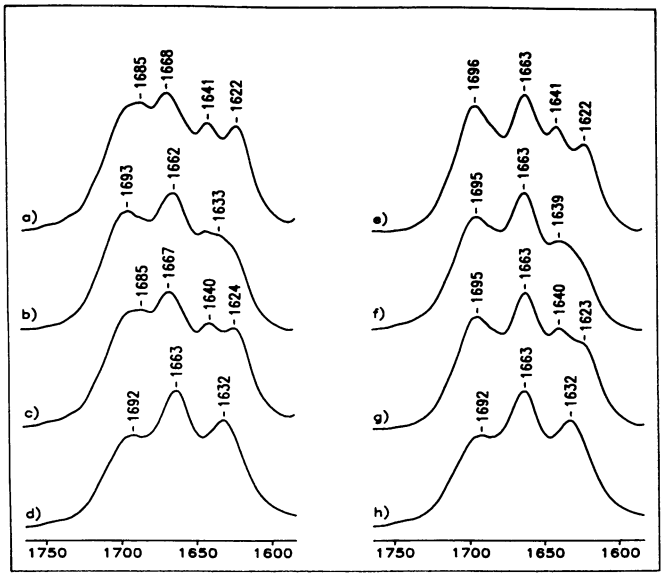

Figure 8. Infrared spectra of ps-D1·D2 (left) and aps-D1·D3 (right) in D_2O solution in the wavenumber region of 1800−1550 cm^{-1}. (**a**) ps-D1·D2; (**b**) complex of D1·D2 with $d(T)_{10}$; (**c**) simulated spectra adding the spectra of the duplex ps-D1·D2 and deoxythymidylate $d(T)_{10}$; (**d**) $d(T)_{10}$; (**e**) aps-D1·D3; (**f**) complex of aps-D1·D3 with $d(T)_{10}$; (**g**) simulated spectra adding the spectra of the duplex aps-D1·D3 and deoxythymidylate $d(T)_{10}$; (**h**) $d(T)_{10}$.

at 378 nm, due to direct contact between the pyrenes at the 5′ ends of the two strands in the ps-duplex (3). Any rearrangement to imperfectly matched aps-DNA would be indicated by a decrease or loss of the excimer fluorescence since the 5′ ends of the two strands in aps-DNA are far from each other. Addition of netropsin (1.8 drug per duplex) was accompanied by a decrease of the excimer fluorescence, which was complete after 16 hours (Fig. 7).

Triple helix formation

The duplex poly(dT)·poly(dA) can form a triple helix with poly(dT) (16, 17). In the triplex poly(dT)·poly(dA)·poly(dT), the third strand poly(dT) is attached to double helical poly(dT)·poly(dA) by hydrogen bonds of the Hoogsteen type. The thymines of the third strand are hydrogen-bonded to the adenines of the initial double strand at N7 and at the hydrogen of the N6 amino group that is not involved in the A·T Watson−Crick base pairing. These hydrogen-bonding sites for the binding of the additional thymine strand are the same for an initial ps-DNA or aps-DNA duplex. Thus in the $(dA)_{10}\cdot(dT)_{10}$ part of the duplex of ps-D1·D2 as well as of aps-D1·D3, triple helix formation by addition of equimolar amounts of $(dT)_{10}$ should be possible in principle. Formation of a DNA triple helix of this type T·A·T is indicated in the infrared spectra by disappearance of vibration bands at 1622 cm^{-1} and 1641 cm^{-1} (18, 19). In the duplexes ps-D1·D2 and aps-D1·D3, 40% of the base pairs constitute a tract of $(dA)_{10}\cdot(dT)_{10}$, which should be able to form a triple helix with $(dT)_{10}$. Addition of $(dT)_{10}$ to aps-D1·D3 induced spectral changes in agreement with that expected for formation of a triplex involving 40% of the whole sequence: the intensity of the band at 1622 cm^{-1} decreased significantly, but the intensity of the 1641 cm^{-1} band was considerably reduced as well (Fig. 8). Addition of $(dT)_{10}$ to ps-

D1·D2 induced several spectral changes. Again, the intensities of the infrared bands at 1622 cm^{-1} and at 1641 cm^{-1} decreased as expected for a 40% involvement in a triplex (Fig. 8). The apparent triplex formation of ps-DNA, however, was accompanied by shifts of the two carbonyl stretching bands form 1685 cm^{-1} to 1693 cm^{-1} and from 1668 cm^{-1} to 1663 cm^{-1}. The same shifts were observed for the interaction of ps-DNA and minor-groove binding drugs. Addition of netropsin with a ratio of 0.2 drug/base pair did not affect the spectrum of the triplex formed by D1·D3 (aps-DNA) and $(dT)_{10}$. In contrast, the band at 1622 cm^{-1} in the spectrum of the triplex of ps-D1·D2 and $(dT)_{10}$ reappeared partially in presence of netropsin (spectra not shown).

DISCUSSION

The reverse Watson–Crick hydrogen-bonding scheme of parallel-stranded DNA is supported by the infrared spectra

Duplex formation of ps-DNA is accompanied by a shift of both C2=O2 and C4=O4 stretching vibration of thymine; the band involving mainly the C2=O2 stretching vibration is shifted from 1696 cm^{-1} (aps-DNA) to 1685 cm^{-1}, and the band involving mainly the C4=O4 vibration from 1663 cm^{-1} to 1668 cm^{-1} (± 1 cm^{-1}) for D1·D2 and D3·D4, respectively (Table 1). In the dG·dC-containing pair, ps-D5·D6 and aps-D5·D7, the respective C2=O2 vibration shifts from 1698 cm^{-1} to 1690 cm^{-1}. In the spectra of all six double-helical systems listed in Table 1, bands occur at 1641 cm^{-1} and at about 1622 cm^{-1}. Increase of temperature dissociates the two strands of a duplex, as shown for example, in the infrared spectra of the ps-D3·D4 as a function of temperature (Fig. 3). The vibration band at 1641 cm^{-1} disappears completely. This band is a sensitive indicator of duplex formation in aps-DNA as well as in ps-DNA (Table 1). At 52°C, however, the dissociation of the duplex ps-D3·D4 into the two single strands D3 and D4 is incomplete as shown by the coexistence of the bands at 1685 cm^{-1} and at 1697 cm^{-1}. The spectrum of the 'melted' duplex aps-D3·D4 should be comparable to the added spectra of the two single strands D3 and D4. The spectra of the single strands, however, are strongly dependent on the concentration. Single strands can form mismatched 'duplexes' favored at higher concentrations but may be also able to form intramolecular partially base-paired structures through loops, hairpins etc. In the spectra of the single strands, the band at 1641 cm^{-1} disappears (Table 1) or is strongly diminished in the more dilute solution. This band can be attributed to the formation of base-paired A·T structures. Base pairing of aps-DNA is of the Watson–Crick type, i.e. the C4=O4 but not C2=O2 of thymine is involved in hydrogen-bonding. The base pairing scheme of ps-DNA, however, is different as indicated by the shift of both C=O vibrations of thymine. Hoogsteen base pairing would change the contact sites of adenine of A·T but not of thymine (16). We can exclude Hoogsteen base pairing for ps-DNA. However, reverse Watson–Crick as well as reverse Hoogsteen base pairing inverts the engagement of the two C=O groups in thymine; C2=O2 is now involved in hydrogen bonding but not C4=O4. Hydrogen bonding is expected to decrease the electron density of the C=O double bond that would lower the force constant and concomitantly the frequency of the C=O stretching vibration. Therefore, the stretching vibration of the released C=O has a higher frequency than the engaged C=O. Consequently, in reverse Watson–Crick or reverse Hoogsteen base-pairing of dA·dT, the frequency of the stretching vibration of C2=O2 is expected to decrease, but the frequency of the stretching vibration of the C4=O4 should increase compared to the Watson–Crick base pairing. The observed shifts agree with the expected behavior. A resolution between reverse Watson–Crick and reverse Hoogsteen base-pairing can be achieved by inspection of other vibrations. In Hoogsteen and reverse Hoogsteen base pairing, the exocyclic amino group and N7 of adenine are involved in base pairing. Infrared studies of triple helices have shown that a band at 1622 cm^{-1}, involving bending motions of the exocyclic amino group, disappears (19), and another band at 1483 cm^{-1} assigned to an adenine vibration involving N7 (20, 21) is affected when a third $d(T)_n$ strand is bound to the $d(T)_n \cdot d(A)_n$ duplex. In ps-DNA, however, the bands at 1622 cm^{-1} and at 1483 cm^{-1} do not change in intensity when compared to aps-DNA (Figs. 1 and 2). These results exclude reverse Hoogsteen base pairing but strongly support reverse Watson–Crick base pairing in ps-DNA. The infrared results are in complete agreement with conclusions drawn from a Raman spectroscopic study (9), in which other significant spectral changes in the region of 1150–1400 cm^{-1} were reported. By contrast, we could find no significant differences between the infrared spectra of aps-D1·D3 and ps-D1·D2 in D_2O (not shown) as well as in H_2O solution outside the carbonyl stretching vibration region (Fig.2). All the marker bands characteristic of B-DNA (22) are found in the infrared spectra of aps- as well as of ps-DNA at 1425, 1344, 1328, 1281, 895 and 841 cm^{-1}. On the other hand, the marker bands for A-DNA at 1418, 1401, 1335, 1274, 879, 866, and 808 cm^{-1} (22), are absent in the spectra of both DNAs (Fig. 2). We conclude that the structural parameters reflected in the Raman spectra on the one hand and in the infrared spectra on the other hand are quite different. Subtle differences in the base geometry obviously are more sensitively reflected in the Raman than in the infrared spectra. Infrared spectroscopy, on the other hand, is superior for detecting changes in base pairing that are reflected in the region of the double-bond vibrations between 1500 cm^{-1} and 1800 cm^{-1}.

Parallel-stranded DNA is more sensitive against dehydration than antiparallel-stranded DNA

The infrared spectra of DNA films are changed by the RH to which they are exposed (23, 24). Dependent on the base composition and on the counterions, conformational transitions of DNA can occur as a consequence of dehydration, as for example in the B-A or the B-C transitions (19). Poly(dA)·poly(dT) never assumes the A-form even at low values of RH (25). However, as shown by the infrared linear dichroism of oriented DNA films, rigorous dehydration is accompanied by a loss of all structural order (26). We assign tentatively the decrease of the band at 1055 cm^{-1} accompanying progressive dehydration to the loss of ordered DNA structure. It can be seen by inspection of the infrared spectra of films of aps-D1·D3 and ps-D1·D2, that the ordered B-like structure of ps-DNA collapses at much less dehydration (higher values of RH) than the structure of aps-DNA. In Fig. 4, the spectrum of ps-DNA at RH = 76% is shown to be similar to the spectrum of aps-DNA at RH = 47%. We conclude that ps-DNA is stable only at a high degree of hydration, and is very sensitive to dehydration. That is, the ordered structure of ps-DNA collapses even at a very mild reduction of the water content. As was reported previously, reduction of the water activity by addition of high amounts of NaCl (1.6–5M) destabilized ps-D1·D2 and ps-D5·D6

significantly more than aps-D1·D3 (27). This finding seems to agree with our results.

Minor groove binding drugs induce cooperative dissociation of ps-DNA and a rearrangement to imperfectly matched aps-DNA

The interaction of ps-D1·D2 with the three non-intercalating and minor-groove binding drugs netropsin, distamycin A and Hoechst 33258 is accompanied by significant changes in the infrared spectrum of ps-DNA. The most striking change is the shift of both C=O stretching vibrations from 1685 cm^{-1} to 1697 (1694) cm^{-1}, and from 1668 cm^{-1} to 1663 cm^{-1} (Fig. 5). By contrast, no changes in the spectra of aps-D1·D3 are observed in this region. However, the intensity of the bands at 1328 cm^{-1} and 1304 cm^{-1} decreases as a consequence of drug binding in H_2O solution of both ps-DNA and aps-DNA (not shown). Recently it was shown that the interaction of these drugs with poly(dA)·poly(dT) and poly(dA-dT)·poly(dA-dT) is reflected differently in the infrared spectra. Binding of netropsin or Hoechst 33258 to alternating A-T sequences induces a complex band at 1685 cm^{-1}, but no shifts of the infrared bands between 1800−1600 cm^{-1} were observed with poly(dA)·poly(dT) (14, 28). However, distamycin A shifts the C2=O2 stretching band from 1696 cm^{-1} to 1703 cm^{-1} (19). The aps-DNA D1·D3 is more like poly(dA)·poly(dT) and less like poly(dA-dT)·poly(dA-dT). The drug-induced shifts of both C2=O2 and C4=O4 stretching vibrations of ps-DNA approaches the respective band positions of the aps-DNA. There are at least two explanations: (i) ps-DNA is dissociated by the drugs and converted into imperfectly matched aps-DNA; (ii) the shifts are due to formation of drug-DNA complexes, leaving the DNA in parallel-stranded duplex form.

According to the first mechanism, ps-DNA has to dissociate into two single strands and reform an aps-DNA duplex with several mismatches, an example of which is the following structure involving two strands of D1 and one of D2. The underlined 17 base pairs are matched, the other 8 base pairs are mismatched.

(D1) 5′ AAAAAAAAAATAATTTTAAATATTT AAAAAA-
AAATAATTTTTAAATATTT 3′
(D2) 3′...AAATATTTAAAATTATTTT-TTTTTT... 5′

In this case, the ps-DNA duplex is expected to be destabilized by addition of drugs with preferential interaction with such alternative structures. We have measured the UV melting curve of ps-DNA in the presence of netropsin (input ratios 0.01−0.2 drug/base pair) under the same conditions as in the infrared measurements but at much lower DNA concentration (~0.1 mM). The melting temperature of ps-DNA increased monotonously from 44°C to 53°C with increasing netropsin concentration (data not shown). A direct interaction of netropsin with ps-DNA might be expected to show a biphasic melting curve reflecting the contribution from the uncomplexed ps-DNA (at lower temperatures) and ps duplexes with bound netropsin complex (at higher temperatures). However, biphasic melting is not expected if netropsin induces a rearrangement of ps-DNA into mismatched aps-DNA, in which case numerous species should occur. This situation would appear comparable with the experimental results but the latter do not provide unambiguous evidence in favor of a drug-induced rearrangement. The crucial experiment was the effect of netropsin addition on the excimer fluorescence of the ps-D3·D4 duplex that was labeled with pyrene covalently attached to the 5′-end of each strand via an alkyl linker. The pyrene excited state dimer, the excimer, has a characteristic unstructured fluorescence in the region of 430−600 nm. Its formation requires that the two pyrene partners are in close proximity. Thus the excimer signal can be used to reveal the existence of a parallel-stranded duplex (3). It is not observed if the duplex is disrupted or if the strands adopt an antiparallel orientation. The fluorescence spectra presented in Fig. 7 display a significant decrease of the excimer fluorescence upon addition of netropsin to pyrene labeled ps-D3·D4 with kinetics in the hour range at 7°C. Slow kinetics is expected for the required dissociation of the ps duplex and optimization of the mismatched structure well below the melting temperature. Remeasurement of the sample after 16 hours of equilibration showed a total lack of excimer fluorescence. These results suggest that netropsin induces a conformational change of the ps duplex to an aps secondary structure with mismatches. Such a ps-to-aps transition explains the similarity of the infrared spectra of the ps-DNA and aps-DNA drug complexes as discussed above. This result is in accordance with the proposed drug-induced rearrangement to a mismatched aps-DNA and explains the extreme similarity of the infrared spectra of the drug complexes of ps-DNA and aps-DNA discussed above. Several drug-induced spectral changes in the 1500−1300 cm^{-1} region (14, 21) are almost identical in ps-DNA and aps-DNA (not shown). The surprising result that the drugs also induce significant shifts of the carbonyl vibrations even at a very low drug/DNA ratio (Fig. 6) can be interpreted by a drug-induced dissociation of ps-DNA. One netropsin per ps-DNA duplex is sufficient to induce the transition from perfectly matched ps-DNA into imperfectly matched aps-DNA. The three drugs are known to be minor-groove binding ligands; ps-DNA, however, has no distinct minor groove; both grooves are of nearly equal size (7). The high binding affinity of the minor-groove binding drugs to aps-DNA and particularly to AT binding sites (10) is the driving force for the observed transition. The energy stabilization due to complex formation of these drugs with aps-DNA is greater than due to the loss of the ps-DNA structure. Thus, the apparent stabilization of ps-DNA by netropsin observed in the UV melting experiment may be due to the gain of binding energy of the (imperfect) aps-DNA-netropsin complex that overcompensates the drug-induced destabilization of ps-DNA. At 7°C, the optimal matching of Watson−Crick base pairs in the rearranged aps-DNA requires several hours (Fig. 7). Our conclusion drawn from of infrared and fluorescence spectroscopy results is supported by circular dichroism studies of the netropsin binding to ps-D1·D2 and cross-linked ps-D1·D2 (Ch. Zimmer and I. Förtsch, personal communication). The results of these studies are in excellent agreement with our findings of a drug-induced rearrangement of ps-DNA to a mismatched aps-DNA. In a recent report on the netropsin binding to the same ps and aps duplexes studied here no netropsin induced changes of the ps conformation were noted (29). The results were based on CD measurements and exploited the induced Cotton effect of netropsin binding. The CD spectra of the ps and aps conformation can be distinguished only in the region of 260−280 nm (11). However, this part of the spectrum changes upon binding of netropsin and it is therefore difficult to assess whether any changes in the DNA conformation take place. The presence of isodichroic points in the CD spectra of ps-DNA and their absence after netropsin addition suggests the presence of more than two species in the aps-DNA-netropsin system in the course of drug titration. When comparing the spectra of ps and aps conformation at saturating drug concentration, they appear to be very similar and we conclude that the CD signal is not suitable for detecting a

ps-to-aps transition induced by netropsin. The difference in binding enthalpy of ps- and aps-DNA disappears in the thermodynamic results of Rentzeperis & Marky (29) at a binding ratio of 1−2 netropsin per duplex, in reasonable agreement with our infrared results shown in Fig. 6 with a plateau reached at addition of approximately one netropsin per duplex.

Parallel-stranded DNA is unable to form a triple helix

Triplex formation is known for several DNA sequences, the triplex scheme T·A·T being one of the best investigated examples (17). In this triplex, a duplex $(dT)_n \cdot (dA)_n$ of the common Watson−Crick type binds a third strand $(dT)_n$ *via* a reverse Hoogsteen base pairing scheme. The N7 of adenine as well as the N6 amino hydrogen of adenine — which is not engaged in the Watson−Crick base pairing — are involved in this base pairing scheme.The thymines of the third T strand are hydrogen bonded to the adenines by the N3 imino protons and the C4=O4 carbonyl. The third T strand is parallel to the A strand (30). In a ps-DNA triplex of the type T·A·T, the third N7 T strand would be parallel to both the A strand and the first T strand. In the ps-DNA duplex, the N6 amino group and the N7 sites are available in principle for binding of the third strand. From this point of view, the possibility exists that ps-D1·D2 can accommodate a third $d(T)_{10}$ strand along its $d(A)_{10} \cdot d(T)_{10}$ stem. Such an event would be expected to lead to a reduction of the intensity of the 1622 cm^{-1} band by 40% since only 10 out of 25 base pairs would be involved in the triplex. Infrared spectra show clearly the formation of a triple helix by the very strong decrease in intensity of the vibration bands at 1622 cm^{-1} and 1641 cm^{-1} (Fig. 8). The band at 1622 cm^{-1} has been assigned to a ND_2 bending vibration of adenine coupled to pyrimidine ring vibrations (31, 19), and the band at 1641 cm^{-1} to double-bond vibrations of the thymine ring (19). As reported previously, the disappearance of these two vibration bands is a strong evidence of triple helix formation involving the N7 of adenine (19). However, the interaction of $d(T)_{10}$ with ps-DNA is also accompanied by a shift of both C=O stretching vibrations to the frequencies characteristic of the respective aps-DNA (Fig. 8). This feature is very similar to that found for the ps-DNA-drug interaction. The question thus arises whether the observed effect in the infrared spectrum is due to triplex formation retaining ps-DNA, or to triplex formation after rearranging the initial ps-DNA to an imperfectly matched aps-DNA. We interpret the changes in the infrared spectra, by analogy to situation pertaining to drug interaction, as suggesting a rearrangement of a mismatched aps-duplex prior to the triplex formation. Again, we assume dissociation of the ps-DNA and rearrangement to imperfectly matched aps-DNA that interacts with $(dT)_{10}$ under formation of a triple-stranded complex aps-$[(dT)_{10} \cdot (dA)_{10}] \cdot (dT)_{10}$. The imperfection of the system is illustrated by the high sensitivity of this triple helix against netropsin. In presence of 0.2 netropsin/base pair, the 1622 cm^{-1} band reappears in the spectrum of the apparent ps-DNA triplex. The spectrum of the triplex of aps-DNA and $d(T)_{10}$, on the other hand, does not show a 1622 cm^{-1} band upon addition of netropsin. The triplex starting from the aps-D1·D3 obviously is more stable than that based on the imperfectly matched and aps rearranged triplex originating from ps-D1·D2. However, fluorescence experiments with 5′-pyrene labeled ps-D3·D4 did not detect a rearrangement to aps-DNA in the presence of $d(T)_{10}$. Apparently, the high DNA concentration used in the infrared experiments is required for rearrangement of ps-DNA to imperfectly matched aps-DNA with subsequent formation of the triple helix.

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