Nonoriented d(CGCGAATTCGCG)$_2$ Dodecamer Persists in the B-Form Even at Low Water Activity

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The self-complementary Drew−Dickerson dodecamer (DDD), d(CGCGAATTCGCG)$_2$, was the first oligonucleotide for which a single-crystal X-ray structure of B-DNA has been detemined,[1−4] and because of its biological importance it remained the focus of biophysical studies up to now. It contains the recognition site of the *Eco*RI restriction enzyme and is frequently used in gene recombination techniques.[5] In the crystal and in aqueous solution it is in the B-form.[1−4,6] Transition of the B-form of DDD into the A-form has been assumed to occur at low water activity,[7−9] because this is the behavior usually observed for B-DNA structures.[10−14] Here we show by FT-IR spectroscopy that the sodium salt of the d(CGCGAATTCGCG)$_2$ dodecamer persists in the B-form even when the water activity is low, that is relative humidity is 65%, and Γ (water molecules per nucleotide) is 6. Our findings are important because they provide the experimental basis for interpretations of molecular dynamics (MD) simulations of DDD in ethanol/water solution[7,8] and of solid-state NMR studies of hydrated DDD fibers.[9] The surprising persistence of the dodecamer's structure in the B-form must be caused by its base sequence.

Hydrated films of nonoriented DDD of required Γ values were obtained by placing aqueous solutions onto AgCl disks and equilibrating these over saturated salt solutions, with 92.5, 84.3, and 65% relative humidity, in the same manner as described in our FT-IR spectroscopic studies of B-DNA's conformer substates.[15,16] These relative humidities are expected to give Γ values of 20, 11, and 6 (see Table 1 in ref 17). The AgCl disks with the equilibrated hydrated films were quickly covered with a second AgCl disk, and then the two disks taped and transferred into the FT-IR spectrometer. The Γ values were further calculated according to Falk et al.,[18] from the ratio (R) of the measured absorbance in the OH stretching band region at ∼3400 cm^{-1} to that of the antisymmetric streching vibration of the PO_2^- group (ν_{as} PO_2^-) at ∼1220 cm^{-1} ($\Gamma = 4.52(R - 0.86)$, $R = 2.2$ and 4.0). This gives Γ values of 14 and 6 for the less hydrated films, and we expect these values to be more accurate because changes in hydration during taping of the films are avoided. Once taped, these R values remain constant. Band area ratios obtained by integration are consistent with the R values. For comparison, three hydrated films of DNA from salmon testes were prepared in the same way, with Γ values of ∼20, 12, and 6, where B-DNA decreases from 100 to ∼50 and ∼10%, and A-DNA increases.[12,19]

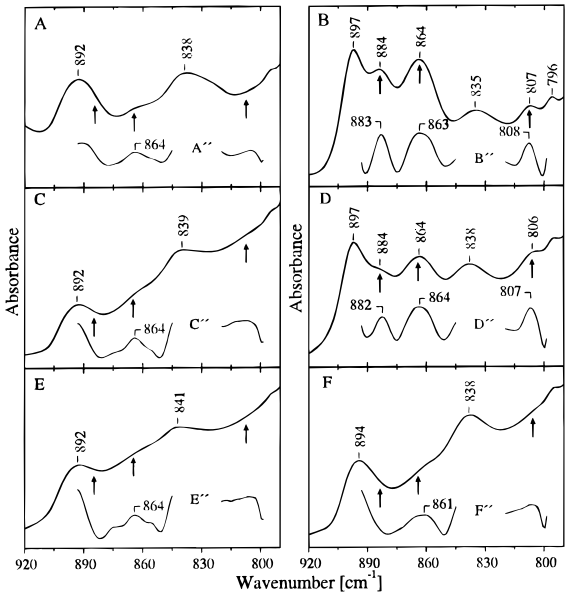

Figure 1. Comparison of the infrared spectral region containing sugar−phosphate backbone vibrations (from 920 to 790 cm^{-1}) of the d(CGCGAATTCGCG)$_2$ dodecamer (DDD, left) with that of DNA from salmon testes (right). Spectra A, C, and E were obtained from hydrated films of DDD containing 6, 14, and ∼20 water molecules per nucleotide, spectra B, D, and F from hydrated films of DNA from salmon testes with 6, 12, and ∼20 water molecules per nucleotide. Note the absence of the A-marker bands at 882, 864, and 806 cm^{-1} (marked) in spectra A, C, and E, and in the second derivatives A″, C″, and E″ (shown inverted, 11-point deconvolution). DDD was obtained from MWG BIOTECH, purified as HPSF (high-purity, salt-free). Thus, excess NaCl was avoided. This is important because NaCl can stabilize the B-form.[25] Spectra were recorded at 290 K. For further experimental details, see refs 15 and 16.

In Figures 1 and 2, we compare infrared spectra of DDD (left) with those of DNA from salmon testes (right). The hydration level of DDD increases from $\Gamma = 6$ (for A) to $\Gamma = 14$ and ∼20 (C and E). The corresponding hydration levels of DNA from salmon testes are $\Gamma = 6$ (for B) and $\Gamma = 12$ and ∼20 (D and F). Infrared marker bands for A-DNA are taken from ref 20 and are indicated by arrows. Figure 1 shows the spectral region containing sugar−phosphate backbone vibrations which contains several characteristic A- and B-DNA marker bands.[20] Infrared A-DNA marker bands are at 882, 864, and 806 cm^{-1} (marked), whereas the B-DNA marker band is at 840 cm^{-1}.[20] In the spectra of DNA from salmon testes (right), the intensity of the three A-DNA marker bands increases in comparison to that of the B-DNA band at ∼840 cm^{-1} on going from F ($\Gamma \approx 20$) to D and B ($\Gamma = 12$ and 6). In hydrated films of DDD, only the B-DNA band at ∼840

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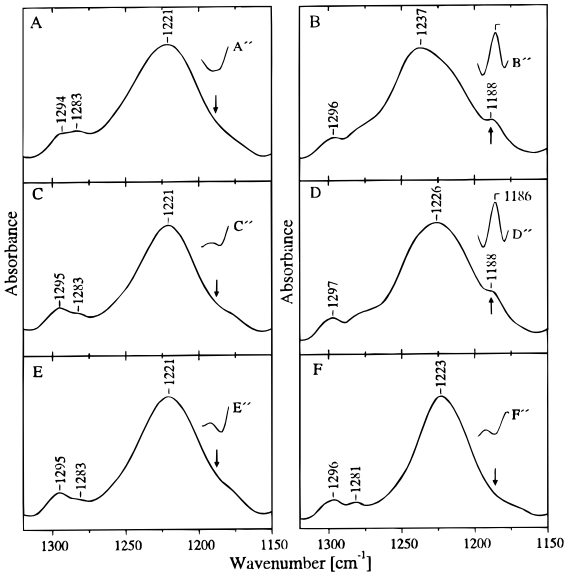

Figure 2. Comparison of the infrared spectral region containing the antisymmetric stretching vibration of the ionic phosphate group (from 1350 to 1150 cm^{-1}) of the d(CGCGAATTCGCG)$_2$ dodecamer (DDD, left) with that of DNA from salmon testes (right). Spectra A, C, and E were obtained from hydrated films of DDD containing 6, 14, and ~20 water molecules per nucleotide, spectra B, D, and F from hydrated films of DNA from salmon testes with 6, 12, and ~20 water molecules per nucleotide. Note the absence of the A-marker band at ~1188 cm^{-1} (marked) in spectra A, C, and E, and in the second derivatives A″, C″, and E″ (shown inverted, 11-point deconvolution).

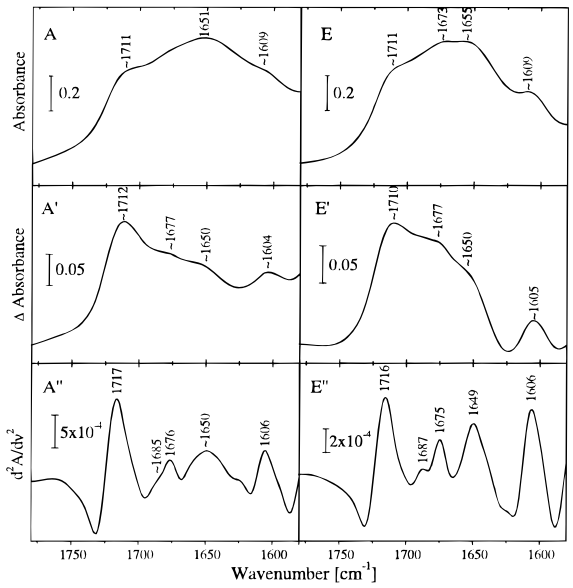

Figure 3. Infrared spectroscopic evidence for double-stranded structure in the d(CGCGAATTCGCG)$_2$ dodecamer (DDD) films via the strong band centered between 1708 and 1718 cm^{-1} (from 1780 to 1580 cm^{-1}).[21−24] Spectra A and E were obtained from DDD films containing ~20 and 6 water molecules per nucleotide (labeled as in Figures 1 and 2), spectra A′ and E′ after subtraction of water's deformation band, and spectra A″ and E″ the second derivatives of A and E (shown inverted, 23-point deconvolution). Vertical bars give the ordinate scales.

cm^{-1} is observable for $\Gamma \approx 20$, 14, and 6 (E, C, and F), and A-DNA marker bands are absent even in the second derivate curves A″ and C″ (inverted). We note that the weak feature at ~860 cm^{-1} in A, C, E, and F is not from a small amount of A-DNA but is observable in infrared spectra consisting of 100% B-DNA (see Figure 6.6 in ref 20).

In Figure 2, we compare for the same hydrated films the spectral region of ν_{as} PO_2^-. In the infrared spectra of DNA from salmon testes, the marker band for A-DNA centered at ~1188 cm^{-1} is absent in F, and it increases with decreasing hydration from D to B. These spectral changes become more clearly observable in the form of their second derivatives inserted as F″, D″, and B″. These spectral changes are absent in both the infrared spectra of DDD (A and C) and their second derivatives (A″ and C″). In the spectra of DNA from salmon testes, a shift of the peak maximum of the broad band from ν_{as} PO_2^- to higher wavenumbers with decreasing hydration (from F to D and B) is a further indicator for conversion of B-DNA (peak maximum at 1225 cm^{-1}) into A-DNA (at 1240 cm^{-1}). This shift is absent in the infrared spectra of DDD (from E to C and A).

The comparison of the infrared spectrum of DDD with that of B-DNA from salmon testes is meaningful because Raman spectra of DDD and of B-DNA from salmon testes show "that both have very similar B-type conformations".[6] In addition, the infrared spectral features of B-DNA's conformer substates are remarkably similar (see Figure 2 in ref 16).

In Figure 3, we show that the double-stranded structure is formed in DDD at high and low water activity. The spectral region of the double-bond stretching vibrations of the bases undergoes on base pairing and stacking interactions the most profound changes, and an intense infrared band centered between 1708 and 1718 cm^{-1} is a characteristic marker band for double-stranded DNA.[21−24] This band is absent in single-stranded DNA.[21,22] In curves A and E of Figure 3, we compare for this spectral region infrared spectra of the hydrated DDD films with $\Gamma \approx 20$ (A) and $\Gamma = 6$ (E). The double-bond stretching vibrations of the bases are superimposed on water's broad deformation band, and a shoulder at ~1711 cm^{-1} in both A and E indicates the double-stranded structure. This shoulder becomes the prominent peak in curves A′ and E′ after subtraction of the water band. In A′ and E′, peak maxima are at the same positions, but the overall shape of the curves differs. We attribute this to incomplete subtraction of the water band: its band shape is altered slightly by interaction of the water with the dodecamer, and thus, the water band cannot be subtracted quantitatively.[22] Curves A″ and E″ are the second derivative curves (shown inverted) of A and E. The peak maximum of the most intense feature is centered at 1717 (A″) and 1716 (E″) cm^{-1}. We thus conclude that the double-stranded structure is formed in the hydrated DDD film with $\Gamma \approx 20$, and that it is preserved at low water activity in the DDD film with $\Gamma = 6$.

Transition of B-DNA to A-DNA can also be induced in aqueous solution by lowering the water activity by addition of sufficient ethanol (e.g., in 85% (v/v) ethanol/water solution).[10−14] Based on these previous studies and our study of hydrated films of low water activity, we expect that the B-form of DDD also persists in 85% ethanol/water solution. This suggests that transitions of DDD from B- to A-DNA in 85% ethanol/water solution observed in MD simulations are driven by the force field.[7,8] On the other hand, the unexpected MD simulation of DDD by Sprous et al.[8] could be consistent with the experiment because they had reported that "the B-structure in the 85% (v/v) ethanol/water remains B-form even after 2.0 ns".

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