Thermal Difference Circular Dichroism of Pf1 Filamentous Virus and Effects of Mercury(II), Silver(I), and Copper(II)†

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ABSTRACT: The circular dichroism (CD) of Pf1 filamentous virus has been examined over the temperature range 0–40 °C, in the absence and presence of Hg(II), Ag(I), and Cu(II). Thermal difference CD spectra were obtained by subtraction of spectra recorded above and below a thermally induced structure transition near 12 °C. The thermal difference spectra look like they arise from shifts in two exciton bands, one centered at 230 nm and the other at 290 nm. The amplitudes on either side of a crossover at 230 nm are 10 times those of a crossover at 290 nm. It is proposed that the difference spectra result from thermally induced shifts in coupled oscillator interactions between Tyr_{40} residues of the coat protein and the guanine and cytosine bases of the DNA. Metal ions can reduce or block these shifts. The changes in ellipticities at 220, 237, and 270 nm induced by changing the temperature have inflections near 12 °C. Ag(I) and Hg(II), which are known to bind to the DNA bases in Pf1, reduce or eliminate the inflections in the thermal profiles, depending on the metal ion type and concentration. Cu(II) ions do not affect the profiles. The spectral changes and the effects of the metal ions indicate intimate contact between the DNA bases and the protein subunits in the virion.

Pf1 is a filamentous virus of *Pseudomonas aeruginosa* (Takeya & Amako, 1966). It genome is a circular, single-stranded DNA molecule that extends along the length of the virus (2.0 μm) within a cylindrical coat of protein, such that there are two antiparallel DNA strands interacting with each other and with the protein. The genome size is about 7400 bases, and in the virion there appears to be very nearly one subunit per nucleotide (Wiseman & Day, 1977). Each protein subunit contains 46 amino acids, only two of which are aromatic, Tyr_{25} and Tyr_{40} (Nakashima et al., 1975), so that the aromatic spectral system consists of one base (any of A, T, C, or G) and two tyrosines. The DNA in Pf1 has the most extended naturally occurring nucleic acid structure, with a long axial nucleotide translation (5–6 Å), and the results of various spectral studies, including absorbance, fluorescence, CD,[1] Raman, and NMR, as well as Ag(I) and Hg(II) binding studies confirm that it is a unique structure (Day & Wiseman, 1978; Day et al., 1979, 1988; Casadevall & Day, 1982, 1983; Thomas et al., 1983; Cross et al., 1983; Marzec & Day, 1983). It has been proposed that the DNA in the Pf1 virion is a two-stranded helix with the phosphates in the center and the bases directed outward, interacting 1:1 with Tyr_{40} side chains and quenching their fluorescence (Day & Wiseman, 1978; Day et al., 1979, 1988b; Marzec & Day, 1983).

The X-ray fiber diffraction patterns of Pf1 virus are dominated by contributions from the protein coat (Marvin et al., 1974, 1987; Makowski, 1984), and no information about DNA structure in the virion has yet been extracted from the patterns, other than its location within a central core region (Marvin et al., 1974; Makowski & Caspar, 1978). The virus is known to undergo a reversible, thermally induced, transition in the helical symmetry of its protein coat in the vicinity of 12 °C which results in marked changes in its fiber diffraction pattern (Nave et al., 1979) and in a number of other physical properties, including its circular dichroism and Raman spectra (Hinz et al., 1980; Thomas et al., 1983; Makowski, 1984). The role of the DNA in the low-temperature protein coat symmetry transition is unknown.

In this paper we report CD spectra that were recorded at temperatures above and below the transition temperature. Thermal difference spectra generated from the data are unusual and are proposed to be examples of coupled electronic transitions of tyrosines and bases. The effects of Hg(II), Ag(I), and Cu(II) on the CD phenomena were also examined, to help establish the utility of these metals as structure probes for the filamentous viruses. The rationale behind the measurements was that since the DNA and protein components of Pf1 are linked in some way (Casadevall & Day, 1983) and since the transition involves a very small change in the packing of close-packed protein subunits (Nave et al., 1979), it would be of interest to examine the effects, if any, of ligands that bind to the DNA bases on the transition in protein coat symmetry.

Materials and Methods

Pf1 virus and its Ag(I) and Hg(II) complexes were prepared by adding small volumes (order of microliters) of stock solutions of $AgNO_3$ or $HgCl_2$ to solutions of Pf1 virus in selected buffers, as described previously (Casadevall & Day, 1982, 1983). Solutions of Pf1 and $CuSO_4$ were prepared in the same way. The parameter m characterizing a given solution is the moles of metal ion added per mole of nucleotide present. CD spectra were recorded in a Cary 60 spectropolarimeter equipped with a CD attachment and calibrated to give +7260 deg cm^2 dmol^{-1} at 290 nm for *d*-camphorsulfonic acid. A Hewlett-Packard 2801 quartz thermometer probe inserted into the solution measured the temperature. Data collection for thermal profiles at the indicated wavelengths required long periods for thermal equilibration at each temperature, but no photodegradation of the metal–virus complexes was observed. Indeed, one sample of Ag–Pf1 complexes at m = 3.0 was

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[1] Abbreviations: CD, circular dichroism; m, ratio of moles of metal ion added per mole of nucleotide.

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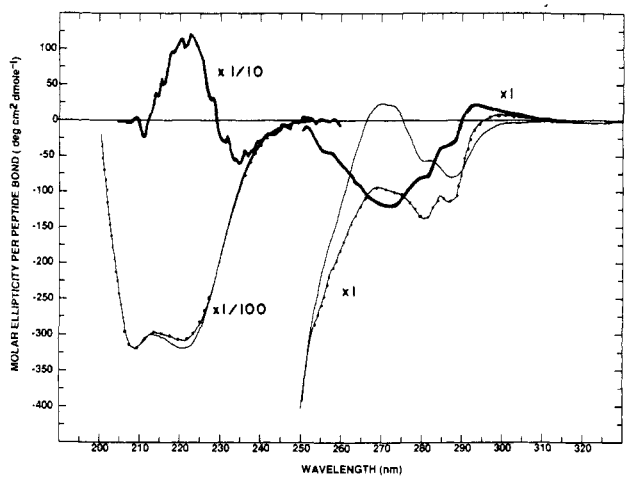

FIGURE 1: CD spectra of Pf1 in 0.1 M sodium phosphate buffer, pH 7, at 26 °C (dotted) and 3 °C (light solid). The optical path was 1.0 cm, and the Pf1 concentration was 0.45 mg/mL. Similar results were observed in 1 and 100 mM potassium phosphate. The difference spectrum (heavy solid) was generated by subtraction of the spectrum at 3 °C from the spectrum at 26 °C. Amplitudes for wavelengths below 250 nm were reduced by factors of 10 or 100 for this combined figure. Molar ellipticities expressed per nucleotide are 45-fold larger (see text).

exposed to the CD beam for about 20 h after which time the ellipticity at 220 nm was within 1 mdeg of that of a fresh sample. Further, no hysterisis effects were observed when temperatures were reached by cooling or warming. Some spectra were converted to digital form with the aid of a digitizing tablet; thermal difference spectra were generated by computer subtraction of such spectra.

Results and Discussion

CD of the Low-Temperature Transition of Pf1. The CD spectrum of Pf1 virus from 200 to 250 nm is like that of an α-helical protein in having local minima at 208 and 220 nm, with molar ellipticities of about −35 000 and −32 000 deg cm^2 dmol^{-1} per peptide bond, respectively (Day & Wiseman, 1978; Casadevall & Day, 1982). Above 250 nm, and over the temperature range from 20 to 40 °C, the CD spectrum of Pf1 has local minima at 280 and 287 nm, each with molar ellipticities of about −100 deg cm^2 dmol^{-1} per peptide bond (or −2250 per tyrosine residue or −4500 per nucleotide). Cooling Pf1 from 26 to 3 °C causes little or no change in ellipticity at 208 nm, a key wavelength for α-helix content estimations, but does cause the changes shown in Figure 1 (see also Figure 2). Thermal difference spectra are obtained as the difference between spectra at two temperatures, but whether the higher is subtacted from the lower or vice versa is arbitrary and immaterial in the present case. The thermal difference spectra show local extrema at approximately 220, 237, 270, and 295 nm, and crossovers occur near 230 nm and near 290 nm. The difference spectrum of Figure 1 looks like it consists of two separate, so-called exciton CD bands [see Cantor and Schimmel (1980) and Tinoco et al. (1980)], one centered at 230 nm and a weaker one centered at 290 nm. The temperature dependence of the ellipticities at 220, 237, and 270 nm are shown in Figure 3 (and the insert of Figure 2B), and for all three wavelengths there are inflection points at about 12 °C. The inflections occur in the temperature range in which Pf1 virus undergoes a well-characterized transition in the symmetry of its protein coat (Nave et al., 1979; Hinz et al., 1980), and we assign the inflections to the transition.

The thermal difference CD spectrum for the transition (Figure 1) is unusual. As stated in the introduction, the

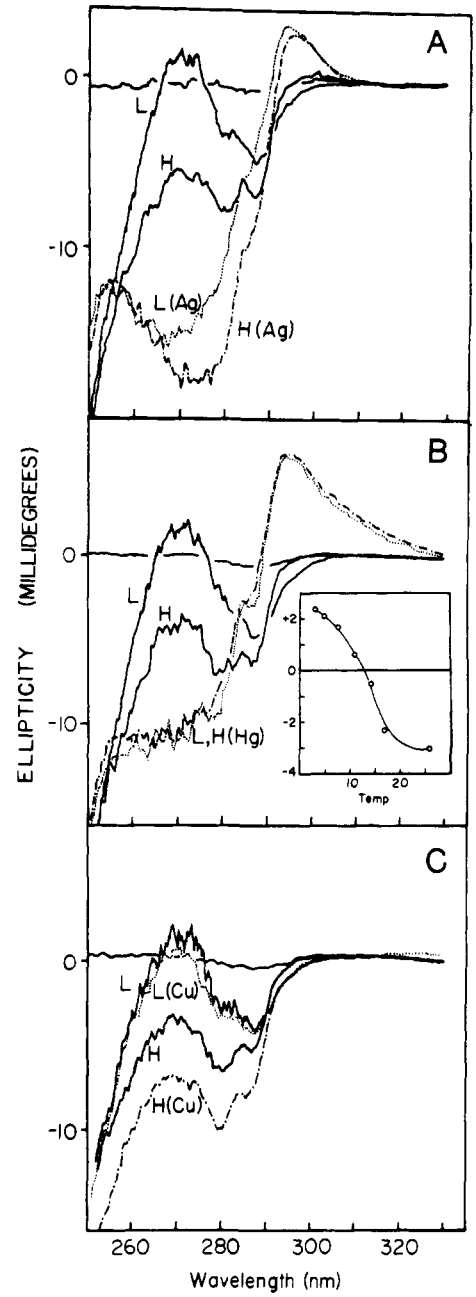

FIGURE 2: CD spectra of Pf1 virus at high (H) temperature (26 °C) and low (L) temperature (3–4 °C) in the presence and absence of Ag(I), Hg(II), and Cu(II). (A) Effect of Ag(I) (m = 0.5) in 100 mM pH 7.0, potassium phosphate buffer. The optical path length was 1.0 cm, and the virus concentration was 0.68 mg/mL. (B) Pf1 with and without Hg(II) (m = 1.0) in 0.15 M sodium borate buffer. The virus concentration was 0.68 mg/mL. Cooling the Hg–Pf1 solutions caused little change if any in the 250–300-nm region [compare L(Hg) and H(Hg)]. (C) Pf1 virus with and without Cu(II) at m = 0.5. The concentration of virus was 0.55 mg/mL [We have included, as the insert in (B), a typical thermal profile at 270 nm in order to show that the maximum change with temperature is near 12 °C. This particular sample was in 150 mM NaCl–15 mM sodium phosphate, pH 7.]

aromatic spectral system consists of one base and two tyrosines, and several arguments have been put forth for the existence of an everted DNA structure in Pf1 that would have the bases directed outward and available for direct interaction with the protein (Day & Wiseman, 1978; Day et al., 1979, 1988b; Marzec & Day, 1983). Although direct proof of such a DNA structure is not available, we are aware of no data that are inconsistent with it. An everted DNA model provides the basis for our proposal as to the source of the present observations.

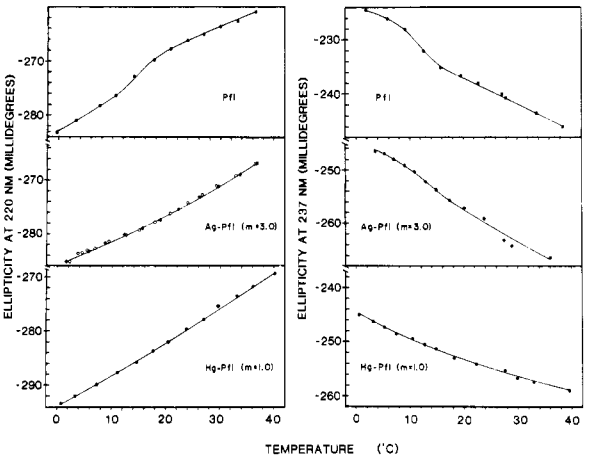

FIGURE 3: Plots of ellipticity versus temperature at 220 and 237 nm for Pf1 virus and its Ag(I) and Hg(II) complexes. The buffer was 150 mM borate, pH 8.6. For the temperature profiles at 220 nm the virus concentration was 0.09 mg/mL, and for the profiles at 237 nm it was 0.40 mg/mL. The optical path length was 1.0 cm. The data at 220 nm are for repeat experiments (open and filled points).

The line of reasoning is as follows. First, the CD negative double maxima at 280 and 287 nm can be reversibly and simultaneously shifted to a broad negative maximum at 305 nm, the shift having a characteristic pK of about 11.5 (unpublished results of L. A. Day and R. L. Wiseman). Titrations monitored by ultraviolet absorbance have shown that one of two tyrosines in the intact virion has a pK value of approximately 11.3 and the other cannot be titrated without disrupting the virion (Day & Wiseman, 1978). Further, the fluorescence of one of the two tyrosines has a pK of about 11.4 (Day et al., 1979). The fluorescent, reversibly titratable tyrosine is Tyr_{25} (Greulich & Wijnaendts Van Resandt, 1984). The fluorescence of the other tyrosine residue, Tyr_{40}, is extensively quenched (Day et al., 1979; Greulich & Wijnaendts Van Resandt, 1984). Therefore, since the pK value of 11.4 ± 0.1 is common to signals monitored by absorbance, fluorescence, and CD, we assign the negative CD maxima at 280 and 287 nm to Tyr_{25}. Tyr_{25} is located near the outer surface of the virus (Nave et al., 1981). The negative maxima are present in the CD spectra both at 3 and at 26 °C, suggesting that the 12 °C structure transition has only a small effect on Tyr_{25} environment.

Contributions from the DNA dominate the ultraviolet absorbance spectrum of the virus (Day & Wiseman, 1978), but there are no obvious DNA features in the CD spectrum; neither are there extrema near the DNA absorbance maximum near 260 nm, nor are there extrema above and below 260 nm from the well-known coupled oscillator, or exciton, interactions typical of A-form or B-form DNA and of base-stacked single-stranded polynucleotides (Ivanov et al., 1974; Tinoco et al., 1980). The absence of assignable DNA features is explained in part by the long axial nucleotide translation of 5–6 Å, which would make electronic interactions between adjacent bases weak.

The multiple features of the thermal difference CD spectrum are generated in a concerted, simultaneous manner. No isolated chromophore in the virus, responding individually to a changed environment, could generate these particular features, whether tyrosine or any of adenine, thymine, guanine, or cytosine. However, two or more chromophores could simultaneously generate them through coupled oscillator mechanisms. If a structure allows alignment of electronic transition dipoles of nearly the same energy, the excited states of the two groups can couple to produce CD bands of opposite signs on either side of a crossover near the common energy, or wavelength, of the transition (Tinoco et al., 1980).

We have considered tyrosine–tyrosine-coupled oscillator models, but these fail for different reasons. Neither Tyr_{40}–Tyr_{40} coupling nor Tyr_{25}–Tyr_{25} coupling is possible because the distance between the same residue on adjacent subunits must be greater than 10 Å on the basis of current models of the Pf1 virion structure (Makowski, 1984; Marvin et al., 1987; Marzec & Day, 1987). Tyr_{25}–Tyr_{40} coupling is difficult to reconcile with the independent alkaline titration behavior of these two residues described above and the fact that Tyr_{25} is located near the outside of the virus (Nave et al., 1981) and Tyr_{40} is in the domain of the subunit close to the DNA (Nakashima et al., 1975). Coupled oscillator interactions between neighboring bases in the DNA structure are unlikely, given the long axial nucleotide translation, and should they exist, they would be expected to be centered near 260 nm rather than near 230 and 290 nm.

Base–tyrosine-coupled oscillator models, however, are plausible. In general, stacking interactions between aromatic protein side chains and the bases of nucleic acids can contribute significantly to the stabilization of nucleoprotein structures (Kumar & Govil, 1984). In particular, the L_a band π–π^* transitions of tyrosine at 228 nm overlap in energy the π–π^* transition of cytosine at 230 nm, the only electronic transition of a base in this region, and the L_b band transitions of tyrosine at 280 nm overlap the guanine transition at 281 nm, the longest wavelength π–π^* of any base (Beavan & Holiday, 1952; Woody, 1977, 1978; Rizzo & Schellman, 1984). Hydrophobic environments in the virion could shift these transitions to longer wavelengths (Yanari & Bovey, 1960); the 275/282-nm absorbance doublet of free tyrosine in aqueous solution asserts itself as a 280/287-nm doublet in the CD spectrum of Pf1. The L_a band and the L_b band transition dipoles of tyrosine are orthogonal to each other (Hooker & Schellman, 1970), and current models of Pf1 DNA structure (Day et al., 1988) allow base planes to stack with tyrosyl aromatic groups. Thus, the geometries and the energies involved would allow the electronic transitions of tyrosine and guanine and cytosine to couple. The difference CD would then result from thermally induced small changes in the mutual orientations of the overlapping base–tyrosine dipoles of an inverted DNA surrounded by protein. This proposed mechanism is not unlike the A-form to B-form transition of conventional DNA whereby small changes in dipole orientations lead to pronounced changes in CD spectra (Ivanov et al., 1974; Johnson et al., 1981).

Effects of Metal Ions on the Low-Temperature Transition of Pf1. We have previously shown by absorbance, CD, and Raman titrations that Hg(II) and Ag(I) bind to the bases of Pf1 virus (Casadevall & Day, 1982, 1983; Day et al., 1988a). Addition of Hg(II) to m = 1.0, the equivalence point established earlier, resulted in a CD spectrum between 250 and 320 nm which showed no variation with temperature (Figure 2B); also, plots of ellipticity versus temperature at 220 nm and at 237 nm showed no inflections (Figure 3). Addition of Hg(II) at 4 °C (m = 1.0) produced the same spectrum as cooling Hg–Pf1 complexes (m = 1.0) from 26 to 4 °C. The reversible binding of Hg(II) to Pf1 abolishes the inflection at the three wavelengths we investigated and, by this criterion, appears to block the transition completely.

Additions of Ag(I) reduce some, but not all, of the features of the transition. Cooling the Ag–Pf1 complexes at m = 0.5 induced a maximum change in molar ellipticity at 280 nm of 4200, very nearly the same as that in the absence of Ag(I) (Figure 2A). Ag–Pf1 complexes for $m > 0.5$ were studied in

0.15 M, pH 8.6, 150 mM sodium borate buffer (Casadevall & Day, 1983). At 220 nm, the plot of ellipticity versus temperature for $m = 1.0$ shows the usual inflection near 12 °C (data not shown), but for $m = 3.0$ there is no inflection (Figure 3). At 237 nm, however, there is still the suggestion of an inflection point at $m = 3.0$. Addition of Ag(I) at 4 °C produced the same spectrum as obtained on cooling the Ag–Pf1 complexes from 26 to 4 °C. At $m = 1.0$, Ag–Pf1 complexes behave much like native Pf1 with respect to the transition by the CD criterion. By $m = 3.0$, sites on the bases are probably saturated. In contrast, addition of Hg(II) to $m = 0.5$, half its equivalence ratio, reduces the amplitude of the inflection at 270 nm to about one-third of that observed in the absence of Hg(II) (data not shown). Thus, structure perturbations caused by Ag(I) appear to be smaller than those caused by Hg(II). This is consistent with our earlier observation that Hg(II) binding causes larger changes in Pf1 sedimentation rates than does Ag(I) binding. Under conditions of reduced inflection amplitudes, for both Ag–Pf1 and Hg–Pf1 solutions, the transition may be partially blocked over the entire population or completely blocked over part of the population.

Addition of Cu(II) to Pf1 in 0.15 M sodium borate, pH 8.6, resulted in increased apparent absorbance throughout the 230–300-nm range, but its wavelength dependence indicated an increase in light scattering rather than, or in addition to, pure absorbance changes, if any (data not shown). Similarly, Cu(II) decreased the ellipticity broadly over the 250–300-nm range without changing the shape of the spectrum (Figure 1C). Cu(II) ions have little effect at 270 nm on the thermally induced structural transition as monitored by CD changes at 270 nm. Further, highly viscous glasses of gels formed when Cu(II) was added to Pf1 solutions in unbuffered 0.1 M NaCl. The observed spectral changes together with the increases in light scattering and solution viscosity strongly suggest that the Cu(II) ions are interacting primarily with the protein coat of Pf1. Given these observations, the Cu(II) ion does not appear to be a useful probe of DNA structure in this virus.

In summary, the CD spectral changes accompanying the thermally induced structure transition near 12 °C are unusual, and we propose shifts in base–tyrosine stacking and electronic coupling as a plausible explanation of the thermal difference CD spectra. The idea is that all the bases of an everted two-strand DNA helix extend out to interact with all the Tyr_{40} side chains of the helical array of protein subunits holding the DNA, but only guanine and cytosine would have electronic transitions with energies close enough to those of the tyrosines to generate the thermal difference CD. The presence of Ag(I) or Hg(II) modifies the thermally induced structure transition, blocking it if sufficient metal ion is added.

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Registry No. Hg, 7439-97-6; Ag, 7440-22-4; Cu, 7440-50-8.

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