Circular Dichroism Study of *Escherichia coli* Initiation Factor 3 Binding to Nucleic Acids†

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ABSTRACT: The circular dichroic spectral features of $(A)_{10-20}$, $(C)_{10-20}$, A_8UGU_6, poly(A), and poly(C), at both neutral and acidic pH values and in the presence and absence of Mg^{2+}, are significantly altered by *Escherichia coli* initiation factor 3 (IF3), implying the occurrence of protein-induced changes in nucleic acid secondary structure. Similarly, the circular dichroic spectral characteristics of helical poly(U), poly(A)·poly(U), and poly(I)·poly(C) are modified by IF3. However, no structural perturbation of poly(A)·poly(U) occurs in the absence of Mg^{2+} by IF3. The oligonucleotides $(A)_{10-20}$ and $(C)_{10-20}$ at both pH 7.5 and 5.5 titrate to an end point of 26 ± 4 nucleotide residues per IF3 [except $(C)_{10-20}$ at pH 5.5 which titrates to 17 ± 1 nucleotide residues per IF3], whereas the hairpin A_8UGU_6 under similar conditions at neutral pH and in the presence of Mg^{2+} titrates to an end point of 56 ± 3 nucleotide residues per IF3, thereby suggesting the presence of multiple binding sites on the protein. By contrast, poly(A) and poly(C) at neutral pH and in the absence of Mg^{2+} titrate to an end point of 13 ± 1 nucleotide residues per IF3. The occurrence of significant light-scattering artifacts precluded a determination of the end point stoichiometry in most other cases. The circular dichroic spectra of *E. coli* tRNA, MS2 RNA, φX174 DNA, and sonicated calf thymus DNA were unaffected by IF3 at physiological concentrations. Addition of an equimolar mixture of IF3 and ribosomal protein S1 titrates the circular dichroism of poly(C) at acid pH as did S1 alone. However, addition of IF3 to mixture of poly(A) and S1 at neutral pH did not result in significant titration of the optical activity until IF3 was in excess over S1, even though filter binding assays indicate normal IF3 binding to the polynucleotide. The possible relation of these observations to the biological function of IF3 is briefly considered.

A primary event in the translation of messenger ribonucleic acid (mRNA) by prokaryotic ribosomes is the formation of an initiation complex between mRNA and the 30S ribosomal subunit. This process is stimulated by initiation factor 3 (IF3)[1] [for reviews see Lodish (1976), Grunberg-Manago & Gros (1977), and Revel (1977)], although under certain conditions this protein has been found to be dispensable (Zipori et al., 1978). The degree of initiation stimulation by IF3 appears to correlate with the extent of secondary structure present in the initiation region of the mRNA, regardless of whether the mRNA is synthetic or naturally occurring (Lodish, 1976; Revel, 1977).

mRNA binding to 30S subunits also requires S1 protein, a protein associated with the small ribosomal subunit (Lodish, 1976; Grunberg-Manago & Gros, 1977; Revel, 1977). S1 protein has been shown to induce alterations in the secondary structures of certain synthetic polynucleotides (Bear et al., 1976).

Since both IF3 and S1 appear to have important roles in promoting mRNA binding to 30S subunits and both bind in close proximity to each other on the 3′ terminus of 16S ribosomal RNA (rRNA) (van Duin et al., 1975; Kenner, 1973), the consequences of their protein–nucleic acid interactions are of interest. In this paper we present the results of studies which examine the effect of IF3 binding on the secondary structures of various well-characterized oligonucleotides and polynucleotides by means of circular dichroism spectroscopy, as has been done for S1 (Bear et al., 1976). We also investigated the effect of IF3 on polynucleotide secondary structure in the presence of S1. Our results reveal the occurrence of a significant stoichiometric alteration in the secondary structure of certain oligonucleotides and polynucleotides induced by the binding of IF3.

Materials and Methods

Solutions. PE buffer contained 10 mM Na_2HPO_4, 1 mM Na_2EDTA, 1 mM DTT, and 5% glycerol, at pH 7.5. TM buffer contained 10 mM $(HOCH_2)_3CHNH_2$·HCl, 10 mM $Mg(OAc)_2$, 1 mM DTT, and 5% glycerol at pH 7.5. CM buffer (for thermal melting studies) contained 10 mM sodium cacodylate, 10 mM $Mg(OAc)_2$, 1 mM DTT, and 5% glycerol, at pH 7.5. Buffers of the same composition, adjusted to pH 5.5, were also prepared. All solutions were filtered through 0.42-μm nitrocellulose filters.

Nucleic Acids. The oligonucleotides $(C)_{10-20}$ and $(A)_{10-20}$ were obtained from Collaborative Research. The polynucleotides poly(A), poly(C), poly(U), poly(A)·poly(U), poly(I)·poly(C), tRNA, MS2 RNA, φX174 DNA, and calf thymus DNA were obtained from Miles Chemicals, P-L Biochemicals, or Worthington Biochemical Corp. All were used without further purification. The synthesis of A_8UGU_6 has been described (Wickstrom & Tinoco, 1974). Concentrations of oligonucleotides, polynucleotides, and naturally occurring nucleic acids were determined spectrophotometrically by using published extinction coefficients.

Preparation of Proteins. IF3 was isolated from early log phase *Escherichia coli* MRE 600 cells (Grain Processing Co.) essentially according to Hershey et al. (1977), in both the Denver and Santa Cruz laboratories. Protein S1 was prepared according to Bear (1978) from the same bacterial source. Protein preparations were at least 90–95% pure as judged from one-dimensional 10% polyacrylamide–$NaDodSO_4$ gels. Purified IF3 gave a specific activity of 7.0 units/mg (Hershey

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[1] Abbreviations used: IF3, initiation factor 3; CD, circular dichroism; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

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Table I: Alteration of Oligo- and Polynucleotide Circular Dichoric Spectra by IF3[a]

sample	buffer	λ_{max} (nm)[b]	shift (nm)[c]	% decrease[c]	λ_{min} (nm)[b]	shift (nm)[c]	nuc/IF3[d]	thermal melt[e]	light scattering[f]
$(C)_{10-20}$	7.5 PE	275	0	30			28	+	−
	7.5 TM	275	0	46			28	+	−
$(A)_{10-20}$	7.5 PE	264	0	38	249	+2	25	+	−
	7.5 TM	264	0	22	249	+3	29	+	−
$(C)_{10-20}$	5.5 PE	286	−6	56	261		17	−	−
	5.5 TM	286	−5	38	260		16	−	−
$(A)_{10-20}$	5.5 PE	262	0	54	240	+6	29	−	−
	5.5 TM	262	0	16	240	+5	26	−	−
A_8UGU_6	7.5 PE	266	+4	30	247	0	*g*	−	−
	7.5 TM	267	0	9	247	0	56	−	−
poly(U)[h]	7.5 TM	262	+10	100				−	+
poly(C)	7.5 PE	276	+4	23			13	+	−
	7.5 TM	276	+9	69				−	+
poly(A)	7.5 PE	264	+8	71	244	+9	12	−	−
	7.5 TM	265	+18	70	248	+6		−	+
poly(A)[i]	7.5 TM	266	−3	80	250	0			−
poly(C)	5.5 PE	287	+16	>84	261	+12		−	+
	5.5 TM	286	+20	>95	262	+12		−	+
poly(C)[j]	6.0 TM	287	0	42	266		14[h]	+	−
poly(A)	5.5 PE	262	>12	100	236	+4		−	+
	5.5 TM	260	+6	68	240	+4		−	+
poly(A)·poly(U)	7.5 PE	264	0	0	245	0			−
	7.5 TM	263	+9	63	245	+2		−	+
poly(I)·poly(C)	7.5 PE[k]	277, 245	0, +2	15			36	−	−
	7.5 TM[k]	277, 245	0, +2	12			36	−	−

[a] IF3 was added incrementally, up to a maximum of ~2 μM, to nucleic acid solutions in 0.5-mL, 1 cm path length cylindrical cells at 6 °C. Spectra were recorded twice, after each addition of IF3. [b] In the absence of protein. [c] Maximum change observed; in the absence of light scattering this corresponds to the end point of the titration. [d] Nucleotide residues per IF3 at lattice saturation. [e] Spectra at 6 °C with added protein were compared with thermally denatured spectra in the absence of protein. (+) indicates identical spectra due to protein or thermal denaturation; (−) indicates nonidentical spectra. [f] (+) indicates the occurrence of light-scattering artifacts (peak flattening, pronounced red shift, peak obliteration, and/or turbidity); (−) indicates no indications of light scattering. [g] No sharp end point was observed. [h] At 2 °C. [i] After the spectrum was measured in the absence of protein, S1 was added to 0.5 μM, followed by additions of IF3. [j] An equimolar mixture of IF3 and S1 was used to titrate poly(C). Stoichiometry is expressed in nucleotide residues per *protein* molecule. [k] To which 0.15 M KCl was added.

et al., 1977). Two-dimensional gels (Mets & Bogorad, 1974; Subramanian, 1974) showed our preparations to migrate like IF3l, as characterized previously (Suryanarayana & Subramanian, 1977). IF3 protein prepared in each of our laboratories proved to be indistinguishable by the criteria of one- and two-dimensional gel electrophoresis, filter binding assays, and their effects on nucleic acid circular dichroism. Protein concentrations were determined by either Coomassie Blue (Bradford, 1976) or Lowry (Lowry et al., 1951) assays, or both, using bovine γ-globulin (Sigma Chemical Co.) as a standard.

Physical Measurements. Ultraviolet absorbance measurements were made on a Beckman Acta V spectrophotometer. CD spectra were recorded at a fixed temperature of 6 °C (unless otherwise stated) with a Durrum-Jasco J-20 spectropolarimeter which had been modified to allow the sample cell to be placed "far" (39 cm) from or "near" (5.5 cm) to the end-window photomultiplier tube, thereby significantly altering the light-collection geometry of the instrument. Calibration was achieved with *d*-10-camphorsulfonic acid (DeTar, 1969). In all cases examined, IF3 did not contribute to the CD between 240 and 320 nm at the employed protein concentrations. Thermal transition curves (in the absence of protein) for the oligonucleotides and polynucleotides employed in this study were also obtained by CD. All solution absorbances were below 1.0. Constant temperature was maintained by the circulation of thermostated fluid through a brass cell holder block. Each spectrum was recorded at least twice. Spectra were digitized by use of an X–Y digitizing tablet interfaced to a Varian 620/L computer, followed by averaging and base line subtraction. The value of $[\theta]$ is given in units of deg·cm^2·dmol^{-1}.

Filter Binding Assays. Nitrocellulose filter assays were performed according to Wickstrom et al. (1980).

Results

Spectral data for IF3 titrations of oligonucleotides, polynucleotides, and naturally occurring nucleic acids are tabulated in Table I. A representative set of CD spectra for the oligonucleotides $(C)_{10-20}$ and $(A)_{10-20}$ at neutral and acidic pH in the presence of increasing amounts of IF3 is shown in Figure 1A–D; titration curves appear in the inserts to these figures. For the neutral forms of $(C)_{10-20}$ and $(A)_{10-20}$ (Figure 1A,B) the uniform reduction in the magnitude of the positive band produced by the addition of IF3 is very similar to that elicited by increasing temperature. In the case of $(A)_{10-20}$ both protein addition and rising temperature result in a slight increase in the negative band at 249 nm coupled with a small red shift. The simplest interpretation is that the binding of these oligonucleotides to IF3 leads to reduced base stacking of the single-stranded structure (Brahms et al., 1966, 1967; Van Holde et al., 1965) with little alteration in the relative base–base geometry. The diminution in optical activity at the end point of the titration is significantly greater in PE than in TM buffers for $(A)_{10-20}$, whereas the opposite effect was noted for $(C)_{10-20}$; the stoichiometry is 28 ± 2 nucleotide residues per IF3 in both buffers.

The negative band at 261 nm in the spectrum of acidic $(C)_{10-20}$ (Figure 1C) is diagnostic of the double-stranded stacked conformation. This is clearly revealed in thermal melting studies which show that this band disappears above the melting temperature and the resulting spectra resemble those of single-stranded $(C)_{10-20}$ at high temperature. However, upon addition of IF3 to the sample at 6 °C the positive

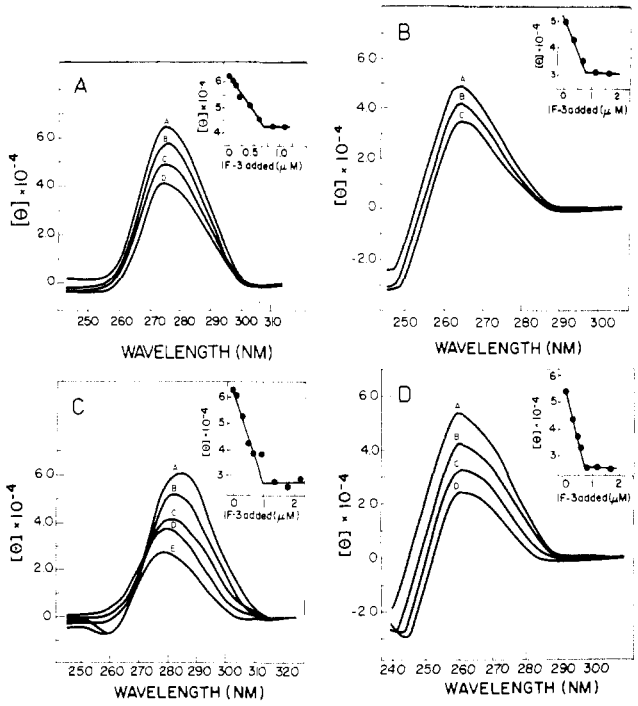

FIGURE 1: CD spectra of oligonucleotides in the presence and absence of IF3 protein. The effect of IF3 on the ellipticity maxima is shown in the inserts. Measurements were taken at 6 °C in PE buffer. (A) For $(C)_{10-20}$ (17.3 μM CMP, pH 7.5), IF3 concentrations are (A) no IF3, (B) 0.1, (C) 0.4, and (D) 0.8 μM. (B) For $(A)_{10-20}$ (16.5 μM AMP, pH 7.5), IF3 concentrations are (A) no IF3, (B) 0.3, and (C) 0.7 μM. (C) For $(C)_{10-20}$ (15.0 μM CMP, pH 5.5), IF3 concentrations are (A) no IF3, (B) 0.3, (C) 0.5, (D) 0.7, and (E) 1.7 μM. (D) For $(A)_{10-20}$ (18.0 μM AMP, pH 5.5), IF3 concentrations are (A) no IF3, (B) 0.3, (C) 0.6, and (D) 1.1 μM.

band blue shifts concomitant with a reduction in amplitude, and the negative band progressively loses intensity (Figure 1C). By contrast, increasing temperature leads to a reduction in amplitude for both the positive and negative bands. We conclude that complexation to IF3 results in disruption of the double-stranded structure (Brahms et al., 1967) concomitant with diminished base–base interaction and an alteration in the relative base–base orientation. The stoichiometry at the sharp breakpoint corresponds to 16 to 17 nucleotide residues per IF3 in both buffers. For acidic $(A)_{10-20}$, a double-stranded structure (Van Holde et al., 1965), the positive 262-nm band uniformly reduces in amplitude concomitant with a red shift and a diminution in amplitude of the negative 240-nm band with increasing temperature. Addition of IF3 elicits a uniform reduction in the positive band, coupled with a 5–6-nm red shift and a slight amplitude increase in the negative band (Figure 1D). We propose that complexation of IF3 to acidic $(A)_{10-20}$ results also in disruption of the double-stranded structure coupled with decreased base–base interaction and a geometry alteration. The stoichiometry for this protein-induced structural change is 26–29 nucleotide residues per IF3 in both TM and PE buffers; however, the maximum decrease in ellipticity is substantially greater in the latter buffer for both oligonucleotides.

The hairpin A_8UGU_6 sustains a uniform reduction in both the positive and negative CD bands with increasing temperature (Wickstrom, 1972), whereas protein addition elicits a uniform reduction in the positive band, coupled with a slight increase in the amplitude of the negative band. The decrease in ellipticity for the positive band is significantly greater in PE than in TM buffer. We suggest that in the presence of IF3 this hairpin structure "melts" to an open single-stranded

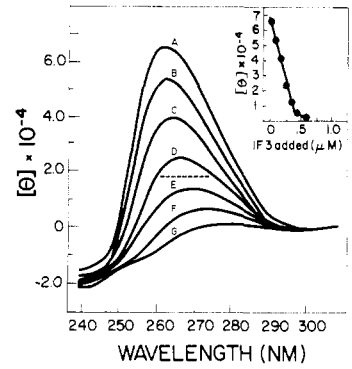

FIGURE 2: CD spectra of poly(U) in the presence and absence of IF3 protein. The effect of IF3 on the ellipticity maxima is shown in the insert. Measurements were taken at 2 °C in pH 7.5 TM buffer. The poly(U) concentration is 13.4 μM (UMP residues); IF3 concentrations are (A) no IF3, (B) 0.08, (C) 0.16, (D) 0.25, (E) 0.34, (F) 0.42, and (G) 0.59 μM. The dashed line indicates the ellipticity maximum of poly(U) at 25 °C in the absence of protein.

form. Furthermore, the titration end point is sharp in TM buffer, whereas in PE buffer no sharp end point was observed. The stoichiometry in TM buffer corresponds to 56 nucleotide residues (3.5 strands) per IF3.

Poly(U) attains a stable secondary structure characterized by a series of hairpin loops which form a double-helical stacked structure at low temperature; a T_m of ~4 °C in 10 mM Mg^{2+} is characteristic of this secondary structure (Thrierr et al., 1971). For poly(U) at 2 °C, the addition of IF3 elicits a reduction in the positive 262-nm band, coupled with a slight red shift (Figure 2, spectra A–D) producing a spectral form very similar to that generated by increasing temperature, except at low nucleotide residue/protein ratios where a more pronounced red shift and "peak flattening" are noted (Figure 2, spectra E and F). At very low nucleotide residue/protein ratios virtual "peak obliteration" is observed (Figure 2, spectrum G). Spectra E–G of Figure 2 have band amplitudes less than that expected for the completely unfolded form. These spectral characteristics are undoubtedly the consequence of light scattering. However, the spectral changes observed for spectra A–D are consistent with the IF3 binding induced disruption of the hairpin duplex polynucleotide structure, i.e., "melting", which is characterized by markedly reduced base–base interaction. Because of the occurrence of light-scattering artifacts a reliable determination of the stoichiometry is precluded.

The neutral form of poly(C), a single-stranded stacked structure (Brahms et al., 1967; Fasman et al., 1964), sustains a decrease and red shift in the positive 276-nm band upon the addition of IF3. The decrease in optical activity is greater in TM than in PE buffer at the titration end point. Heating elicits the same spectral response, and thus it is reasonable to conclude that the effect of complexation to IF3 is a decrease in base stacking comparable to that evoked by an increase in temperature. The stoichiometry at the end point is 13 nucleotide residues per IF3 in PE buffer.

Heating the neutral form of poly(A), a single-stranded stacked structure (Brahms et al., 1967; Adler et al., 1969), leads to a diminution in amplitude and a moderate red shift for both the positive and negative CD bands, whereas addition of IF3 evokes a decrease in the positive band, no change in the negative band, and red shifts in both bands. It therefore seems likely that the base–base geometry is altered, perhaps to a state characerized by reduced base stacking. Similar behavior was noted in both buffers. The stoichiometry is 12 nucleotide residues per IF3.

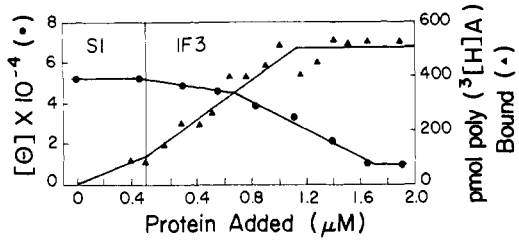

FIGURE 3: Titration of 11.0 μM (nucleotide residues) poly(A) with the addition of S1 protein to 0.5 μM, followed by increasing amounts of IF3. Ellipticity maxima are indicated by ●. Measurements were taken at 6 °C in pH 7.5 TM buffer. Also shown are nitrocellulose filter binding assays of [³H]poly(A) bound to protein (▲) in 50-μL reaction mixtures containing 12 μM labeled polynucleotide and 1 mg/mL bovine serum albumin in pH 7.5 PE buffer at 0 °C, with S1 protein added to 0.5 μM, followed by increasing amounts of IF3.

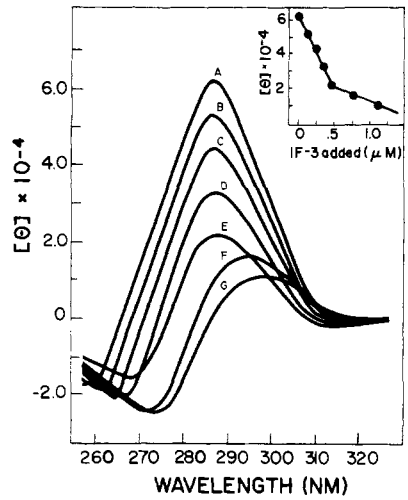

FIGURE 4: CD spectra of poly(C) in the presence and absence of IF3 protein. The effect of IF3 on the ellipticity maxima is shown in the insert. Measurements were taken at 6 °C in pH 5.5 PE buffer. The poly(C) concentration is 15.2 μM (CMP residues); IF3 concentrations are (A) no IF3, (B) 0.16, (C) 0.25, (D) 0.33, (E) 0.41, (F) 0.78, and (G) 1.11 μM.

We also explored the combined effect of IF3 and S1 on the CD spectrum of neutral poly(A) in TM buffer. S1 was first added to 0.5 μM which, as expected, did not alter the CD spectral features of poly(A) (Bear et al., 1976). Titration of the poly(A) and S1 mixture with IF3 (Figure 3) demonstrated that the titration curve was similar to that obtained in the absence of S1, except that the curve was displaced 0.5 μM toward higher protein concentration and had a shallower slope. However, nitrocellulose filter binding assays under identical reaction conditions revealed unimpaired IF3 binding to [³H]poly(A), similar to that observed in the absence of S1 (Wickstrom et al., 1980), except that the slope was less steep (Figure 3). Thus, it appears that the titration, as manifested by alterations in the CD spectrum, is impaired until a stoichiometric addition of IF3 (with respect to S1) has occurred.

A double-helical structure is characteristic of poly(C) under acidic conditions (Brahms et al., 1967). Addition of IF3 produces a uniform reduction in the magnitude of the 286-nm positive band, concomitant with a 10-nm red shift in the negative band at 266 nm (Figure 4, spectra A–E), until a ratio of 34 nucleotide residues per IF3 is attained, below which turbidity appears. At this point the addition of increasing amounts of protein results in a progressive 8–16-nm red shift in the positive band concomitant with "peak flattening"; the negative band also red shifts and increases in magnitude (Figure 4, spectra F and G). Similar effects were observed

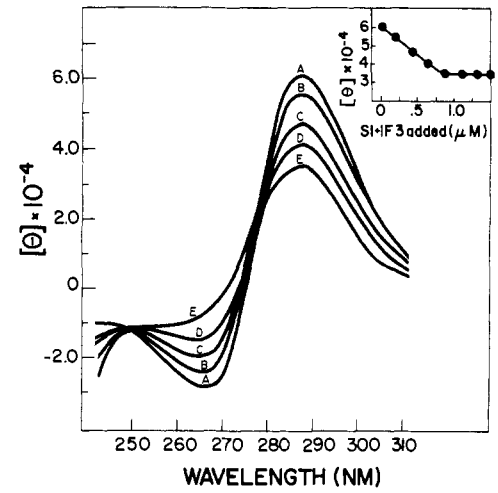

FIGURE 5: CD spectra of poly(C) in the presence and absence of S1 and IF3 proteins. The effect of IF3 plus S1 on the ellipticity maxima is shown in the insert. Measurements were taken at 6 °C in pH 5.5 TM buffer. The poly(C) concentration is 12.0 μM (CMP residues); the total protein concentrations of the equimolar mixtures are (A) no added protein, (B) 0.22, (C) 0.44, (D) 0.66, and (E) 1.10 μM.

in TM buffer. Heating, by contrast, elicits a reduction in amplitude and a blue shift in the positive band and a decrease in the negative band with no wavelength shift. As was the case for $(C)_{10-20}$, we take the negative band to be indicative of the double-stranded structure. Since the negative band remains in the presence of moderate amounts of protein, we propose, unlike for $(C)_{10-20}$, that the double-stranded structure is not disrupted by IF3 but that instead an alteration in base-stacking geometry perhaps coupled with a minor decrease in stacking occurs upon complexation. This is consistent with the observation that IF3 binds more tightly to the poly(C) lattice at pH 5.5 than at pH 7.5 (K. Twombly and T. Schleich, unpublished experiments). S1 protein, by contrast, elicits a reduction in the negative band of acid poly(C) (see, e.g., Figure 5), implying the disruption of the duplex structure (Bear et al., 1976).

To assess the contribution of differentially scattered polarized light to the CD spectrum, we moved the sample cell from its customary "far" position to a distance of 5.5 cm from the end-window photomultiplier tube, thereby drastically changing the solid angle of detection of the light collection geometry, and repeated the protein titration of acidic poly(C) at room temperature. This procedure constitutes an excellent test for the contribution of differential CD scattering effects to the observed CD spectrum (Dorman & Maestre, 1973; Dorman et al., 1973; Schneider, 1973). At high ratios of nucleotide residues/protein (60:1) no difference was detected in the wavelengths or the amplitudes of the positive band of "far" and "near" CD spectra, whereas at lower ratios (14:1) the positive band of "near" spectrum was observed to be shifted 4 nm to the blue and an average 18% lower in amplitude than that of the "far" spectrum. Thus it would appear that differential light-scattering effects contribute to the observed CD spectra, particularly in those cases where turbidity is encountered.

To explore the possible interactions between IF3, S1 protein, and the double-helical poly(C) lattice, we titrated this polynucleotide species at pH 6.0 in TM buffer with an equimolar mixture of the two proteins. The combined presence of S1 protein and IF3 elicits the formation of the single-stranded structure with diminished base stacking, as shown in Figure 5. Clearly, both proteins are active in the presence of the other on the poly(C) lattice as evidenced by an end point of 14

nucleotide residues/*total* protein.

Thermal melting of the acidic double-stranded poly(A) structure (Brahms, 1967; Adler et al., 1969) elicits a reduction in the magnitude and a slight red shift in the positive 262-nm band; the negative band likewise decreases in amplitude and shifts to the red to a position characteristic of the single-stranded structure. The addition of IF3 results in a decrease of the positive band concomitant with a small red shift until moderate nucleotide residue/protein ratios are attained, and the negative band displays a similar change. At low nucleotide residue/protein ratios (35:1 or smaller), which implies a greater binding density, turbidity is readily apparent. Concomitant with turbidity is peak flattening and a pronounced red shift for both bands. At smaller nucleotide residue/protein ratios (e.g., 23:1) the effect is so severe that the band is virtually obliterated. We suggest that prior to the initiation of turbidity the double-stranded structure of poly(A) is disrupted by bound IF3, along with a reduction in base stacking.

For poly(A)·poly(U) IF3 elicits a uniform reduction in amplitude, coupled with a very slight red shift of the positive 260-nm band, whereas the negative 245-nm band decreases in amplitude uniformly. However, at moderately high nucleotide residue/protein ratios (83:1) significant peak flattening and pronounced red shifts are observed for both CD bands, concomitant with the appearance of turbidity. In the absence of the divalent Mg^{2+} ion no alteration in the CD occurs. Increasing temperature, however, results in a reduction of the positive band, while the negative band remains virtually unchanged until the complex is disrupted, whereupon this band sustains a 6-nm red shift and a dramatic increase in amplitude, characteristic of unstacked poly(A). We propose that, prior to the onset of turbidity of this protein–polynucleotide complex, the integrity of the duplex structure is preserved but with somewhat reduced base–base interaction.

In the presence of IF3, the poly(I)·poly(C) duplex CD spectrum sustains a small yet significant uniform reduction in the amplitudes of both positive bands. Similar effects were noted in both PE and TM buffers. We suggest that the binding of IF3 to this duplex structure results in a slight to moderate reduction in base stacking. The stoichiometry at the end point is 18 base pairs per IF3.

Additions of IF3 up to 0.7 μM did not result in altered optical activity spectra for *E. coli* tRNA, MS2 RNA, ϕX174 DNA, and sonicated calf thymus DNA. With the exception of tRNA, higher protein concentrations caused turbidity.

Discussion

Two potential complications must be considered in the interpretation of the protein-induced CD spectral changes. The first is that the binding of IF3 to the nucleic acid lattice results in alterations in protein secondary structure, which produce, in turn, the observed spectral changes. We believe this possibility to be extremely unlikely for the following reasons: (1) the protein concentration is sufficiently low that no contribution to the CD between 240 and 320 nm is evident in the absence of nucleic acid, so that in order to account for the observed spectral changes a drastic increase in the protein CD must occur; (2) in each case the altered CD is characterized by *decreases* in ellipticity and wavelength shifts which are proportional to the amount of protein added; (3) the protein-induced spectral changes are very similar to the spectral characteristics of the particular nucleic acid under consideration and do not resemble those expected for proteins in the wavelength range 240–320 nm.

The second potential complication in the interpretation of polynucleotide–IF3 complex CD spectra arises in those instances where turbidity is encountered and, hence, significant light scattering occurs. The effects of light scattering on CD spectra have been examined both theoretically and experimentally [Dorman & Maestre (1973), Dorman et al. (1973), Schneider (1973), and references cited therein]. CD spectra of biological molecules in suspension typically display bands depressed in amplitude (peak flattened) and red shifted relative to those obtained for the molecularly dispersed sample. Not all bands in a spectrum are affected equally. In severe cases peak obliteration and red shifts of at least 10 nm are observed. These CD spectral distortions arise from three contributions: (1) Duysens absorption flattening which may simply be viewed as a "sieve" effect acting to reduce the amount of transmitted light and thus the "effective" concentration of optically active species; (2) light scattering of the incident beam to regions of space outside the solid angle of detection, resulting also in the reduction of the effective concentration and, hence, the amplitude of the CD signal (this and the previous contribution are frequently referred to as "concentration obscuring" effects); (3) differential scattering of left- and right-handed circularly polarized light which causes red-shifted bands. Contribution 1 is independent of the light-collection geometry since the sieve effect is intrinsic to the sample, whereas contributions 2 and 3 are dependent on the solid angle of light detection. This has, in fact, been observed by the use of large-angle detection and the fluoroscat cell (Dorman & Maestre, 1973; Dorman et al., 1973). We have demonstrated that the positive 287-nm CD band characterisitic of the acidic form of poly(C), which is peak flattened and red shifted by 16 nm, is sensitive to the solid angle of detection; thus it appears that circularly polarized light is scattered differentially. We attribute peak flattening and/or peak obliteration and *pronounced* red shifts to be the consequence of light-scattering artifacts. Yet, even in those systems where turbidity is encountered, conformational information may still be secured because spectral artifacts due to light scattering usually are not manifested until low nucleotide residue/protein ratios are attained, as exemplified by the titration of acidic poly(C) by IF3 (Figure 4). In severe light-scattering situations conformational interpretations may still be made by use of the corresponding oligonucleotides whose complexes with protein do not display turbidity.

The results reported in this paper demonstrate that the near-ultraviolet CD of certain nucleic acid structures is significantly altered upon complexation with IF3, implying that the binding of this protein to the nucleic acid lattice induces a change in its secondary structure. IF3 is known to bind tightly to nucleic acids under the conditions employed in this study (Wickstrom, 1974; Wickstrom et al., 1980; K. Twombly and T. Schleich, unpublished experiments). We would like to be able to extract the detailed conformational features of a nucleic acid from its CD spectrum. The kind of information we can obtain at present includes the following. (1) Does the extent of base stacking change upon complexation? (2) Does the relative geometry of the bases in a base-stacking arrangement change upon complexation? (3) Does strand separation occur in multistrand structures upon complexation?

The approach we have taken for the interpretation of changes in nucleic acid CD induced by protein binding is similar to that used by Greve et al. (1978) for the T4 gene 32 protein binding induced spectral changes in certain polynucleotides. This approach is based on the comparison of protein-induced polynucleotide spectral changes with those mediated by temperature increases alone. The procedure fails if the protein induced spectral changes arise from nonconformational origins such as light-scattering artifacts.

It is well established that the characteristic near-ultraviolet CD arises predominantly because of electronic interactions between the neighboring bases (Brahms & Brahms, 1970; Bush, 1974), although base–sugar interactions within the same residue also make a contribution. Thus the CD of a nucleic acid is sensitive to its conformation in terms of relative base–base geometry and the separation distance between the bases, as well as the glycosyl torsion angle. On theoretical grounds a uniform reduction in the rotational strength of a CD band would imply a reduction in base–base interaction arising from increased base separation or increasing oscillation of the bases; by contrast, a nonuniform reduction in the CD, such as exemplified by concomitant wavelength shifts in band positions, would imply an alteration in base–base geometry, most likely coupled with a diminution in base stacking. While changes in the glycosyl torsion angle make a contribution to the CD, unequivocal assignment to this source is usually not possible.

We have taken the site size, i.e., the number of base residues (or the number of base pairs for a duplex structure) occluded or "covered" by the protein, to be equivalent to the stoichiometric end point determined in the CD titration. In those instances where turbidity was encountered, reliable stoichiometric end-point values could not be obtained. For single-stranded stacked polymers site sizes were found to be 12 to 13 base residues in PE buffer. This value is in accord with the value of 14 ± 1 determined by filter binding assays (Wickstrom et al., 1980). For the duplex poly(I)·poly(C) structure a value of 18 base pairs was obtained in both buffers. These values are consistent with those determined for other nucleic acid binding proteins. In contrast, the stoichiometric end point for the oligomers at neutral pH is equivalent to 28 nucleotide residues per protein molecule, suggesting either that two single-stranded oligomers bind to the same site with partial protein overlap leading to complete structural alteration in each oligomer or that two binding sites are available on the protein for oligomer binding. For duplex $(A)_{10-20}$ binding the stoichiometry is consistent with the binding of one double-stranded structure per IF3, whereas for $(C)_{10-20}$ under acidic conditions the observed stoichiometry implies less than two strands binding per protein molecule. Short helical segments are known to be unstable so that it is quite conceivable that partial protein overlap of an oligomer could result in total structural disorganization. The notion of multiple binding sites is made more plausible by the finding that the hairpin A_8UGU_6 binds with an average stoichiometry of 3.5 strands per protein. It is hard to envisage 4 strands, each about half a site in length binding to one site.

The effect of Mg^{2+} is variable. For the oligomers the presence of Mg^{2+} in the buffer diminishes the optical activity reduction induced by IF3 two- to fourfold, with the exception of neutral $(C)_{10-20}$ where the effect is reversed. In the case of polymeric structures, the presence of Mg^{2+} has little effect on the protein-induced spectral changes observed for neutral poly(A), but a significant effect was noted for neutral poly(C). However, some of this optical change undoubtedly arises from light-scattering artifacts. With heteroduplexes, IF3 will not perturb the structure of poly(A)·poly(U) in the absence of Mg^{2+}, while for poly(I)·poly(C) the presence of this divalent ion has apparently little effect. The basis of the Mg^{2+} effects is unclear at present. The behavior of the oligomers in the presence of this divalent ion suggests a simple ionic strength inverse dependence of binding, perhaps arising from a competition for binding sites, while in the case of particular polymeric structures a requirement for Mg^{2+}, as evidenced by enhanced optical activity changes, appears to be present. Complicated, although not identical, Mg^{2+}-dependent effects have also been observed for *Caulobacter crescentus* IF3 binding to polynucleotide structures (Leffler & Szer, 1974).

In contrast to ribosomal protein S1, IF3 displays less specificity in its ability to alter the secondary structures of nucleic acids. This is shown by the fact that S1 does not perturb the secondary structures of the neutral and acidic forms of poly(A), the duplexes poly(A)·poly(U) and poly(I)·poly(C), and the hairpin A_8UGU_6 (Bear et al., 1976). As shown in this paper, IF3 affects the conformation of all of these nucleic acid structures. The observation that S1 does not induce conformational alterations in preexisting duplex structures [except poly(C)] is consistent with the notion that this protein serves to stabilize rRNA–mRNA during initiation (Steitz et al., 1977; Kolb et al., 1977; Draper & von Hippel, 1979). Conversely, the observation that IF3 destabilizes certain duplex structures suggests it probably does not participate in the stabilization of such base pairing but rather may facilitate this interaction, as has been suggested previously (Wickstrom, 1974; Szkopinska et al., 1975). We do not yet understand why IF3 has such a strong effect on poly(A)·poly(U) in TM buffer but no apparent effect in PE buffer; both experiments were performed under conditions quite far from those where triple-strand formation occurs (Stevens & Felsenfeld, 1964). The divalent ion Mg^{2+} exerts a stimulatory effect on the binding of *C. crescentus* IF3 to certain synthetic and naturally occurring nucleic acid structures (Leffler & Szer, 1974). This IF3 has no apparent base specificity and displays good binding to single-stranded stacked structures but weaker binding to duplex and unstacked structures. While we have found that *E. coli* IF3 does exert a conformational alteration effect on a variety of synthetic polynucleotide structures, no apparent effect has been observed on naturally occurring nucleic acids with very stable secondary structures.

The question naturally arises as to the functional significance of nucleic acid conformational alterations induced by IF3 binding. The in vivo concentration of free and ribosomally bound IF3 in the *E. coli* cell has been reported to be ~2 μM (Howe et al., 1978). Typically our experiments are conducted with ~12 μM nucleic acid (base residues) and for this concentration ~0.9 μM protein is necessary to achieve a stoichiometric end point. Thus it would appear that the effects described in this paper are well within the range characteristic of biologically relevant concentrations. Additional experiments are required to establish the biological importance of the nucleic acid conformational alteration behavior of IF3.

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Fluorescence Studies of Platelet Tubulin†

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ABSTRACT: Temperature-related changes in tubulin conformation were investigated by several fluorospectroscopic methods utilizing steady-state fluorescence techniques. Tubulin was isolated from human platelets by three cycles of temperature-dependent polymerization–depolymerization. 8-Anilino-1-naphthalenesulfonate (ANS) was used as a probe of polarity of tryptophanyl fluorescence in tubulin. The microtubule protein displayed definite differences at 4 and 37 °C. While the total number of ligand sites was the same, differences in the binding affinity of ANS were noted. Acrylamide, a discriminating and efficient quencher of intrinsic protein fluorescence, also revealed temperature-dependent changes in the exposure of tryptophanyl residues of tubulin. ANS-induced quenching was associated with a transfer of energy from tryptophanyl residues to this fluorophor which could be used to calculate a critical distance between the acceptor–donor pair. The temperature-related changes were not found to be contingent upon the presence of polymerized tubulin.

The optical properties of 8-anilino-1-naphthalenesulfonate (ANS) make it a useful probe of polarity in many proteins. ANS, which exhibits virtually no fluorescence in water, fluoresces strongly in nonpolar solvents or when absorbed to certain proteins, lipids, or membranes (Weber & Laurence, 1954; Brand & Gohlke, 1972; Gally & Edelman, 1965; Stryer, 1965; Einarsson, 1976). Although the properties of fluorophors in general are determined by time-dependent processes occurring in their immediate microenvironment, inferences can be made concerning certain structural details of the molecules to which they are attached.

Quenching of intrinsic protein fluorophors can also be utilized to gain information on the configuration of the polypeptide chains especially on the exposure of fluorescent residues such as the amino acid tryptophan. Since the fluorophor is contained in proteins usually only in small numbers, its fluorescence makes it well suited for topographical studies using low molecular weight substances as potential quenchers of the fluorescent emission from its excited indole ring.

Tubulin, a protein serving a variety of functions in different cells, maintains the discoid shape of platelets in its polymerized

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