Physical Studies of the Interaction of a Calf Thymus Helix-Destabilizing Protein with Nucleic Acids†

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ABSTRACT: UP1, a calf thymus protein that destabilizes both DNA and RNA helices, dramatically accelerates the conversion of the inactive conformers of several small RNA molecules to their biologically active forms [Karpel, R. L., Swistel, D. G., Miller, N. S., Geroch, M. E., Lu, C., & Fresco, J. R. (1974) *Brookhaven Symp. Biol. 26*, 165–174]. Using circular dichroic and spectrophotometric methods, we have studied the interaction of this protein with a variety of synthetic polynucleotides and yeast $tRNA_3^{Leu}$. As judged by perturbations in polynucleotide ellipticity or ultraviolet absorbance, the secondary structures of the single-stranded helices poly(A) and poly(C), as well as the double-stranded helices poly[d(A-T)] and poly(U·U), are largely destroyed upon interaction with UP1 at low ionic strength. This effect can be reversed by an increase in $[Na^+]$: half the UP1-induced perturbation of the poly(A) CD spectrum is removed at 0.05 M Na^+. The variation of poly(A) ellipticity and ultraviolet absorbance with $[UP1]/[poly(A)]_p$ is used to determine the length of single-stranded polynucleotide chain covered by the protein: 7 ± 1 residues. A model is presented in which the specificity of UP1 for single strands and their concomitant distortion are a consequence of maximal binding of nucleic acid phosphates to a unique matrix of basic residues on the protein. Analogous to the effect on polynucleotides, UP1-facilitated renaturation of yeast $tRNA_3^{Leu}$ follows the partial destruction of the inactive tRNA's secondary structure. At the tRNA absorbance maximum, UP1 effects a hyperchromic change of 10%, representing one-third of the secondary structure of the inactive conformer. This change is also clearly observable as a perturbation of the tRNA's circular dichroism spectrum.

Helix-destabilizing proteins bring about the denaturation of nucleic acid helices by selectively binding to single strands. The conformations of nucleic acid single strands are often altered upon binding to these proteins, as indicated, for example, by electron microscopic evidence for chain lengthening (by T4 bacteriophage gene 32 protein; Delius et al., 1972) or shortening (by the *Escherichia coli* helix-destabilizing protein; Sigal et al., 1972). Spectrophotometric and circular dichroism techniques have been used to monitor protein-induced helix destabilization as well as distortion of ordered single strands (Jensen et al., 1976). These methods, as well as fluorescent (Kelly & von Hippel, 1976; Kelly et al., 1976), density gradient (Jensen & von Hippel, 1977), and filter binding (Banks & Spanos, 1975) approaches, have been utilized to explore quantitative and qualitative aspects of helix-destabilizing protein–nucleic acid interactions.

UP1[1] is one of several proteins isolated from calf thymus which after passage through native DNA–cellulose selectively bind to denatured DNA–cellulose in a buffer containing 0.05 M Na^+ and resist elution by dextran sulfate (Herrick & Alberts, 1976a). Under low to moderate ionic strength conditions, UP1 lowers the T_m of both DNA and RNA helices, indicating that this protein has a strong affinity for single-stranded RNA as well as DNA (Herrick & Alberts, 1976b). This affinity is likely related to the ability of UP1 to bring about the renaturation of inactive yeast $tRNA_3^{Leu}$, *E. coli* $tRNA^{Trp}$, and *E. coli* 5S RNA under conditions where there is little or no spontaneous renaturation (Karpel et al., 1974, 1976).

Although at least some of the in vivo functions of several phage-coded gene products, such as the T4 gene 32 protein, have been established by genetic methods (Champoux, 1978), the physiological roles of most of the helix-destabilizing proteins which have been isolated have not been established. UP1 could conceivably play a role in DNA replication since it stimulates the homologous DNA polymerase-α (Herrick et al., 1976), but its apparently exclusive location in calf thymus cytoplasm [it was not found in sucrose-isolated nuclei; Karpel et al. (1976) and unpublished results] as well as its demonstrated RNA helix-destabilizing and renaturing properties suggests the possibility of its involvement in RNA physiology.

A full understanding of helix-destabilizing proteins such as UP1 will require delineation of their effect on nucleic acid structure. The studies reported here concentrate on the effects of this protein on the secondary structures of double helical and single-stranded nucleic acids. Circular dichroism (CD) techniques, in particular, can be singularly useful for characterizing protein-induced nucleic acid conformational changes. At wavelengths above 240 nm, we show that UP1 displays virtually no CD and thus does not interfere with the spectra of nucleic acids in this range. Any alterations in the magnitude and position of CD spectra at wavelengths greater than 240 nm can therefore be ascribed to changes in nucleic acid structure rather than in protein structure. Alterations in the ultraviolet absorption of the nucleic acids, although less dramatic than the CD perturbations, confirm these conformational changes. We have utilized UP1-induced changes in the CD spectrum of poly(A) to measure the length of polynucleotide occluded (covered) by protein. The dependence of the interaction on $[Na^+]$ can be analyzed by the method of Record et al. (1976) in terms of the number of ion pairs formed between each protein molecule and the polynucleotide chain residues to which it is bound.

We have also employed CD and ultraviolet techniques to better understand the conformational changes which accom-

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[1] Abbreviations used: UP1, calf thymus helix-destabilizing (formerly termed *unwinding*) protein 1; T_m, melting temperature of a nucleic acid helix; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid), random coiled form; poly(U·U), poly(uridylic acid), double helical form; poly(C), poly(cytidylic acid); poly[d(A-T)], poly(deoxyribonucleic acid) consisting of alternating adenylic and thymidylic acid residues; $NaDodSO_4$, sodium dodecyl sulfate.

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pany UP1-facilitated $tRNA_3^{Leu}$ renaturation. This tRNA is one of a number of RNA molecules which, upon removal of Mg^{2+}, assume biologically inactive conformations (Fresco et al., 1966; Sueoka et al., 1966; Aubert et al., 1968). Upon restoration of sufficient Mg^{2+} to stabilize the active ("native") conformers, renaturation is extremely slow, so that the inactive forms are metastable. The measured half-life of $tRNA_3^{Leu}$ renaturation is ~11 h at 25 °C (Lindahl et al., unpublished results; Hawkins et al., 1977); extrapolation of the renaturation Arrhenius plot to 0 °C yields a half-life of several years at this temperature (the activation energy for renaturation is ~60 kcal/mol). Oligonucleotide binding studies indicate that the tertiary structure of the inactive conformer is significantly different from that of the active form (Uhlenbeck et al., 1974), and tritium-exchange studies suggest that a very open (rapidly exchanging) structure is an intermediate in the renaturation process (Webb & Fresco, 1973). It is thus reasonable to conclude that renaturation of $tRNA_3^{Leu}$ is dependent on temporary destruction of incorrect secondary and/or tertiary structure in the inactive conformer followed by refolding of the molecule to the native form.

Under conditions where inactive $tRNA_3^{Leu}$ spontaneously renatures extremely slowly (0 °C), addition of stoichiometric amounts of UP1 brings about conversion to the native form in a matter of minutes (Karpel et al., 1974, 1976; unpublished results). In a gel electrophoresis system, UP1 was shown to form a complex with inactive $tRNA_3^{Leu}$ (Karpel et al., 1976; unpublished results). The studies reported below are concerned with the interrelationship of the helix-destabilizing properties of UP1, its RNA renaturing capabilities, and the apparent requirement that at least some of the inactive tRNA secondary and/or tertiary structure be destroyed in order to bring about renaturation.

Materials and Methods

Protein. UP1 was prepared as previously described (Herrick & Alberts, 1976a). In the majority of experiments, the high salt eluting fraction of UP1, roughly corresponding to the "basic" fraction of Herrick & Alberts (1976a), was utilized, although a somewhat more acidic fraction gave essentially the same results in several experiments. The protein was at least 90% pure as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1970). After purification, the protein was dialyzed against a buffer containing 5 mM Tris, pH 8.8, 1 mM EDTA, 0.1 mM DTT, and 10% (v/v) spectroquality glycerol (Eastman) and kept frozen at −80 °C until just prior to use. After thawing, storage for several weeks at 0 °C did not alter the activity of the protein, as judged by the assays described herein. The UP1 concentration was determined spectrophotometrically by using $\epsilon_{1\%}^{280} = 4.45$ (Herrick & Alberts, 1976a) with a small scattering correction obtained from a log A_λ vs. log λ plot (Wetlaufer, 1962) and an assumed molecular weight of 21 000 ± 2000. This molecular weight and uncertainty was based on comparison of the electrophoretic mobility of UP1 on the Laemmli (1970) $NaDodSO_4$–polyacrylamide gel system with those of protein standards and is consistent with the observation of Herrick & Alberts (1976a), who have previously showed that UP1 runs as a 19 000-dalton monomer on a calibrated gel permeation column.

Nucleic Acids. Poly(A), poly(U), poly(C), and poly[d(A-T)] were obtained from P-L Biochemicals. With the exception of poly(A), synthetic nucleic acids were dissolved in 1 mM Tris, pH 7.9, 0.1 mM EDTA, 0.1 mM DTT, and 10% (v/v) glycerol and used without any further purification. The poly(A) used in the titration and $[Na^+]$ dependence experiments was dissolved in this buffer and chromatographed on an 80 × 1.5 cm Sephadex G-100 column in order to remove any short poly(A) segments which might have slightly altered UP1 binding properties. Yeast transfer RNA_3^{Leu} was a gift from Dr. J. R. Fresco. It was dialyzed against 0.01 M cacodylate, pH 7.5, 0.05 M KCl, and 0.1 mM EDTA, then heated to 75 °C for 2 min to break up any aggregates, and quickly cooled. This material was inactive in an aminoacylation assay (Karpel et al., 1975) but could be charged either by addition of Mg^{2+} to 0.01 M followed by heating to 60 °C for 5 min or by treatment with UP1 as previously described (Karpel et al., 1974, 1976). Nucleic acid concentrations were determined spectrophotometrically in the 1 mM Tris buffer (see above) by using the following extinction coefficients (in L mol^{-1} cm^{-1}): poly(A), $\epsilon_{260} = 1.00 \times 10^4$, which was found to be, within experimental error, the same as ϵ_{257} in 0.195 M Na^+ (Blake et al., 1967); poly(U), $\epsilon_{261} = 9.43 \times 10^3$ (Blake et al., 1967); poly(C), $\epsilon_{268} = 6.30 \times 10^3$ (Wang & Kallenbach, 1971); poly[d(A-T)], ϵ_{260} (= ϵ_{262}) = 6.65×10^3 (Inman & Baldwin, 1962); inactive yeast $tRNA_3^{Leu}$, $\epsilon_{258} = 7.48 \times 10^3$, which was calculated from a molecular weight of 29 500 (based on its sequence; Kowalski et al., 1971) and a $\epsilon_{258}^{1\%}$ of 215.5 for the inactive (formerly termed "denatured") conformer (Adams et al., 1967). Unless otherwise stated, all other chemicals utilized were of reagent or comparable grade.

Circular Dichroism Studies. A JASCO J-40 spectropolarimeter was modified to accept 1 cm path length, semimicro rectangular (4-mm inside width) stoppered quartz cuvettes in a thermostatable cell holder. Temperatures within the cuvettes were determined with the aid of a thermistor probe (Yellow Springs Instruments). By use of an aperture of approximately 6 × 1 mm, samples as small as 400 μL could be measured. In the UP1–$tRNA_3^{Leu}$ experiments, a self-masking (black quartz-walled) microcell (2-mm inside width) was employed, which permitted a reduction in sample size to 100 μL. Aside from displaying somewhat lower signal to noise ratios, the CD spectra of several polynucleotides and *d*-10-camphorsulfonic acid taken under these conditions were identical with those taken with 1 cm inside width cuvettes utilizing a larger aperture. Wavelength and CD scale calibration were periodically checked according to the J-40 manual by using respectively holmium oxide and neodynium glass and 0.142% (6.10×10^{-3} M) recrystallized *d*-10-camphorsulfonic acid as standards.

UP1–polynucleotide mixtures were prepared by combining 200 μL of polynucleotide + polynucleotide buffer [1.0 mM Tris, pH 7.9, 0.10 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol] with 200 μL of protein + protein buffer [5.0 mM Tris, pH 8.8, 1.0 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol] to give 3.0 mM Tris, 0.55 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol, pH 8.25 ± 0.15 ("1:1 buffer"). In the poly(A)–UP1 titration experiment, an initial mixture corresponding to the lowest $[UP1]/[poly(A)]_p$ was prepared and its CD spectrum was then determined. Aliquots of a poly(A) solution of the same residue concentration and in the same buffer as the initial mixture were then successively added, such that the $[UP1]/[poly(A)]_p$ was decreased while the absolute $[poly(A)]_p$ remained invariant. After each addition, the mixtures were determined to be at equilibrium, since the CD maxima were unchanged with time. In the experiment determining the $[Na^+]$ dependence of the UP1–poly(A) interaction, a UP1–poly(A) mixture in 1:1 buffer was prepared and successive aliquots of an identical mixture in 2.0 M NaCl were added. An analogous experiment performed in the absence of UP1 showed a small (7%) increase in the poly(A)

264-nm CD maximum when the [Na^+] was raised from ~0 to 0.025 M. The ellipticity of the UP1–poly(A) mixture prior to addition of NaCl was corrected for this change. Above 0.025 M NaCl, there was no further change in the poly(A) CD maximum in the absence of UP1. In other experiments dealing with [Na^+] or [Mg^{2+}] dependence, aliquots of added concentrated NaCl or $MgCl_2$ solutions were often sufficiently small so there was effectively no dilution of the protein–nucleic acid mixture. When dilution was significant, this is accounted for generally by comparing nucleic acid in the presence and absence of protein (e.g., Figures 4 and 5).

$tRNA_3^{Leu}$–UP1 mixtures were prepared in the same buffer used in previous studies (Karpel et al., 1974, 1976): 50 parts protein buffer, 10 parts tRNA buffer (0.010 M cacodylate, pH 7.0, 0.050 M KCl, and 0.10 mM Na_2EDTA), and 5 parts 0.36 M KCl to give 3.8 mM Tris, 1.5 mM cacodylate, 0.78 mM Na_2EDTA, 0.077 mM DTT, 0.035 M KCl, and 7.7% (v/v) glycerol, pH 8.2 ± 0.2.

Spectrophotometric Studies. A Cary 219 scanning spectrophotometer, outfitted with thermostatable cell holders and a microcell adapter accessory, was utilized for studies on the effect of UP1 on the $tRNA_3^{Leu}$ absorbance spectrum. A 400-μL UP1–$tRNA_3^{Leu}$ mixture was prepared in the buffer described above and split between two matched self-masking semimicro cuvettes; one cuvette was placed in the sample holder the other in the reference position. By use of the auto-base line feature of the spectrophotometer, a flat (deviation <0.0005*A*) base line was established between 320 and 220 nm on a full-scale sensitivity of 0.1*A*. Aliquots of a concentrated (3.6 M) solution of KCl were added to one cuvette while simultaneously the same volumes of H_2O were added to the other. Thus, initially, the tRNA in both cuvettes was interacted with UP1 under conditions which would produce a significant conformational change and lead to near total renaturation upon addition of Mg^{2+}. However, with the addition of increments of KCl to 0.50 M, conditions were created under which the complex is destabilized (Karpel et al., unpublished results). (Mg^{2+} would also have the same effect; however, significant changes below 250 nm in the background absorbance of the buffer were observed upon addition of $MgCl_2$. The effect of KCl on buffer absorbance was less and was subtracted from the UP1–$tRNA_3^{Leu}$ difference spectra.) Thus, in the absence of significant perturbation of protein absorbance, the difference spectrum of a UP1–RNA mixture under favorable vs. unfavorable complex-forming conditions is representative of the tRNA hyperchromic change upon complex formation.

Results

Effect of UP1 on Poly(A) Secondary Structure. At the ellipticity maximum of poly(A), 264 nm, virtually no circular dichroism is exhibited by UP1, and any changes in the spectrum must therefore be associated with the polynucleotide. Thus, the reduction in magnitude of both the positive and negative poly(A) CD peaks by UP1 (Figure 1) reflects changes in the secondary structure of the polynucleotide. Such changes have generally been interpreted as being indicative of the disruption of base stacking (Jensen et al., 1976). In support of this, we find that when interacted with UP1, poly(A) exhibits a hyperchromic change at 260 nm of as much as ~20% at 20 °C ([UP1]/[poly(A)]$_p$ = 0.14). In the absence of protein, this hyperchromic effect would result from an increase of temperature to ~75 °C. Although this protein-induced change could conceivably reflect a perturbation in the UP1 ultraviolet absorbance spectrum, the vastly greater A_{260} of poly(A) in this experiment (0.250) relative to that of UP1

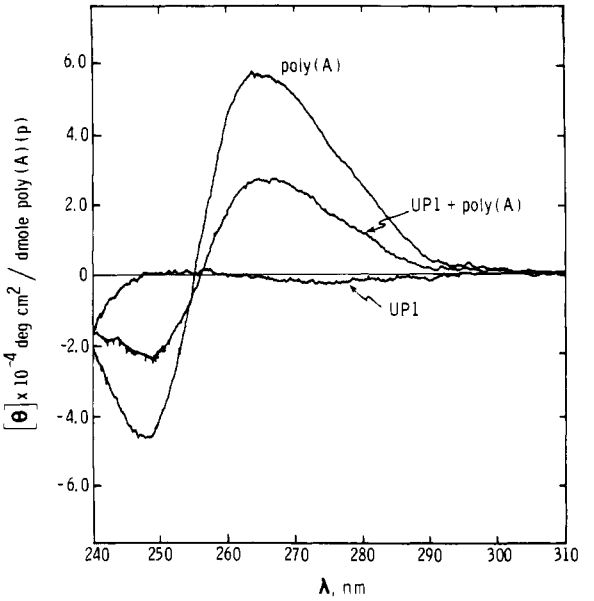

FIGURE 1: Effect of UP1 on the CD spectrum of poly(A); 3.0 mM Tris-HCl, 0.55 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol, pH 8.25, 20 °C. For the poly(A) and poly(A) + UP1 spectra, [poly(A)] = 2.50 × 10^{-5} M (p); for the UP1 and poly(A) + UP1 spectra, [UP1] = 3.6 × 10^{-6} M.

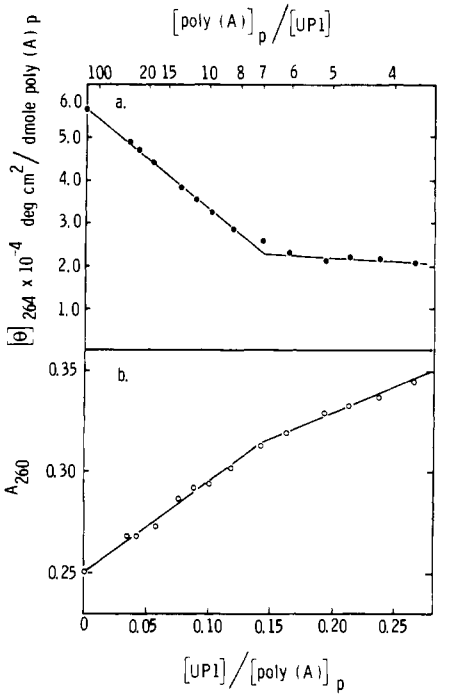

FIGURE 2: Determination of the length of single-stranded polynucleotide chain occluded by UP1, 20 °C. Buffer and [poly(A)]$_p$ were the same as in Figure 1. [UP1] was varied between 0 and 6.7 × 10^{-6} M. (a) Variation of poly(A) molar (p) ellipticity ([Θ]$_{264}$) with [UP1]/[poly(A)]$_p$. (b) A_{260} of the data points shown in (a).

(≤0.038) would suggest that any such perturbation was minor compared to changes in the polynucleotide's absorbance.

Titration of Poly(A) Ellipticity by UP1: Determination of Occluded Binding Site Size. The significant diminution of the poly(A) CD spectrum maximum at 264 nm by UP1 can serve as a probe of the extent of protein saturation of the polynucleotide lattice. As seen in Figure 2, the ellipticity at this wavelength decreases linearly with increasing [UP1]/[poly(A)]$_p$ up to 0.14; beyond this point there is very little change in [Θ]$_{264}$. The end point of 7.0 nucleotides/UP1 indicates that each protein molecule occludes 7 residues when

bound to the polynucleotide. This result is in agreement with the electron microscopic data of Herrick et al. (1976).

A plot of the change in A_{260} with increasing [UP1]/[poly(A)]$_p$ is also shown in Figure 2. Although the increase in absorbance is relatively small when compared to the uncertainty in measurement of $\pm 0.003A$, the plot gives an end point of 6.9 nucleotide residues/protein molecule, confirming the CD results. About one-third of the maximum ΔA_{260} can be attributed to the increase in concentration of UP1 from 0 to 6.7×10^{-6} M ([poly(A)]$_p$ is constant). Between a [protein]/[polynucleotide]$_p$ ratio of 0 and the end point of 0.14, the $\Delta A_{260} = 0.065 \pm 0.006$. Of this value, 0.023 is attributable to protein absorbance, leaving an apparent poly(A) hyperchromicity of 0.042 ± 0.006, $17 \pm 2\%$. Beyond the end point ([protein]/[polynucleotide]$_p$ between 0.14 and 0.27), about two-thirds of the ΔA_{260} is attributable to the increase in [UP1]. If corrected for protein absorbance, the slope in the plot to the right of the end point would be only one-third that seen in Figure 2.

Were the binding between UP1 and polynucleotide infinitely strong, the portion of the plot at [UP1]/[poly(A)]$_p > 0.14$ would be perfectly horizontal. The very slight slope in the CD plot beyond the end point is probably indicative of less than perfect saturation by UP1 of overlapping polynucleotide sites (McGhee & von Hippel, 1974). Since the affinity of protein for polynucleotide is ionic strength dependent (see below), UP1 was dialyzed against a buffer of extremely low ionic strength ($\lesssim 0.001$) with a view toward concomitantly lowering the ionic strength during the titration (which was ~ 0.004; the concentration of monovalent cations is ~ 0.003 M). Under these conditions, the protein showed signs of aggregation, as judged by a significant increase in light scattering at 320 nm. It was therefore impossible to perform a titration under conditions of presumably greater protein–poly(A) affinity. Titrations run in buffers of higher ionic strengths, e.g., 0.007, yielded end points between 7 and 8 nucleotide residues/UP1, consistent with the results of Figure 2 (data not shown). As would be predicted, the slopes of the plots beyond the end points were somewhat greater than that observed in 2.5 mM Tris, pH 8.2. However, the variation of $\leq \pm 1$ residue in the end point supports a value of 7 ± 1 residues for the occluded site size.

[Na^+] Dependence of the UP1–Poly(A) Interaction. A number of previous studies suggested that the interaction of UP1 with single-stranded nucleic acids is strongly dependent on ionic strength. Herrick & Alberts (1976b) showed that the T_m depression by UP1 of poly[d(A-T)] or poly(A-U) decreases with increasing [Na^+] or [Mg^{2+}]. UP1-accelerated $tRNA_3^{Leu}$ renaturation, although requiring Mg^{2+}, is inhibited if sufficient Mg^{2+} is present when UP1 initially interacts with inactive $tRNA_3^{Leu}$ (Karpel et al., unpublished results). A similar effect is observed at sufficiently high [K^+]. In order to directly assess the influence of ionic strength on UP1–single-stranded nucleic acid interaction, we studied the effect of [Na^+] on the CD spectrum of poly(A) in the presence of UP1.

Since these earlier studies had indicated a decreased UP1–nucleic acid interaction with increased [Na^+], a [UP1]/[poly(A)]$_p$ of 0.092, which is somewhat lower than the end point, was chosen. In this manner, the increase of poly(A) ellipticity with [Na^+] would be near-maximal under conditions where all the protein molecules would be bound to polynucleotide (see Discussion). The results are shown in Figure 3. The ability of UP1 to depress the $[\Theta]_{264}$ of poly(A) is greatest in the absence of NaCl.

With increasing [NaCl], the interaction is decreased, as indicated by the increase in poly(A) ellipticity. At or above

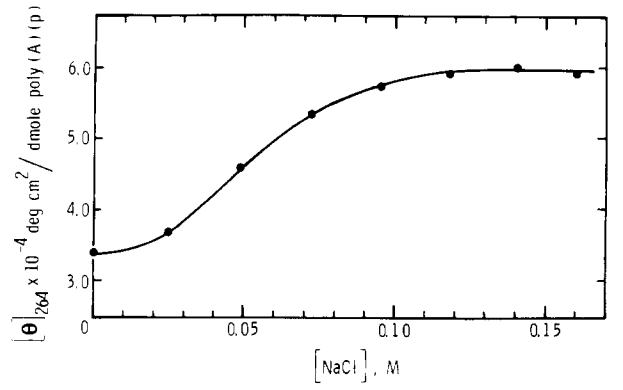

FIGURE 3: [Na^+] dependence of the UP1–poly(A) interaction; 3.0 mM Tris-HCl, 0.55 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol, pH 8.25, 20 °C. $0 \leq$ [NaCl] ≤ 0.16 M. [Poly(A)] = 2.84×10^{-5} M (p); [UP1] = 2.6×10^{-6} M.

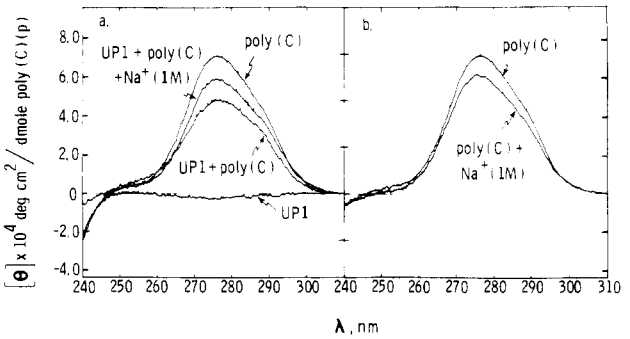

FIGURE 4: Effect of UP1 on the CD spectrum of poly(C); 3.0 mM Tris-HCl, 0.55 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol, pH 8.25, 20 °C; [NaCl] was as indicated. [Poly(C)] = 2.50×10^{-5} M (p); [UP1] = 6.6×10^{-6} M where applicable. (a) Poly(C), UP1, UP1 + poly(C), UP1 + poly(C) + NaCl (1.0 M). (b) For comparison, CD spectra of poly(C) in the absence and presence of added NaCl (1.0 M).

0.12 M NaCl there appears to be no interaction since the plot is flat and the ellipticity is virtually identical (96%) with that of poly(A) in the absence of protein. The midpoint of the sigmoidal transition is 0.052 M NaCl. The interaction may be less than absolutely maximal at the lowest ionic strength studied; the slope of the plot may not be quite 0 at that point (see Discussion). But, as noted above, aggregation of UP1 occurs at extremely low ionic strengths, thus precluding observation of protein–poly(A) interaction below $\mu \sim 0.004$. The shape of the sigmoidal plot in Figure 3 would suggest that the theoretical limiting $[\Theta]_{264}$ (at $\mu \rightarrow 0$) is not much less than the data point at ~ 0 M NaCl, so that the true midpoint of the transition is probably very close to 0.05 M NaCl.

Effect of UP1 on Poly(C) Secondary Structure. In competition experiments, poly(A) was shown to be more effective than poly(C) in its ability to inhibit UP1-induced $tRNA_3^{Leu}$ renaturation (Karpel et al., 1976; unpublished results), suggesting that poly(A) has a greater affinity for UP1. This is supported by the effect of UP1 on the circular dichroism spectrum of poly(C) (Figure 4). When the small negative circular dichroism at 277 nm of UP1 was taken into account, interaction of the protein with poly(C) reduces the polynucleotide's maximum at this wavelength by 17%. In contrast, the same [UP1]/[polynucleotide]$_p$ (0.26) reduced the poly(A) CD maximum at 264 nm by more than 50% (Figure 2). As with poly(A), high [Na^+] disrupts the interaction between polynucleotide and UP1. Although the dependence of the protein's interaction with poly(C) on [Na^+] was not explored in detail, it is seen in Figure 4 that addition of NaCl to 1.0

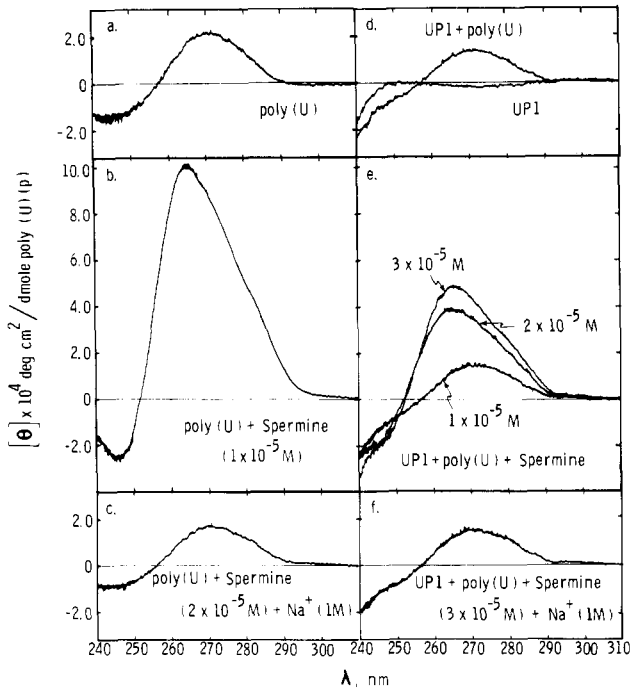

FIGURE 5: Effect of UP1 on the CD spectra of the poly(U) random coil and the poly(U·U) double helix; 3.0 mM Tris-HCl, 0.55 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol, pH 8.25, 15 °C. [Spermine] and [NaCl] were as indicated. [Poly(U)] = 2.50 × 10^{-5} M (p). Where applicable, [UP1] = 6.1 × 10^{-6} M. (a) Poly(U) (coil). (b) Poly(U) + spermine (1 × 10^{-5} M) [poly(U·U) helix]. (c) Poly(U) + spermine (2 × 10^{-5} M) + NaCl (1.0 M) (coil). (d) UP1 + poly(U) (coil). (e) UP1 + poly(U) + spermine, as indicated. (f) UP1 + poly(U) + spermine (3 × 10^{-5} M) + NaCl (1.0 M) (coil).

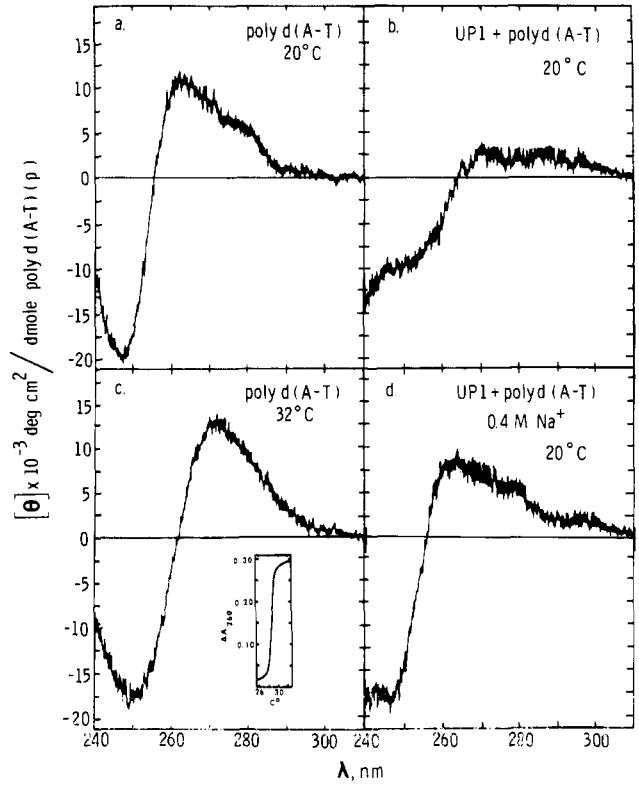

FIGURE 6: Effect of UP1 on the CD spectrum of poly[d(A-T)]; 3.0 mM Tris-HCl, 0.55 mM Na_2EDTA, 0.10 mM DTT, and 10% (v/v) glycerol, pH 8.25; [poly[d(A-T)]] = 1.78 × 10^{-5} M (p). [UP1] = 2.6 × 10^{-6} M, where applicable. (a) Poly[d(A-T)] double helix, 20 °C. (b) Poly[d(A-T)] + UP1, 20 °C. (c) Denatured poly[d(A-T)], 32 °C. (Inset) Denaturation of poly[d(A-T)], ΔA_{260} vs. °C. T_m = 29 °C. (d) Poly[d(A-T)] + UP1 + Na^+ (0.4 M), 20 °C.

M yields a spectrum identical with that of a 400-μL solution of poly(C) alone at the same [Na^+]. The interaction between UP1 and poly(C) is therefore fully reversible by high concentrations of salt.

Effect of UP1 on Poly(U) and Poly(U·U). Poly(U), which in the absence of highly charged cations (such as spermine, 4+) exists as a random coil with no secondary structure (Richards et al., 1963), is the most effective polynucleotide inhibitor of UP1-induced $tRNA_3^{Leu}$ renaturation (Karpel et al., 1976; unpublished experiments). As seen in parts a and d of Figure 5, very little change in the CD spectrum of the poly(U) coil is brought about by interaction with UP1 at 15 °C ($[UP1]/[poly(U)]_p$ = 0.24). However, the protein clearly alters the conformation of the poly(U·U) double helix (Zimmerman, 1976) which is formed in the cold in the presence of spermine or other highly charged ions (Szer, 1966; Thrierr et al., 1971) and possesses a CD spectrum very different from that of the coil (Figure 5; Thrierr et al., 1971).

When spermine is added (to 1 × 10^{-5} M) to a solution of the poly(U) coil, the poly(U·U) helix forms readily (parts a and b of Figure 5). But when the same level of spermine is added to the UP1–poly(U) mixture, there is virtually no change in the CD spectrum, indicating that UP1 depresses the T_m of poly(U·U) below the temperature of the experiment, 15 °C (parts d and e of Figure 5). When the spermine level is increased to 2 × 10^{-5} M, some formation of helix occurs, as evidenced by an increase in magnitude of the positive CD peak and the shift of that peak to lower wavelengths (Figure 5e). At 3 × 10^{-5} M spermine, the helix is still somewhat less than half-formed; the T_m therefore is presumably slightly below 15 °C. The effects of UP1 and spermine are in competition with each other. UP1 binds more tightly to the coil, thus lowering the T_m of the poly(U·U) helix. On the other hand, spermine has a higher affinity for the helix, thus raising the melting transition. The interactions of both protein and cation with respectively the coil and helix are strongly electrostatic; when an aliquot of concentrated NaCl is added (to 1 M) to the poly(U·U) helix (in 2 × 10^{-5} M spermine) and to the UP1–poly(U)–spermine (3 × 10^{-5} M) mixture, the resulting solutions give virtually identical CD spectra, indicative of the coil (parts c and f of Figure 5). [Although 1 M Na^+ can itself stabilize the poly(U·U) helix, the T_m under these conditions is below 15 °C (Michelson & Monny, 1966).]

Effect of UP1 on Poly[d(A-T)]. When certain proteins, such as the product of T4 gene 32, lower the T_m of DNA helices, the CD spectrum of the resulting denatured DNA is significantly different from that of heat-denatured DNA, indicating that the denatured DNA conformation is distorted when bound to protein (Jensen et al., 1976). However, ribonuclease A, which also destabilizes DNA helices, has little effect on denatured poly[d(A-T)], since its CD spectrum upon RNase-effected denaturation is very similar to that of heat-denatured poly[d(A-T)] (Jensen & von Hippel, 1976). Since under low ionic strength conditions UP1 effectively lowers the T_m of poly[d(A-T)] (Herrick & Alberts, 1976b), it was of interest to determine whether this behavior is accompanied by a perturbation in the single-stranded DNA's CD spectrum.

The results of this experiment are shown in Figure 6. In accord with previous reports (Jensen & von Hippel, 1976), heat denaturation causes a shift in the poly[d(A-T)] CD maximum (from 262 to 272 nm), minimum (247 to 249 nm), and crossover (255 to 261 nm) (parts a and c of Figure 6). At $[UP1]/[poly[d(A-T)]]_p$ = 0.15, conditions under which this synthetic DNA is completely melted at 20 °C in a similar buffer (Herrick & Alberts, 1976b), the CD spectrum seen in

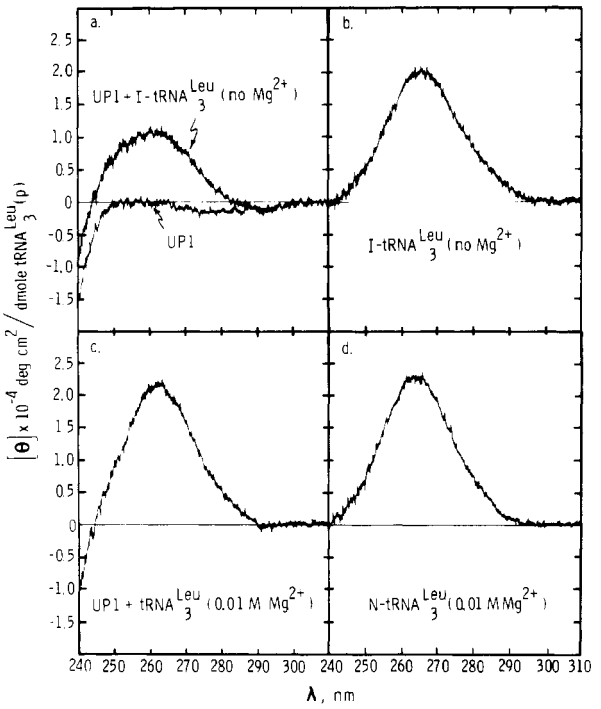

FIGURE 7: Effect of UP1 on the CD spectrum of yeast $tRNA_3^{Leu}$; 3.8 mM Tris-HCl, 1.5 mM cacodylate, 0.78 mM Na_2EDTA, 0.077 mM DTT, 0.035 M KCl, and 7.7% (v/v) glycerol, pH 8.2, 15 °C. [$tRNA_3^{Leu}$] = 4.37×10^{-5} M (p). (a) UP1 (6.6×10^{-6} M) + inactive $tRNA_3^{Leu}$ and UP1 alone. (b) Inactive $tRNA_3^{Leu}$. (c) UP1 + $tRNA_3^{Leu}$ + Mg^{2+} (0.010 M). (d) Heat-renatured (native) $tRNA_3^{Leu}$ in 0.010 M Mg^{2+}.

Figure 6b is very different from that of either heat-denatured or native poly[d(A-T)]. The poly[d(A-T)] CD maximum is now significantly reduced in magnitude, with the crossover point shifted to 264 nm. A corresponding hyperchromic change of ~35% at 260 nm was observed, indicating that denaturation of the poly[d(A-T)] was complete. When an aliquot of concentrated NaCl was added to the UP1–poly[d-(A-T)] mixture, so that the final [Na^+] = 0.4 M, a CD spectrum similar to that of native poly[d(A-T)] in the absence of protein was obtained (Figure 6d). The crossover is identical, and, allowing for the dilution upon NaCl addition, the magnitude of the CD peak is restored to ~87% of that of the native poly[d(A-T)] in the absence of protein. A similar result was found for a UP1 + poly[d(A-T)] mixture in 0.2 M NaCl (data not shown). The results show that analogous to its effect on polyribonucleotides, UP1 distorts the conformation of single-stranded DNA upon binding in a low ionic strength environment. Upon addition of NaCl, the complex dissociates, as evidenced by restoration of the native DNA spectrum. The similarity in the effect of UP1 on RNA and DNA single strands is consistent with the observation that this protein depresses the T_m of poly(A-U) to a degree comparable to that of poly[d(A-T)] (Herrick & Alberts, 1976b).

Secondary Structural Changes in $tRNA_3^{Leu}$ during Its UP1-Induced Renaturation. The ability of UP1 to destroy synthetic polynucleotide secondary structure is paralleled by the protein's effect on $tRNA_3^{Leu}$, thus providing a rationale for UP1-induced renaturation. The effect of UP1 on the CD spectrum of $tRNA_3^{Leu}$ at 15 °C is shown in Figure 7. At this temperature, the half-life of spontaneous renaturation is about 1 week (Lindahl et al., unpublished results). The presence of UP1 at a molar ratio to tRNA (p) of 0.15 [optimal for renaturation; Karpel et al. (1974) and unpublished results] reduces the positive CD peak of inactive tRNA by about 50%

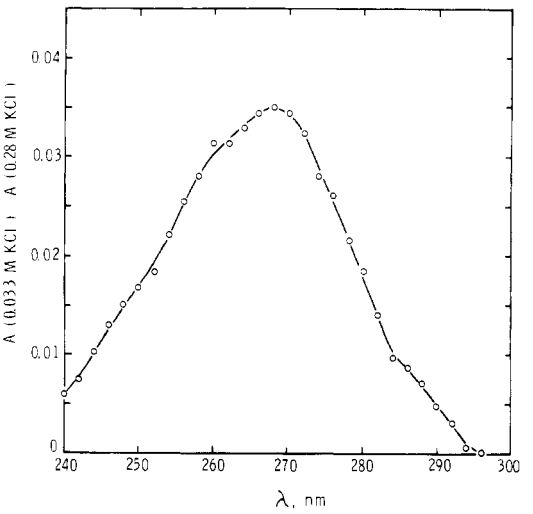

FIGURE 8: Denaturation spectrum of yeast $tRNA_3^{Leu}$ in the presence of UP1; 3.8 mM Tris-HCl, 1.5 mM cacodylate, 0.78 mM Na_2EDTA, 0.077 mM DTT, and 7.7% (v/v) glycerol, pH 8.2, 15 °C. [$tRNA_3^{Leu}$] = 4.37×10^{-5} M (p). [UP1] = 6.6×10^{-6} M. The spectrum was obtained as the difference between the absorbance of two mixtures of UP1 + $tRNA_3^{Leu}$ which were identical, except that one cuvette contained 0.033 M KCl (interactive conditions) and the other cuvette contained 0.28 M KCl (noninteractive conditions).

(parts a and b of Figure 7). A major conformational change involving a significant loss of secondary structure (see below) is thus effected upon binding the protein. When $MgCl_2$ is added to this mixture, to a final concentration of 0.01 M, the resultant CD spectrum is virtually identical with that of heat-renatured $tRNA_3^{Leu}$ in 0.01 M Mg^{2+}, indicating that renaturation has occurred (parts c and d of Figure 7). (The kinetics of the Mg^{2+}-induced change of the tRNA CD spectrum in the presence of UP1 were too rapid to follow at 15 °C. At 0 °C, we found in a preliminary CD experiment that the $t_{1/2}$ of this change is 5–10 min.) The sequential effects of UP1 and Mg^{2+} on the circular dichroism of $tRNA_3^{Leu}$ parallel earlier studies using aminoacylation as an assay for tRNA conformation, where the rate-limiting step for renaturation appears to be the Mg^{2+}-induced breakup of the tRNA–protein complex (Karpel et al., 1976).

Direct evidence for loss of tRNA secondary structure when complexed to UP1 is provided by ultraviolet difference spectroscopy. In this experiment, a UP1–$tRNA_3^{Leu}$ mixture under the same conditions as in the CD experiment described above was split between two matched cuvettes and KCl was sequentially added to one of the cuvettes (see Materials and Methods). An absorbance change of 5% at 260 nm is observed when [K^+] was raised to 0.12 M (from 0.035 M), and it was 8% at 0.20 M. At [K^+] ≥ 0.28 M, the hyperchromic effect at 260 nm was 10%. The difference spectrum at 0.28 M KCl is shown in Figure 8. The maximum absorbance change is seen to occur at 268 nm; this perturbation represents 14% of the tRNA absorbance at this wavelength. An aliquot of the concentrated KCl solution was added to the cuvette to which H_2O had previously been added and the same volume (30 μL) of H_2O was added to the cuvette which had previously received KCl aliquots, so that the [KCl] was now identical (0.44 M) in both cuvettes. The resulting difference spectrum was virtually flat above 240 nm, indicating full reversibility of the UP1-induced effect.

Conceivably, part of this hyperchromic effect at 268 nm may be due to changes in the protein's 280-nm absorbance maximum. Under the conditions of the experiment, the ratio of tRNA absorbance to protein absorbance is 2.6 at 268 nm,

whereas at 260 nm this ratio is 4.0. Given the dramatic change in the CD spectrum of $tRNA_3^{Leu}$ upon interaction with UP1 and the relatively low protein absorbance at 260 nm, it seems likely that the hyperchromic effect at this wavelength seen upon complex formation is largely representative of changes in tRNA secondary structure. Since the hyperchromic effect upon destroying the secondary structure of tRNA by heat denaturation is 25–30%, the UP1-induced change is indicative of destruction of $\sim 1/3$ of the $tRNA_3^{Leu}$ secondary structure.

A hyperchromic change of 10% (based on tRNA absorbance) at 260 nm was also observed with another, somewhat different protocol using concentrations of protein and RNA comparable to those of the above experiment. Equal amounts of inactive $tRNA_3^{Leu}$ were added to two cuvettes at 15 °C; one contained UP1 and the other was devoid of protein but contained the identical buffer. The increase in absorbance at 260 nm was 10% higher in the cuvette containing UP1; at 268 nm, the difference was 14%. A small aliquot of a concentrated $MgCl_2$ solution was added to each cuvette so as to bring the $[Mg^{2+}]$ in both to 0.016 M, conditions which bring about the rapid renaturation of $tRNA_3^{Leu}$ after having been complexed to UP1. After the contribution of protein was taken into account, the tRNA absorbances at 260 nm were within 1% of each other. In the absence of protein, $tRNA_3^{Leu}$ would not significantly renature at 15 °C and should have a slightly higher ($\sim$3%) absorbance at 258 nm relative to the native conformer (Adams et al., 1967). The UP1-renatured $tRNA_3^{Leu}$ A_{258} was 2% less than the A_{258} of the inactive conformer in the other cuvette; this difference is probably fortuitous and is within the experimental error of the experiment. Nevertheless, these results qualitatively agree with the conclusion that a significant portion of the secondary structure of inactive $tRNA_3^{Leu}$ is destroyed upon interaction with UP1.

Discussion

UP1-Induced Destruction of Nucleic Acid Secondary Structure: A Proposal for the Molecular Basis of Selective Binding to Single Strands. The perturbation of single-stranded nucleic acid circular dichroism spectra by UP1 is indicative of the distortion of the polynucleotide chain. Such distortion is also brought about by a number of other helix-destabilizing proteins: e.g., gene 32 protein (Jensen et al., 1976) and the *E. coli* binding protein (Sigal et al., 1972) as well as S1 ribosomal protein from the latter organism (Szer et al., 1976; Bear et al., 1976). Since the binding of UP1 to poly(A) and other polynucleotides is accompanied by a significant hyperchromic effect, it is likely that in the distorted conformation the bases are unstacked relative to their orientation in the unperturbed structure. The protein-induced distortion of the polynucleotide backbone very likely results from optimization of binding interactions between the two macromolecules. In the case of UP1, this observation along with other relevant results forms the basis of the following model of this protein's interaction with and specificity for single-stranded nucleic acids.

Although direct interaction with the nucleic acid heterocyclic bases could be responsible for enhanced and selective binding of a protein to single strands (relative to double helices), this is not the case for UP1. An oxidized single-stranded polynucleotide devoid of its base [poly(ribosylurea phosphate)] prepared from poly(C) binds more tightly to UP1 than does poly(C) (Karpel et al., unpublished results), and the reactivity of adenine residues toward chloroacetaldehyde in UP1–denatured DNA complexes is comparable to free denatured DNA reactivity (Kohwi-Shigematsu et al., 1978). UP1 must therefore interact solely with the polynucleotide backbone, and the strong ionic strength dependence of this interaction reported above (and see below) suggests that electrostatic interactions between basic amino acid residues and phosphate groups are a significant if not major factor in the binding between protein and nucleic acid.

Electrostatic interactions have been shown to be of importance in the binding of a number of different proteins to nucleic acids, both single and double stranded (Record et al., 1978). In the case of those interactions involving double helical nucleic acids, the regular array of phosphates in the helix restricts electrostatic binding to a complementary array of basic amino acid residues. However, phosphate–phosphate distances in random coiled single strands can be extended or compressed (within limits) without a significant expenditure of energy, allowing the polynucleotide chain to form close contacts between its phosphates and a matrix of positive groups at the surface of the protein. This suggests a structural basis for the selective recognition of single strands: the distances between the positive charges on the helix-destabilizing protein are sufficiently varied so that a random coil could most easily bind its phosphates to the basic amino acids. Nucleic acids possessing a regular array of phosphate groups (with a repeating distance between negative charges) would have to undergo conformational changes in order to bring these groups into close contact with the interacting basic residues of the protein. Double helical nucleic acids would denature, hence, the T_m depressing effect of helix-destabilizing proteins. The phosphate–phosphate distances in single strands possessing some helical structure, such as poly(A), are more constrained than they are in totally random coils, hence, the disruption of poly(A) and poly(C) secondary structure seen upon interaction with UP1 and other proteins. An analogous effect is seen in the hyperchromicity of single-stranded natural DNAs upon interaction with UP1, although here the secondary structure disrupted largely consists of double-stranded intramolecular hairpins (Herrick & Alberts, 1976b). Consistent with this observation, electron microscopy shows that the contour length of fd DNA is increased by about 20% upon interaction with UP1 (Herrick et al., 1976).

Estimation of Binding Parameters. An approximation of the binding constant of UP1 to poly(A) may be obtained by applying the extended Scatchard approach of McGhee & von Hippel (1974) to the titration results of Figure 2. This method takes into account the statistical effect of protein ligand binding to overlapping sites on a nucleic acid lattice for both interacting (cooperative, their eq 10) and noninteracting (noncooperative, their eq 15) ligands. From a plot of ν, the binding density of bound ligand per total lattice residues, vs. ν/L (where L = concentration of free ligand), the association constant, K, occluded site size, n, and cooperativity parameter, ω, can in principle be determined. If it is assumed that the $[\Theta]_{264}$ obtained at maximal $[UP1]/[poly(A)]_p$ ($[\Theta]_{satn}$) represents essentially complete saturation of the polynucleotide, ν_i can be calculated by interpolating $[\Theta]_i$ values between $[\Theta]_{satn}$ and $[\Theta]$ in the absence of UP1. Knowing $[UP1]_{total}$, total $[poly(A)]_p$, and ν, we can in principle calculate L. However, at $[protein]/[polynucleotide]_p$ ratios below the theoretical saturation point (assuming $n = 7$), we find that $[UP1]_{bound} \sim [UP1]_{total}$, and thus L cannot be determined with certainty. At ratios beyond the theoretical saturation point, the calculated ν values are between 0.133 and 0.143, too small a variation to obtain meaningful binding parameters.

The only data that can effectively be used to obtain K is the $[\Theta]$ observed at the end point, which represents a depression in ellipticity of 85% of the maximal value. If the

interaction is noncooperative in nature, eq 10 of McGhee & von Hippel (1974) yields $K = 6 \times 10^7$ M^{-1}. This number must be treated as an upper limit for the following reasons. First, we do not know if the apparent $[\Theta]_{satn}$ observed (2.15×10^4 deg cm^2 dmol^{-1}) represents the maximal depression of poly(A) ellipticity by UP1. Although it is likely that the true $[\Theta]_{satn}$ is not significantly lower than the observed value, a decrease in ν of 10% would lower K by an order of magnitude. Second, any curvature in the $[\Theta]$ vs. $[UP1]/[poly(A)]_p$ plot (little if any is apparent) would tend to overestimate the value of n. With n reduced to 6, K could also be an order of magnitude lower than the calculated value. Third, although on the basis of previous results it is unlikely that the interaction of UP1 with single-stranded nucleic acids is strongly cooperative (Herrick & Alberts, 1976b; Herrick et al., 1976), the results of this or previous studies cannot rule out the existence of moderate cooperativity. Thus, with n set at 7 and ω at 50, use of eq 15 of McGhee & von Hippel (1974) yields $K = 1 \times 10^5$ M^{-1}. Finally, the experimental uncertainty in $[\Theta]_{264}$ ($\pm 0.1 \times 10^4$ deg cm^2 dmol^{-1}) will also be reflected in K, although the resulting variation (2×10^7 to 1×10^8 M^{-1}) will be relatively small. Consistent with the observed reduction of binding with increasing $[Na^+]$, data obtained from titrations run at higher ionic strengths yielded lower values for ν and K.

Dependence of UP1–Poly(A) Binding on $[Na^+]$: Estimation of the Number of Ion Pairs in the Interaction. Record et al. (1976) have developed an approach by which the number of ion pairs, i.e., charge interactions, involved in the binding of a protein ligand to a polynucleotide lattice may be calculated from the dependence of the binding constant on monovalent cation concentration. They showed that

$$-\frac{\partial \log K}{\partial \log [M^+]} = k + m'\psi \qquad (1)$$

where K is the association constant, $[M^+]$ is the monovalent cation concentration, k is the number of anions displaced by the interactions, m' is the number of ion pairs formed, and ψ is the fraction of counterion bound in the thermodynamic sense per lattice charge and is characteristic of the particular polynucleotide.

The data of Figure 3 can be used with eq 1 to calculate K, with the following assumptions: first, under the experimental conditions of excess polynucleotide, prior to addition of NaCl essentially all the protein is bound to the polynucleotide; second, since $[\Theta]_{264}$ is invariant at $[Na^+] > 0.12$ M, no UP1 is associated with poly(A) under these conditions. Using the calculated value for K of 6×10^7 M^{-1} under low ionic strength ($\sim$0 M Na^+) conditions, we estimate that more than 99% of the protein is bound to polynucleotide. If K were an order of magnitude smaller, 94% of the protein would be bound. At intermediate $[Na^+]$, it is thus assumed that $[UP1]_{bound}$ varies proportionately with $[\Theta]_{264}$ between these limits, permitting calculation of ν, L, and therefore K via eq 10 of McGhee & von Hippel (1974). A plot of log K thus obtained vs. log $[Na^+]$ is linear (not shown), with a slope of 4.65. Record et al. (1976) have determined ψ to be equal to 0.78 for poly(A) and if k were known, m' could be calculated. Since k has not been determined for UP1, only an upper limit can be assigned: $m' \leq 6.0$. Since oligonucleotides with as few as three phosphates bind UP1 (Karpel et al., 1976), it is likely that m' is considerably smaller than 6.0. A possible source of error in this calculation is the implicit assumption of noncooperative binding. Using eq 15 of McGhee & von Hippel (1974) and assuming ω to be invariant with $[Na^+]$, we find that the slope of the log K vs. log $[Na^+]$ plot decreases with increasing ω. With ω set at 50, the slope is 2.55, which would yield $m' \sim 3$ in the absence of counterion release.

UP1-Induced tRNA Denaturation and RNA Conformational Interchange. Our demonstration that UP1-facilitated $tRNA_3^{Leu}$ renaturation proceeds through an intermediate in which part of the tRNA secondary structure is destroyed is consistent with the observed high activation barrier for spontaneous renaturation (Fresco et al., 1966; Hawkins et al., 1977; Lindahl et al., unpublished results). When bound to the inactive tRNA conformer, the secondary structural impediment to renaturation is either removed or at least sufficiently reduced so that upon addition of Mg^{2+} renaturation is rapid relative to the duration of the subsequent determination of the tRNA's conformation. The circular dichroic and spectrophotometric data presented here do not provide any information about the location of the UP1 molecule(s) on the inactive tRNA; however, the protein's lack of base specificity would presumably allow it to bind to any single-stranded region on the tRNA molecule or to bring about the denaturation of any helical region (regions of relatively low G-C content would probably be more easily denatured than those of high G-C content).

Although most attention has been paid to the involvement of helix-destabilizing proteins in various aspects of DNA function, there is increasing evidence that protein-mediated RNA conformational interchange occurs in vivo. In particular, *E. coli* ribosomal S1 protein (Draper & von Hippel, 1978a,b) and rabbit reticulocyte EIF-3 (Ilan & Ilan, 1977) may function as temporary helix destabilizers at the level of protein synthesis. In this respect, UP1-assisted $tRNA_3^{Leu}$ renaturation could serve as an excellent model for the action of these proteins, since the effect is to bring about a conformational change rather than to permanently denature the RNA. Thus, for example, the formation of an initiation complex may require the destruction of "incorrect" secondary structure on the message and/or the 16S (or 18S) RNA, so as to expose complementary residues for the formation of base pairs between the two molecules (Shine & Delgarno, 1974). The results presented here clearly show that structure formation, in the form of native yeast $tRNA_3^{Leu}$, can be assisted by the denaturing action of a helix-destabilizing protein.

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Investigation, by Cross-Linking, of Conformational Changes in F-Actin during Its Interactions with Myosin†

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ABSTRACT: The hypothesis that the subunits of F-actin rotate during interaction with myosin and ATP has been tested by using the specific cross-linking reagent *p*-phenylene-*N*,*N*′-bis(maleimide) (PM). The insertion of cross-links between F-actin subunits does not change the ability of the F-actin to activate the ATPase of either myosin subfragment-1 (S-1) or heavy meromyosin, and its ability to superprecipitate with myosin is unimpaired. We conclude that large-scale rotations of actin subunits are not required for activity. The cross-linking of F-actin by PM is, however, inhibited in a non-cooperative fashion by S-1 binding, suggesting that a small local change in actin structure may accompany the binding of S-1 or that S-1 sterically blocks the cross-linking by binding near the contact region between actin subunits.

The sliding of thick and thin filaments during muscle contraction is thought to be brought about by tilting of the myosin heads attached to actin (Huxley, 1969; Huxley & Simmons, 1971). Although in Huxley's original hypothesis the tilting was supposed to take place by rotation of the heads over the surface of the actin subunits, it is equally plausible that the contact between actin and myosin is unchanged and that tilting results from rotation of the actin subunits (Huxley, 1974).

The interactions between myosin and actin during the hydrolysis of ATP in solution are thought to mimic the cross-bridge cycle (Lymn & Taylor, 1971). Therefore, the hypothesis that actin subunits rotate during the cross-bridge cycle may be tested by modifying F-actin so as to prevent rotation of the subunits and then observing whether this impairs the ability of F-actin to activate myosin ATPase and to form a superprecipitating mixture. In an earlier paper (Knight & Offer, 1978), we showed that the reagent *p*-phenylene-*N*,*N*′-bis(maleimide) (PM)[1] forms cross-links between a cysteine

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