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Circular Dichroism Study of the Interaction between T4 Gene 32 Protein and Polynucleotides†

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ABSTRACT: The interaction of bacteriophage T4 gene 32 coded protein (DNA melting- or unwinding protein) and the synthetic polynucleotides poly(dA), poly(dT), poly(dA)·poly(dT), and poly[d(A-T)]·poly[d(A-T)] was examined by means of circular dichroism spectroscopy. The protein induced strand separation of the double-stranded molecules at temperatures far below the regular melting temperatures. This denaturation is reversible for poly[d(A-T)]·poly[d(A-T)] but irreversible for poly(dA)·poly(dT). In the complexes formed with the protein the single polynucleotide has a conformation in which the bases are stacked. This conformation closely resembles that of one strand of a poly[d(A-T)]·poly[d(A-T)] molecule at high LiCl concentration. To arrive at this conclusion qualitative rules for the interpretation of the polynucleotide circular dichroism spectra were derived. These rules are: (a) Upon strand separation the minimum in the CD spectrum near 250 nm shifts by about 3 nm to the red. (b) The degree of base stacking in a single-stranded polynucleotide follows from the depth of the minimum in the circular dichroism spectrum near 250 nm. (c) The type of conformation of the single-stranded polynucleotide can be determined from a comparison of the long wavelength part of the circular dichroism spectra of single- and double-stranded polynucleotides. This part of the spectrum has the same shape for a double-stranded and a single-stranded molecule provided the conformations of the separate strands are equal.

Bacteriophage T4 gene 32 coded protein (gp32) was first isolated by Alberts & co-workers (Alberts et al., 1968; Alberts, 1970; Alberts & Frey, 1970). It binds tightly and cooperatively to single-stranded DNA. It facilitates both the denaturation and the renaturation of DNA. Genetic studies have demonstrated that this protein is essential for genetic recombination (Berger et al., 1969; Tomizawa et al., 1966), DNA replication (Epstein et al., 1963), repair of radiation damaged DNA (Wu & Yeh, 1973), and protection of replicating T4 chromosome from degradative activities (Curtis & Alberts, 1976; Mosig & Bock, 1976). In vitro gp32 enhances the rate at which T4 DNA polymerase utilizes single-stranded DNA temperatures by five- to tenfold (Huberman et al., 1971). gp32 is also one of the essential components of the reconstructed DNA replicating apparatus (Alberts et al., 1975; Morris et al., 1975).

It has been suggested that gp32 binds to the phosphate groups of polynucleotides or DNA rather than to the bases so that the bases in the complexes are in exposed positions (Alberts & Frey, 1970; Huberman et al., 1971; Kelly et al., 1976). Sedimentation behavior of gp32 fd DNA complexes indicated

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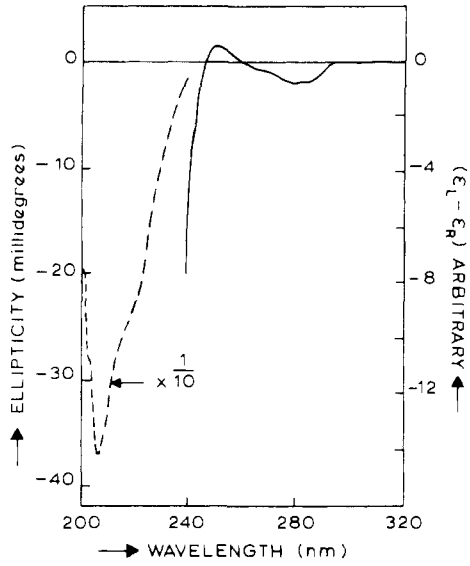

FIGURE 1: CD spectrum of gp32 in buffer I at 0.4 mg per mL. This is the highest protein concentration used in the experiments. The CD is given both as the measured ellipticity and in an arbitrary scale. The latter gives the contribution of the protein to the CD signal of a polynucleotide mixture (nucleotide to protein ratio 6) expressed per mole of nucleotide. The CD is small in the wavelength region shown in the following figures ($\lambda > 240$ nm). Using the latest basis vector set of Dr. Yang's laboratory, it was calculated that the protein contains 21.6% α helix; 25.8% β-pleated sheet; and 52.6% random coil.

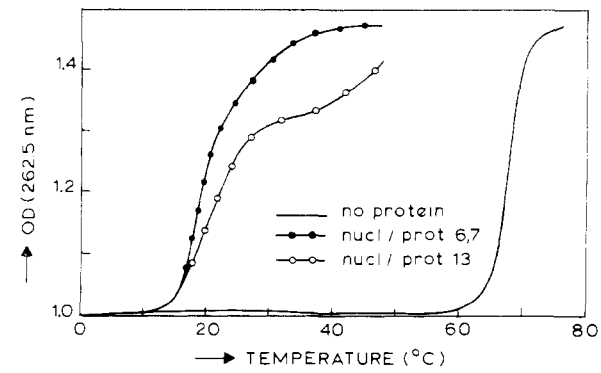

FIGURE 2: Denaturation of poly[d(A-T)]·[d(A-T)] in buffer II in the absence and presence of gp32. The nucleotide to protein ratios used were 6.7 and 13. In the presence of protein the polynucleotide melts about 40 °C below the regular melting temperature.

that the complex has a structure which is more extended (or rigid) than free fd DNA (Alberts & Frey, 1970). Delius et al. (1972) have shown that the contour length of gp32 fd DNA complex spread with cytochrome *c* and 30% formamide on a 10% formamide hypophase was approximately 3.0 μm (about 4.6 Å per nucleotide). The length of free fd DNA spread in the same way was 1.9 μm (2.9 Å per nucleotide). If one assumes that the base-base distance in the gp32 complex is the same in solution as in cytochrome *c* film, it can be concluded that the base-base distance in the complex is 35% longer than that in (B form of) double-stranded DNA and over 50% longer than that in the single-stranded DNA.

It is a well-known fact that circular dichroism (CD)[1] is very sensitive to secondary structure of polynucleotides. Therefore we decided to study by means of CD the complexes formed between gp32 and poly(dA), poly(dT), poly(dA)·poly(dT), and poly[d(A-T)]·poly[d(A-T)]. We conclude that gp32 keeps the single-stranded polynucleotides in a conformation which is almost equal to that of one strand of a double-stranded poly[d(A-T)]·poly[d(A-T)] molecule at high LiCl concentration. In the next paper (Greve et al., 1978), we report the CD spectra of complexes formed with gp32*-I, a limited proteolysis product of gp32.

Materials and Methods

All polymers were purchased from P-L Biochemicals. The catalogue numbers, batch numbers, and extinction coefficients are: poly(dA) 7836, 526-35, 8600 L mol^{-1}; poly(dT) 7834, 508-68, 8520 L mol^{-1}; poly(dA)·poly(dT) 7860, 526-26, 6000 L mol^{-1}; poly[d(A-T)]·poly[d(A-T)] 7870, 508-101, 6600 L mol^{-1}. The lyophilized polymers were dissolved in buffer and stored frozen. gp32 was prepared and purified as described by Moise & Hosoda (1976). To determine the gp32 concentration we assumed that an OD (280 nm, 1 cm) of 1 corresponds to 0.91 mg/mL and that gp32 has a molecular weight of 35 000 (Alberts & Frey, 1970). CD spectra were measured with a Cary 60 spectropolarimeter equipped with a 6001 unit. The computerized data collecting system has been described (Brunner & Maestre, 1975). CD data are presented as the CD per mole of monomer, $\epsilon_L - \epsilon_R$, where ϵ_L and ϵ_R are the extinction coefficients for left and right circularly polarized light, respectively. CD measurements of complexes were made by mixing cold (4 °C) gp32 and polynucleotide solutions in the desired ratio. After mixing, the solution was put in a cooled CD cuvette and cooled further to 1 °C. After equilibration the 1 °C spectrum was measured. Then the temperature was increased by about 5 °C and after equilibration (about half an hour) the next spectrum was measured. This procedure was repeated until spectra had been measured at all desired temperatures. The buffers used are: (buffer I) 10 mM KCl, 0.1 mM EDTA, 2 mM Tris (pH 7.8); (buffer II) buffer I plus 10 mM $MgSO_4$.

Results

The purpose of this investigation is to study the structure of a polynucleotide in a gp32-polynucleotide complex. To do so we want to compare the CD spectrum of the free polynucleotide with the CD spectrum of the polynucleotide as it is in a complex. (Hereafter referred to as "complexed polynucleotide".) Therefore we measured CD spectra of gp32-polynucleotide mixtures. The desired complexed polynucleotide spectra were then calculated by subtracting the CD spectrum of gp32 from the CD spectra of the mixtures. Herein it was assumed that the CD spectrum of gp32 itself does not change upon complexation. The error possibly introduced by this assumption will be small since we concentrated on the polynucleotide spectrum from 240 to 300 nm where the gp32 CD contribution is very small (Figure 1). Moreover, as will be shown below, the spectra obtained in this way look like typical polynucleotide spectra. Since it was found that gp32 itself denatures between 50 and 60 °C (Jensen et al., 1976; Moise & Hosoda, manuscript in preparation), measurements were done at temperatures below 50 °C only.

Optical Density Measurements. Alberts & Frey (1970) reported that gp32 complexed single-stranded DNA is fully hyperchromic. Therefore the denaturation of double-stranded DNA by gp32 can be followed by OD measurements. Figure 2 shows that our gp32 preparation denatured poly[d(A-T)]·poly[d(A-T)] in buffer II at more than 40 °C below its regular melting temperature. Similar curves were obtained for poly(dA)·poly(dT) indicating that gp32 also denatured this polynucleotide. The ratio between the number of nucleotides

[1] Abbreviations used: CD, circular dichroism; gp32, T4 gene 32 protein.

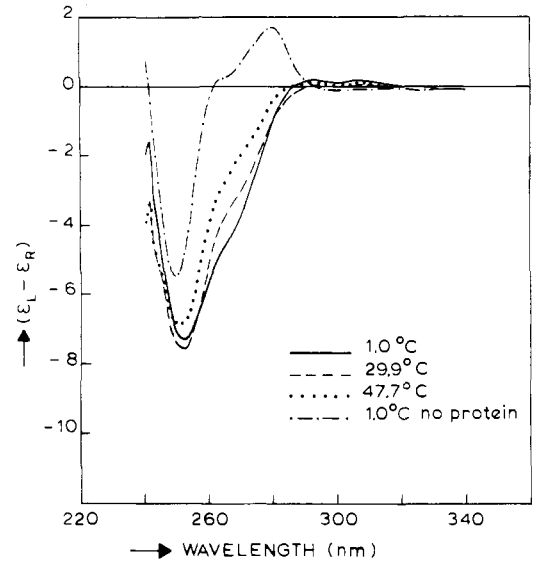

FIGURE 3: CD spectra of complexed poly(dA) in buffer I at 1.0, 29.9, and 47.7 °C. The nucleotide to protein ratio is 8.5. The spectrum of free poly(dA) in the same buffer at 1 °C is also shown.

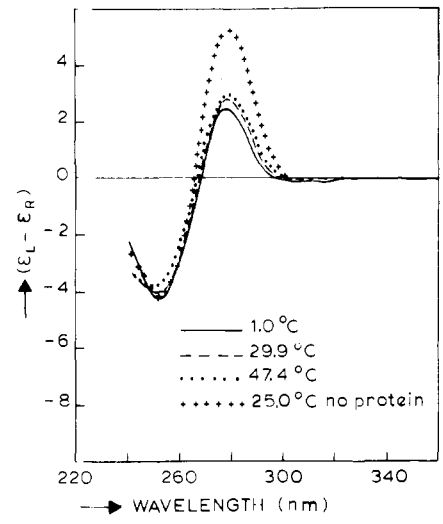

FIGURE 4: CD spectra of complexed poly(dT) in buffer I at 1.0, 29.9, and 47.4 °C. The nucleotide to protein ratio is 8.5. The spectrum of the free poly(dT) measured at 25.0 °C in the same buffer is shown for comparison.

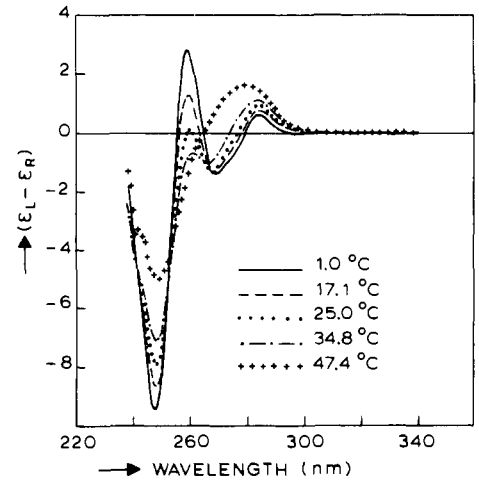

FIGURE 5: CD spectra of poly(dA)·poly(dT) in buffer I at a nucleotide to protein ratio of 11. Due to incomplete complexation the temperature dependence resembles that of free poly(dA)·poly(dT) (Greve et al., 1977).

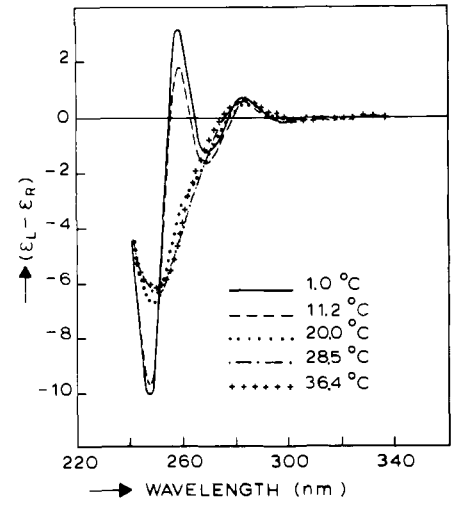

FIGURE 6: CD spectra of poly(dA)·poly(dT) in buffer I at a nucleotide to protein ratio of 3.5. The 1 and 11.2 °C spectra are almost equal to the CD spectra of uncomplexed poly(dA)·poly(dT) measured at the same temperatures. At 20 °C the polynucleotide is partially strand separated. At 28.5 and 36.4 °C the polymer is completely strand separated. The CD minimum has then shifted to 251 nm. Little change is observed in the positive CD band upon complexation.

and the number of protein molecules in the solution (nucleotide to protein ratio) apparently has to be 6.7 or lower to achieve near or complete denaturation. According to Alberts & Frey (1970), the nucleotide to protein ratio in the fully saturated fd DNA–gp32 complex was approximately 10. The Jensen et al. (1976) kinetic experiments have shown that a nucleotide to protein ratio of 7.5 was needed for complete denaturation of poly[d(A-T)]·poly[d(A-T)]. To achieve saturation, allowing for a possible variety in specific activity of the gp32 preparation, we measured the reported CD spectra at least at two different nucleotide protein ratios.

OD measurements showed that by complexation at a nucleotide to protein ratio of 8.5 in buffer I, the OD of poly(dA) increased by 16 ± 3%, whereas that of poly(dT) decreased by 6 ± 3% (at the wavelength of maximum absorption).

Circular Dichroism Spectra. Poly(dA) and Poly(dT). CD spectra of complexed poly(dA) and complexed poly(dT) in buffer I are shown in Figures 3 and 4, respectively. Spectra of the free polynucleotides are also shown. Essentially the same CD spectra were obtained at the nucleotide to protein ratios of 8.5 (as presented in the figures) and 6 (data not presented). Apparently the complexation between these single-stranded polynucleotides and gp32 is already complete at 1 °C. Upon complexation a reduction of the positive CD band is found for both polymers. The negative CD band is little influenced for poly(dT), whereas the magnitude becomes greater for poly(dA). The CD spectrum (and therefore the conformation) of complexed poly(dA) is more temperature dependent than that of complexed poly(dT). This agrees with the results of Bobst & Pan (1975) who showed that poly(dT) is bound three orders of magnitude stronger than poly(dA).

Poly(dA)·Poly(dT). CD spectra of poly(dA)·poly(dT) measured at the nucleotide to protein ratios of 11 and 3.5 in buffer I are shown in Figures 5 and 6, respectively. Apparently the ratio 11 is not low enough to obtain saturation. To obtain the low nucleotide to protein ratio of 3.5, we kept the gp32 concentration constant and lowered the poly(dA)·poly(dT) concentration to avoid excessive self association of gp32 at

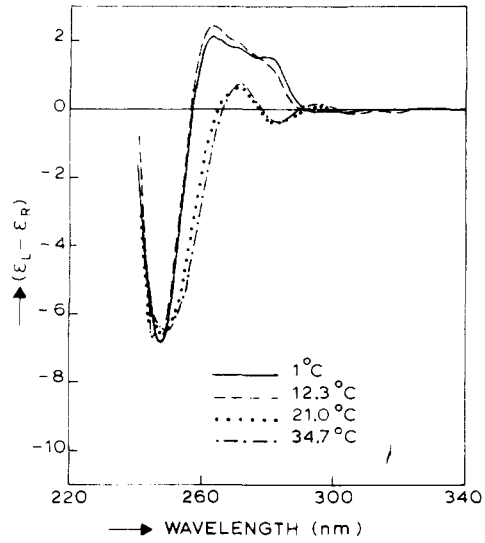

FIGURE 7: CD spectra of poly[d(A-T)]·poly[d(A-T)] in buffer II at a nucleotide to protein ratio of 6.7. The spectra measured at 1.0 and 12.3 °C are equal to those of uncomplexed poly[d(A-T)]·poly[d(A-T)]. At 21.0 °C the polymer is partially strand separated. At 34.7 °C the CD minimum has shifted to 250 nm and the polynucleotide is denatured. Upon complexation with gp32 the positive CD band is greatly reduced.

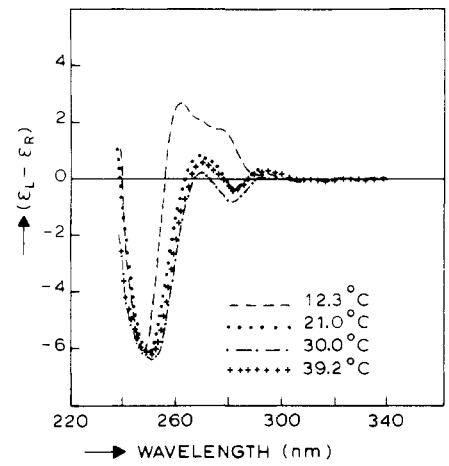

FIGURE 8: CD spectra of poly[d(A-T)]·poly[d(A-T)] in buffer II at a nucleotide to protein ratio of 3.5. The long wavelength CD minimum which is introduced by the complexation is somewhat more pronounced than was found at the nucleotide to protein ratio of 6.7. The further characteristics of the complexation are the same as explained in Figure 7.

higher concentration (Carrol et al., 1975). Consequently some scattering was present in the solution and the CD spectra are somewhat noisier. Both at the nucleotide to protein ratio 11 and 3.5 the CD spectra measured at 12 °C and lower are within experimental error equal to those measured for poly(dA)·poly(dT) in the absence of gp32 (Greve et al., 1977). Above this temperature large changes in CD and OD took place at both ratios. Therefore it is likely that above 12 °C strand separation occurred. However, the CD spectrum never became equal to that of thermally denatured poly(dA)·poly(dT) (see Greve et al., 1977). When poly(dA)·poly(dT), complexed at 20 °C or above, was cooled to 1 °C, the CD spectrum found was not the same as measured directly after mixing with gp32 at low temperature. Apparently no renaturation occurred, even when the sample was cooled for 3 days and 0.1 M $MgSO_4$ was added.

Poly[d(A-T)]·poly[d(A-T)]. Figures 7 and 8 show CD spectra of poly[d(A-T)]·poly[d(A-T)] in the presence of gp32

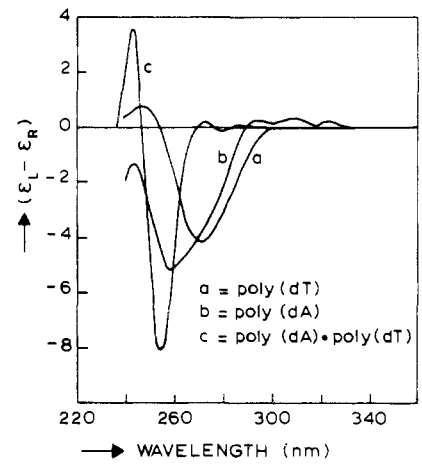

FIGURE 9: Difference CD spectra—complex CD spectrum minus the gp32 spectrum and minus the uncomplexed polymer spectrum. (a) Complexed poly(dT)-gp32-poly(dT), CD spectra at 1 °C, buffer I. (b) Complexed poly(dA)-gp32-poly(dA) spectra at 1 °C, buffer I. (c) Complexed poly(dA)·poly(dT)-gp32-poly(dA)·poly(dT), see text for conditions.

at nucleotide to protein ratios of 6.7 and 3.5 in buffer II. The spectral changes observed at the lower ratio go slightly further than at the higher ratio, indicating that at the ratio 6.7 the polynucleotide was almost saturated with gp32. Again measurements at the lower ratio are somewhat noisy because of the low polynucleotide concentration. From a comparison of these spectra with temperature-dependent CD spectra of uncomplexed poly[d(A-T)]·poly[d(A-T)] (Greve et al., 1977), it is clear that at temperatures below 12.3 °C no spectral changes are detected. At higher temperatures the positive CD band is greatly reduced, whereas the magnitude of the negative CD band does not change much. This CD change and the accompanying increase in OD are likely to represent strand separation. In contrast to what was found for poly(dA)·poly(dT) it appeared that complete renaturation occurred when complexed poly[d(A-T)]·poly[d(A-T)] was cooled to 1 °C or when 0.1 M $MgSO_4$ was added at 25 °C.

When poly[d(A-T)]·poly[d(A-T)]-gp32 mixtures were studied in the absence of Mg^{2+} (in buffer I at 1 °C) CD and OD spectra were observed which were similar to those found in buffer II above 12.3 °C. Clearly complexation is strongly dependent on ionic strength.

Difference CD Spectrum. Figure 9 presents difference CD spectra showing the total change induced by protein binding. The difference spectrum was calculated by subtracting the CD spectrum of free protein and the CD spectrum of free polynucleotide from the CD spectrum of the complexed polynucleotide. The spectra shown are: (a) complexed poly(dT)-gp32-poly(dT) was measured at 1 °C in buffer I; (b) complexed poly(dA)-gp32-poly(dA) was measured at 1 °C in buffer I; (c) complexed poly(dA)·poly(dT)-gp32-poly(dA)-poly(dT) was measured at 28.5 °C in buffer I, so it is strand separated, the other spectra at 1 °C buffer I.

These CD difference spectra clearly show that the total CD changes which are induced are polynucleotide specific. If a major contribution to the changes in CD spectrum upon complexation would have come from an alteration in protein structure more resemblance between the induced changes would have been obtained. Moreover the creation of the extrema which are obtained in these difference spectra, and which should contain information about the location of the optical transitions which are affected by the complexation,

agree well with those obtained before for adenine and thymine containing polynucleotides (Greve et al., 1977).

Discussion

From the reported CD spectra it is clear that upon complexation with gp32 structural changes may occur in: (a) the gp32 protein; (b) the secondary structure of both double-stranded and single-stranded polynucleotides. In the first case it can be argued that, although the gp32 protein has a small CD signal in the 260 to 300 nm region this signal can increase in magnitude upon complexation with the DNA polymer. However, the measurement shows that there is no change in the rest of the protein spectrum in particular in the 200 to 230 nm region. Furthermore, the difference spectra (uncomplexed minus complexed, Figure 9) have maxima and minima at wavelengths which are apparently specific for the polynucleotide used and not the protein (see Greve et al., 1977). To attribute all the CD changes to the protein alone is an extreme view since it has been shown that the double-stranded polymers are denatured by complexation with gp32. Such denaturation or melting will produce a change in the CD spectrum as shown by Greve et al. (1977). For this reason we attribute most of the measured CD changes to the secondary structure of the polymers and not to the protein. To determine what kind of structural transitions occur, we have to know how to interpret the observed spectral changes. Unfortunately it is not yet possible to calculate the secondary structure of a polynucleotide exactly from its CD spectrum. Therefore any discussion of polynucleotide conformation as determined from CD spectroscopy will be of a qualitative nature. Important questions to be answered in such a discussion for the polynucleotide gp32 interaction are: (a) Does strand separation occur in solution? (b) Are the polynucleotide bases stacked inside the protein-polynucleotide complex? (c) If the bases are stacked, what is the polynucleotide conformation? In the following section we will deal with these questions by developing qualitative rules for the interpretation of the CD spectra. It is important to develop such rules since they may also be useful for the determination of polynucleotide structure in other (protein) complexes.

Strand Separation. No strand separation occurs for poly[d(A-T)]·poly[d(A-T)] (in buffer II) and poly(dA)·poly(dT) (in buffer I) below about 12 °C as the CD and OD spectra remain unaltered. At first sight the CD spectra observed at temperatures above 12.3 °C do not clearly indicate whether strand separation occurs or not. The CD spectra of the complexed polynucleotides are not equal to those of the corresponding heat-denatured polynucleotides. This is particularly true for poly[d(A-T)]·poly[d(A-T)] where the intensity of the minimum at 247 nm is hardly diminished upon complexation and where much structure is present in the spectrum above 270 nm. From a comparison of CD spectra of the double-stranded and heat-denatured polynucleotides (Greve et al., 1977), it appears that for both poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] the position of the minimum around 250-nm shifts to the red upon denaturation. For poly(dA)·poly(dT) this shift is from 247 nm to 250 nm, for poly[d(A-T)]·poly[d(A-T)] from 247 nm to 251 nm. We now suggest that the position of this minimum can be used to determine whether the polynucleotides are double- or single-stranded inside a protein complex. Upon complexation with gp32 we observed a shift in the minimum from 247 to 251 nm for poly[d(A-T)]·poly[d(A-T)] (Figures 7 and 8) and from 247 to 251 nm for poly(dA)·poly(dT) (Figure 6). We therefore conclude that strand separation occurs and that the polynucleotides are strand separated

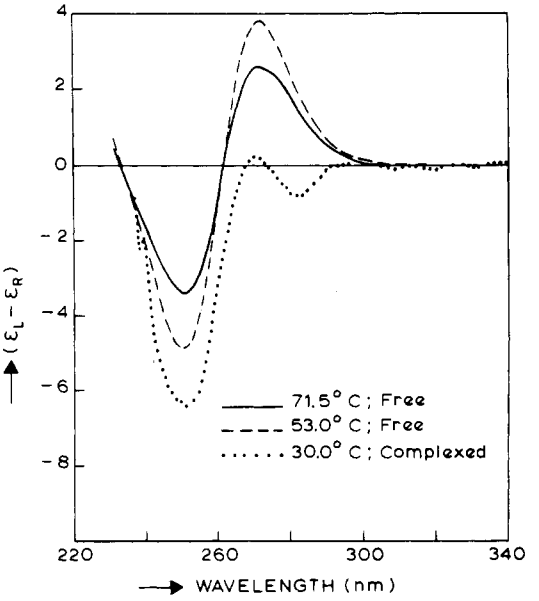

FIGURE 10: CD spectra of free and gp32-complexed single-stranded poly[d(A-T)]. The spectra of the free polynucleotide were obtained by denaturing poly[d(A-T)]·poly[d(A-T)] in buffer I at 53 °C and in buffer II at 71.5 °C. The protein complexed spectrum was measured in buffer I at 30 °C (nucleotide to protein ratio 3.5). The CD spectra of the free polynucleotides are similar in shape to that of double stranded poly[d(A-T)]·poly[d(A-T)] in the B conformation. The CD spectrum of the protein complexed polynucleotide is similar in shape to that of double-stranded poly[d(A-T)]·poly[d(A-T)] at high LiCl concentration.

when fully complexed. This conclusion—and thereby the use of the position of the minimum as indicator for double or single-strandedness—is confirmed by the following observations. Firstly, when CD spectra of complexed (single-stranded) poly(dA) and complexed (single-stranded) poly(dT) are averaged, the spectrum obtained is equal to that measured for complexed poly(dA)·poly(dT) under corresponding conditions. Secondly when poly[d(A-T)]·poly[d(A-T)] was mixed with gp32 at 35 °C in 1 mM KCl, 2 mM Tris (pH 7.8), 0.1 mM EDTA (under these conditions the polynucleotide is heat denatured), the CD spectrum obtained for the complex was essentially the same as found for the complex at higher ionic strength. Apart from this evidence obtained from CD spectra, a firm confirmation of strand separation comes from the measured increase in OD, which is almost the same as found in a regular melting experiment.

Conformation of the Complexed Polynucleotides. It is well known that the CD of a polynucleotide is mainly due to interactions between optical transitions on the bases (DeVoe, 1964, 1965). In general these interactions will be changed upon a conformational change of the polymer. Therefore the CD spectrum of a single-stranded polynucleotide should be different for each different conformation the polymer assumes. That different CD spectra are experimentally found for single-stranded polynucleotides is illustrated for poly[d(A-T)] in Figure 10, where CD spectra of heat-denatured and gp32-complexed poly[d(A-T)]·poly[d(A-T)] are compared. Apparently (at least) two types of conformational changes can occur for single-stranded polynucleotides, namely: (a) changes which leave the overall shape of the CD spectrum unchanged and yield a uniform reduction of the whole CD spectrum. This type of conformational change is found upon increase in temperature (or change in ionic conditions) for heat-denatured poly[d(A-T)]·poly[d(A-T)] (see Figure 10), poly(dT), and heat-denatured calf thymus DNA (Greve et al., 1977, and unpublished results). On theoretical grounds this uniform re-

duction in intensity can only be explained if the strength of the coupling between the optical transitions decreases, whereas the relative orientation of the transition moments stays the same. Such a reduction will for instance occur if the helix is slightly stretched out while the relative orientations of the base planes are kept equal. Alternatively one could imagine that the bases oscillate relative to each other in planes which lie more or less perpendicular to the helix axis. A uniform reduction in CD will then occur when the amplitudes of the oscillations are increased upon heating. (b) Conformational changes which affect the shape of the CD spectrum are the second type. This type of change in CD spectrum must result from a conformational change of the polynucleotide by which the orientation of the base planes relative to each other is altered. Such a change is observed when CD spectra of gp32-complexed and free single-stranded polynucleotides are compared as was done for poly[d(A-T)] in Figure 10. Therefore the conformation of the gp32-complexed polynucleotide must be of a nature which is different from that of the free polynucleotide in solution.

From Figure 10 it is striking that a great similarity exists between the CD spectra of single-stranded poly[d(A-T)] in the two different conformations (heat denatured and gp32 complexed) on one hand and DNA in the B- and C-, respectively, DNA conformations on the other hand (Ivanov et al., 1973). This similarity will be discussed further in a forthcoming paper (Greve, Maestre, & Levin, manuscript in preparation). There it will be shown that from theoretical calculations it follows that the CD spectrum of single-stranded poly[d(A-T)] in the A-, B-, or C-DNA conformation has a shape which is similar to that of double-stranded poly[d(A-T)]·poly[d(A-T)] in the A-, B-, and C-, respectively, DNA conformation. By this we mean with "B-DNA conformation" of single-stranded poly[d(A-T)] that the polynucleotide has a conformation which is equal to that of one strand of the double-stranded poly[d(A-T)]·poly[d(A-T)] in the B-DNA conformation. The differences between A-DNA, B-DNA, and C-DNA CD spectra are most clearly expressed in the long wavelength part of the CD spectrum. Therefore the shape of this part of the experimentally obtained spectrum shows what conformation the single stranded poly[d(A-T)] has. From a comparison of the CD spectra of poly[d(A-T)] shown in Figure 10 with CD spectra of poly[d(A-T)]·poly[d(A-T)] in different conformations (Studdert et al., 1972; Brahms et al., 1976), we therefore conclude that free single-stranded poly[d(A-T)] has a B-DNA conformation whereas gp32-complexed poly[d(A-T)] has the same conformation as one strand of poly[d(A-T)]·poly[d(A-T)] at high LiCl concentrations. For DNA it is generally assumed that high LiCl concentrations induce a C-DNA conformation. However, Brahms et al. (1976) showed that the C conformation of poly[d(A-T)]·poly[d(A-T)] has a CD spectrum which is different from that of the polymer at high LiCl concentrations. For a further classification of the conformation of gp32-complexed poly[d(A-T)], therefore, more information is needed about the conformation of the double-stranded polymer in high LiCl concentrations.

It is evident that a comparison between single-stranded and double-stranded polynucleotide CD spectra cannot be made for poly(dA) and poly(dT). From the reported CD spectra, however, it is clear that the main change in CD spectrum induced by complexation with gp32 is similar to that observed upon complexation of poly[d(A-T)], namely, a reduction of the long wavelength CD band. We therefore assume that, when complexed, these polymers have a conformation which is the same as that of gp32-complexed poly[d(A-T)].

Base Stacking. As argued above, the fact that CD spectra of free poly[d(A-T)] measured under different conditions have the same shape but different magnitudes of the peaks, must be due to a reduction in coupling between the optical transitions. This means that the relative degree of base–base interaction (or base stacking) can be deduced from the relative magnitudes of either the positive or the negative CD peak of the polynucleotide measured under different conditions. Apparently such a comparison cannot be made directly between free and gp32-complexed poly[d(A-T)] as the shapes of the CD spectra are different. However, the CD spectrum of gp32-complexed poly[d(A-T)] has a minimum at the same wavelength (251 nm) as free poly[d(A-T)] (Figure 9). This suggests that this minimum arises due to the same coupling between optical transitions as present in free and gp32-complexed poly[d(A-T)]. It is then clear that the base stacking is least for the polynucleotide in the most flexible conformation (i.e., free in buffer with Mg at high temperature) and greatest in the gp32-complexed form. This must be a result of the fact that the protein keeps the single stranded polynucleotide in a conformation in which the positions of the base planes are rigidly fixed relative to each other.

Applying the same criterion to poly(dA) and poly(dT), it follows that upon complexation with gp32 the base stacking respectively increases and stays almost the same as in the free polynucleotide in buffer.

Backbone Stretching. Delius et al. (1972) report that upon complexation with gp32 the fd DNA is stretched out by about 35% relative to the B conformation. The interaction between the optical transitions is a dipole–dipole interaction which is strongly dependent on the distance between the dipoles. This means that the mentioned stretching would have to decrease the CD enormously, which is clearly not observed for our polynucleotides. Therefore uniform stretching can be ruled out. A non-uniform stretching might still agree with the optical measurements. One could imagine that upon complexation the sugar phosphate backbone between two bases is stretched whereas little or no stretching occurs on both sides of this deformed place. Such a conformation could have a large CD as Cantor et al. (1970) showed that di- and trinucleotides do have a large CD. However, the stretching of the sugar phosphate backbone at locally deformed places has to be unlikely large to explain a 35% increase in length of the whole helix. Therefore we prefer the interpretation given above. Even if non-uniform stretching occurs the discussion above is valid albeit that it should then be applied to the conformation of the non-stretched regions. It should be mentioned that the increase in OD of poly(dA) upon complexation does point to a decrease in interaction between transition moments. We did not find such an increase for poly(dT). Therefore we believe that this increase in OD reflects the fact that free poly(dA) in solution has a complex structure with probably a peculiar base stacking. (For poly(dA) conformation, see Brahms & Brahms, 1970.)

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Circular Dichroism Studies of the Interaction of a Limited Hydrolysate of T4 Gene 32 Protein with T4 DNA and Poly[d(A-T)]·Poly[d(A-T)]†

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ABSTRACT: gp32*I is a protein with a molecular weight of 27 000. It is obtained by limited hydrolysis of T4 gene 32 coded protein, which is one of the DNA melting proteins. gp32*I itself appears to be also a melting protein. It denatures poly[d(A-T)]·poly[d(A-T)] and T4 DNA at temperatures far (50-60 °C) below their regular melting temperatures. Under similar conditions gp32*I will denature poly[d(A-T)·poly[d(A-T)] at temperatures ~12 °C lower than those measured for the intact gp32 denaturation. For T4 DNA gp32 shows no melting behavior while gp32*I shows considerable denaturation (i.e., hyperchromicity) even at 1 °C. In this paper the denaturation of poly[d(A-T)]·poly[d(A-T)] and T4 DNA by gp32*I is studied by means of circular dichroism. It appears that gp32*I forms a complex with poly[d(A-T)]. The conformation of the polynucleotide in the complex is equal to that of one strand of the double-stranded polymer in 6 M LiCl. In the gp32*I DNA complex formed upon denaturation of T4 DNA, the single-stranded DNA molecule has the same conformation as one strand of the double-stranded T4 DNA molecule in the C-DNA conformation.

The lowering of the melting temperature of double helical polynucleotides by T4 gene 32 protein (gp32)[1] and other DNA melting proteins is explained thermodynamically: the protein has a high affinity for the single-stranded form of the polynucleotide and shifts the equilibrium between single- and double-stranded form toward (protein bound) single-stranded form. Jensen et al. (1976) demonstrated that the melting of poly[d(A-T)]·poly[d(A-T)] by gp32 closely followed the thermodynamically predicted pattern. In spite of its denaturing activity toward poly[d(A-T)]·poly[d(A-T)] gp32 did not denature T4 DNA (Alberts & Frey, 1970). Jensen et al. (1976) concluded that gp32 could not denature T4 DNA and several other naturally occurring DNAs because the destabilization of the double helical form by gp32 is kinetically blocked.

By limited proteolysis of gp32 a subunit named gp32*I can be obtained. It has a molecular weight of 27 000 and is formed by removal of the so called A peptide (molecular weight about 8000) from the COOH-terminal end of gp32 (Moise & Hos-

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[1] Abbreviations used: CD, circular dichroism; gp32, T4 gene 32 coded protein; gp32*I, limited hydrolysate of T4 gene 32 protein.

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