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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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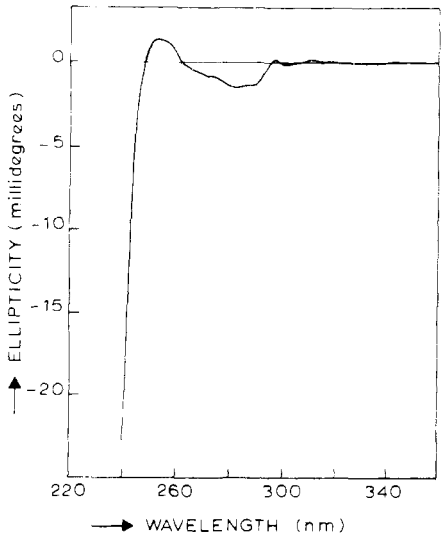

FIGURE 1: CD spectrum of gp32*I in buffer II. The protein concentration is 0.459 mg per mL and the light path is 1 cm. CD is given as the measured ellipticity in millidegrees. The spectrum is shown only at wavelengths greater than 240 nm. Below 240 nm the CD is so strong that no reliable subtraction of protein CD from CD spectra of protein DNA mixtures can be made.

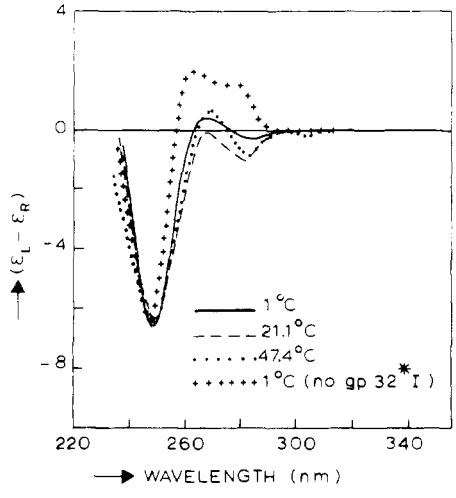

FIGURE 2: CD spectra of poly[d(A-T)]·poly[d(A-T)] complexed with gp32*I at a nucleotide to protein ratio of 5.4 in buffer II. In these and all following figures, the CD contribution due to gp32*I has been subtracted. For comparison a spectrum of the polynucleotide measured in the absence of protein at 1 °C is shown.

oda, 1976, and manuscript in preparation). It appears that gp32*I is a far stronger melting protein than gp32. Under conditions where the original gp32 could not denature T4 DNA, gp32*I denatured this DNA almost completely (Hosoda et al., 1974).

The removal of the A peptide unit from gp32, and the apparent elimination of the kinetic block in the T4 DNA melting mechanism thereby, seems not only of great interest for the protein induced melting of DNA in vitro, but it may also have profound meaning for the role of gp32 in vivo. Moise & Hosoda (1976) proposed that the A peptide is a regulatory peptide which controls the dual functioning of gp32: melting protein and renaturing protein. In the regular conformation the A peptide acts as an inhibitor of melting activity. The gp32 then behaves as a promoter of renaturation and not as a melting protein. This is probably very important to prevent the uncontrolled melting of the T4 chromosome in vivo. Only at a certain position within the DNA replication apparatus is the A peptide displaced from its regular position by interaction with other protein components (activation of gp32). In this activated form gp32 behaves as a melting protein and contributes to the replicating fork movement. This model implies that the interaction between the activated form of gp32 and T4 DNA in vivo is more comparable to the interaction between gp32*I and DNA in vitro than to the interaction between gp32 and DNA in vitro. Therefore we decided to study the interaction of gp32*I with T4 DNA and poly[d(A-T)]·poly[d(A-T)] by means of circular dichroism (CD) spectroscopy. In a preceding paper (Greve et al., 1978), we already reported the results of a CD study of the interaction between gp32 and some polynucleotides. In this paper it will be shown that the conformation of poly[d(A-T)] in the complex with gp32 is slightly different from the conformation of poly[d(A-T)] in the complex with gp32*I. The conformation of the gp32*I complexed single-stranded poly[d(A-T)] closely resembles that of one strand of the corresponding double-stranded molecule at high LiCl concentrations. GP32*I complexed T4 DNA resembles one strand of the double-stranded T4 DNA molecule in the C-DNA conformation.

Experimental Procedure

The gp32*I protein was prepared as described by Moise and Hosoda (manuscript in preparation). To determine the protein concentration we assumed that gp32*I has a molecular weight of 27 000 and that one optical density unit (280 nm, 1 cm) corresponds to a weight concentration of 0.91 mg/mL. The latter relation is the same as used for gp32 and was not checked independently. Consequently the ratio between the number of nucleotide monomers and the number of protein molecules in the solution (nucleotide to protein ratio) may be slightly in error. This is not essential for the interpretation of the data. T4 DNA was obtained by phenol extraction of T4 phage. The extinction coefficient used is 6440 L mol^{-1}. Poly[d(A-T)]·poly[d(A-T)] was purchased from P-L Biochemicals, catalogue number 7,870; batch number 508-101; extinction coefficient 6600 L mol^{-1}. The relative concentrations of DNA and protein will be given as the ratio between the number of nucleotides and the number of protein molecules present in the mixture (nucleotide to protein ratio). Measurements with T4 DNA were made in 10 mM KCl, 0.1 mM EDTA, 2 mM Tris, pH 7.8 (buffer I). For the measurements with poly[d(A-T)]·poly[d(A-T)] 10 mM $MgSO_4$ was added to this medium (buffer II). The measurement procedure and the determination of CD spectra of complexed DNA and polynucleotide spectra have been described before (Greve et al., 1978). In all figures showing CD spectra of poly[d(A-T)]·poly[d(A-T)] or T4 DNA in the presence of gp32*I, the CD contribution due to the protein has been subtracted. CD spectra will be given 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.

Results and Discussion

*GP32*I.* In Figure 1 the CD spectrum of gp32*I is shown for the wavelength region from 240 to 340 nm. The CD spectrum is almost equal to the one reported for gp32 (Greve et al., 1978). Because of the high protein CD at wavelengths smaller than 240 nm, the DNA CD spectra in the following section will be given at wavelengths greater than 240 nm only.

*CD Spectra of gp32*I Complexed Poly[d(A-T)]·Poly[d(A-T)].* CD spectra of poly[d(A-T)]·poly[d(A-T)] in the presence of gp32*I were measured at nucleotide to protein ratios of 5.4 (Figure 2) and 2.7 (Figure 3). At both ratios all

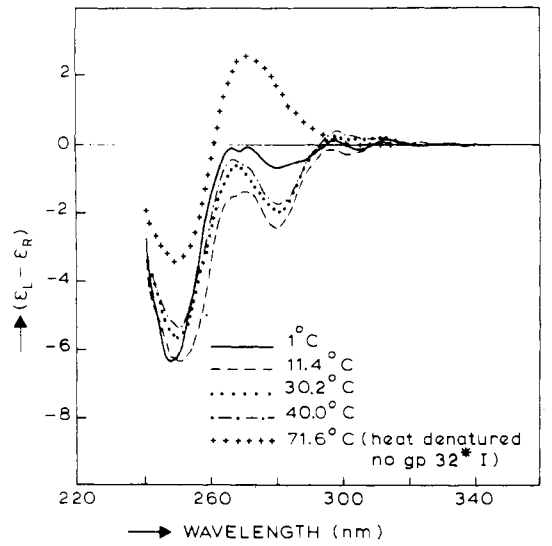

FIGURE 3: CD spectra of poly[d(A-T)]·poly[d(A-T)] complexed at a nucleotide to protein ratio of 2.7 in buffer II. For comparison a spectrum of the heat denatured polymer measured at 71.5 °C is shown.

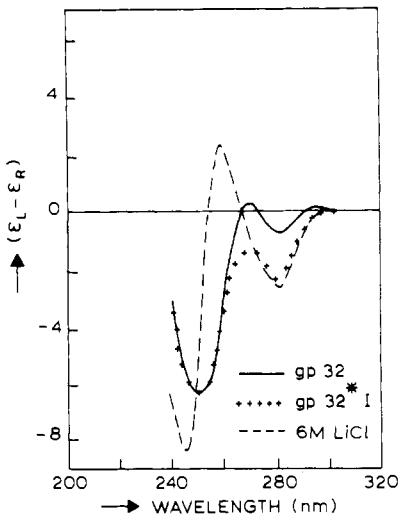

FIGURE 4: Comparison of CD spectra of poly[d(A-T)]·poly[d(A-T)] complexed with gp32 and gp32*I, respectively, in buffer I. (—) Complex with gp32 at nucleotide to protein ratio of 3.5 at 30 °C. (+++) Complex with gp32*I at nucleotide to protein ratio of 2.7 at 11.4 °C. (c) Poly[d(A-T)]·poly[d(A-T)] in 6 M LiCl (---) buffer I, 25 °C.

spectra measured are different from the spectrum of poly[d(A-T)]·poly[d(A-T)] in the absence of protein. This indicates that, contrary to what was found for the complexation with gp32, at 1 °C already complexes with gp32*I are formed in which the polynucleotide structure is not the same as in the double-stranded form. Apparently the nucleotide to protein ratio of 5.4 is not sufficient to saturate the poly[d(A-T)]·poly[d(A-T)] completely with protein since at a ratio of 2.7 further changes are found in the CD spectrum. However, even at the latter, very low, ratio the CD spectrum of the polynucleotide never becomes equal to that of heat denatured poly[d(A-T)]·poly[d(A-T)]. Instead the amplitude of the minimum near 250 nm hardly diminishes upon complexation. To check whether the complexation between gp32*I and poly[d(A-T)]·poly[d(A-T)] is reversible, we added 0.1 M $MgSO_4$ to a sample which had been heated to 47.4 °C at a nucleotide to protein ratio of 5.4. The CD spectrum which was then measured at 1 °C was equal to that of uncomplexed poly[d(A-T)]·poly[d(A-T)]. Apparently the protein-polynucleotide complex had completely dissociated and double-stranded polynucleotide had been formed.

*Conformation of gp32*I Complexed Poly[d(A-T)].* From the CD spectra shown in Figures 2 and 3 it follows that at all temperatures used complexes are formed between poly[d(A-T)]·poly[d(A-T)] and gp32*I. In all these complexes the conformation of the polynucleotide is different from the conformation of the double-stranded or heat denatured polymer in solution. To determine the nature of the conformational changes which have occurred, we will use the criteria which were derived to interpret CD spectra of gp32 complexed poly[d(A-T)] (Greve et al., 1978), namely:

(I) Whether the polynucleotide is double- or single-stranded can be determined from the location of the minimum near 250 nm. For double-stranded molecules this minimum lies at 247 nm, whereas for single-stranded molecules the minimum is found at 251 nm.

(II) The degree of interaction between the optical transitions in the base planes can be determined from the depth of the CD minimum at 251 nm.

(III) The conformation of the single-stranded polynucleotide can be deduced from a comparison of the long wavelength CD spectra (above 270 nm) of double-stranded and single stranded polymer.

Applying criterion I it follows from Figure 2 that at a nucleotide to protein ratio of 5.4 the polynucleotide is partly strand separated at 1 °C, strand separated at 21.1 °C and again partly strand separated at 47.4 °C. The CD spectrum measured at 21.1 °C indicates that the conformation of the complexed single-stranded poly[d(A-T)] is only slightly different from the conformation of poly[d(A-T)] complexed with gp32 at a nucleotide to protein ratio of 3.5 at 30 °C. Since the latter conformation has been discussed at length (Greve et al., 1978), we will concentrate here on the conformation of the complex formed with gp32*I at the nucleotide to protein ratio 2.7. At this ratio the polynucleotide is also only partly strand separated at 1 °C as the CD minimum is found at 248 nm. At 11.4 °C, however, this minimum has shifted to 251 nm and strand separation is complete. In Figure 4 the 11.4 °C spectrum is compared with the spectrum of the gp32-poly[d(A-T)] complex measured at a nucleotide to protein ratio of 3.5 at 30 °C. Under these conditions the polynucleotide is completely strand separated by gp32 (Greve et al., 1978). Clearly both location and depth of the 251-nm minimum are equal for the gp32 and gp32*I complexes. By applying criterion II it follows that the interaction between the optical transitions in the base planes of single-stranded poly[d(A-T)] is comparable in the gp32 and the gp32*I complexes. Therefore, just as found for the gp32 complex, the nucleotide bases in the gp32*I complex are stacked and kept rigidly in position relative to each other. Moreover the single-stranded polynucleotide in the gp32*I complex is not stretched out as compared with one of the strands of a double-stranded molecule. At 280 nm a pronounced minimum ($\Delta\epsilon = -2.5$) is found in the CD spectrum of gp32*I complexed poly[d(A-T)]. In the presence of 6 M LiCl in buffer I (room temperature), poly[d(A-T)]·poly[d(A-T)] (Figure 4) develops a similar long wavelength CD minimum, namely, $\Delta\epsilon = -2.6$ at 282.5 nm (Studdert et al., 1972; Greve et al., manuscript in preparation). By applying criterion III we interpret this similarity of the long wavelength CD spectra as meaning that the conformation of the single-stranded poly[d(A-T)] complexed with gp32*I is almost the same as that of one of the strands of poly[d(A-T)]·poly[d(A-T)] at 6 M LiCl. The long wavelength CD spectrum of gp32 complexed poly[d(A-T)] resembles more that of poly[d(A-T)]·poly[d(A-T)] at 3.5 M LiCl. When the temperature of the

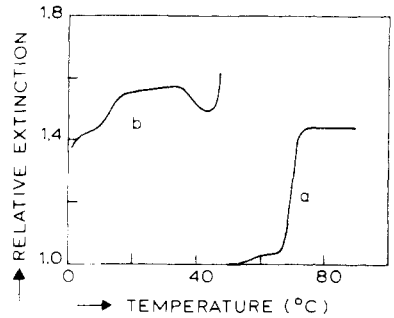

FIGURE 5: Relative OD as a function of temperature for (a) T4 DNA; (b) T4 DNA plus gp32*I at nucleotide to protein ratio of 2.2. In this case aggregation occurred at about 47 °C. The OD of the native protein has been subtracted. Measurements were made in buffer I. No correction for light scattering was applied.

gp32*I poly[d(A-T)] complex is increased above 11.4 °C at the nucleotide to protein ratio of 2.7, a change in structure of the complexed poly[d(A-T)] occurs. This follows from the change in CD spectrum which is detected upon raising the temperature to 30 or 40 °C. The observed change can be described as a lowering of the magnitude of the CD signal at all wavelengths. It must therefore be due to a gradual change in structure of the complex which causes a uniform decrease of all the optical interactions. As the positions and relative magnitudes of the CD peaks remain equal, this conformational change must leave the relative orientations of the base planes almost the same. For these reasons we propose that at the higher temperatures a slight uniform stretching of the bound polynucleotide occurs. It should be stressed that the overall increase in length must be small since CD is strongly dependent on nucleotide spacing. A similar change in CD spectrum was not observed on raising the temperature of the complex formed at nucleotide to protein ratio 5.4. This may indicate that the change in structure is caused by binding of extra protein.

*Denaturation of T4 DNA by gp32*I.* In Figure 5 melting curves of T4 DNA in the presence and absence of gp32*I are compared. The nucleotide to protein ratio was 2.2. Apparently gp32*I induces denaturation of T4 DNA at temperatures far below the regular melting temperature. In the presence of gp32*I 54% hyperchromicity was reached at about 17 °C. No correction was applied for the scattering by the (very high molecular weight) gp32*I T4 DNA complex. This may be the reason that the hyperchromicity reached is somewhat higher than in the case of heat denaturation. Above 37 °C the gp32*I partly comes off the DNA. The protein itself denatures above 45 °C.

*CD Spectra of gp32*I Complexed T4 DNA.* CD spectra of T4 DNA in the presence of gp32*I at nucleotide to protein ratios of 6.0 and 2.2 are shown in Figures 6 and 7, respectively. In these measurements differential CD scattering was observed, probably caused by the high molecular weight of the complex. The general behavior of the complexation is the same as found for poly[d(A-T)]·poly[d(A-T)], namely: (a) At both nucleotide to protein ratios the 1 °C CD spectrum is already different from that of the native T4 DNA. (b) The spectral changes observed at the lower nucleotide to protein ratio of 2.2 go further than those measured at the nucleotide to protein ratio of 6.0. (c) At both nucleotide to protein ratios the CD spectra of complexed T4 DNA are different from that of heat denatured T4 DNA.

Contrary to what was found for poly[d(A-T)]·poly[d(A-T)], we do not believe that T4 DNA is completely strand separated at the nucleotide to protein ratio of 6.0. In the first place the hyperchromicity in this case reached only 22%. Secondly, the

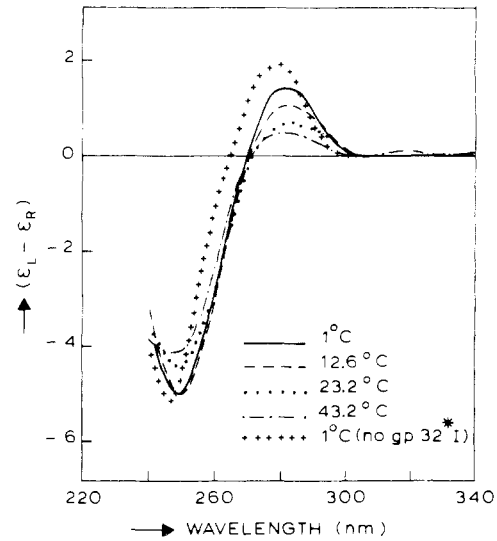

FIGURE 6: CD spectra of complexed T4 DNA at nucleotide to protein ratio of 6.0 in buffer I. For comparison a spectrum of T4 DNA measured in the absence of gp32*I at 1 °C is shown.

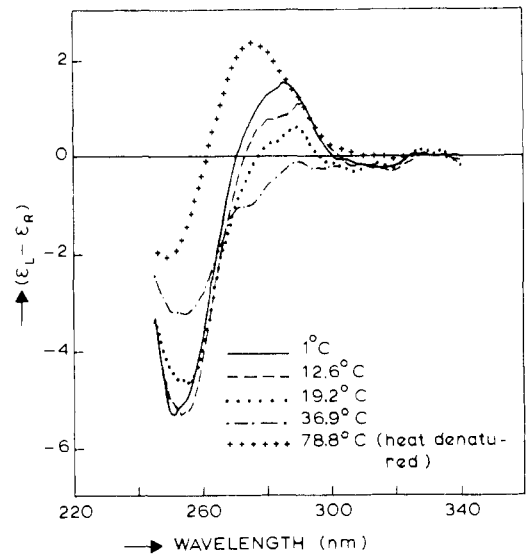

FIGURE 7: CD spectra of complexed T4 DNA at nucleotide to protein ratio of 2.2 in buffer I. For comparison a spectrum of heat denatured T4 DNA measured in the absence of gp32*I at 78.8 °C is shown.

differences in CD spectra between the complexes found at nucleotide to protein ratios of 6.0 and 2.2 are of a different kind than in the case of poly[d(A-T)]·poly[d(A-T)]. For the latter polymer only a difference in the long wavelength spectrum was found, whereas the CD minimum was at 251 nm at both nucleotide to protein ratios used. For T4 DNA not only the long wavelength part of the spectra are different, but the CD minima are also located at different wavelengths. Therefore we suggest that at the higher nucleotide to protein ratio the T4 DNA is only partially strand separated. It seems most logical to assume that the DNA is preferentially denatured in the A-T rich regions. This partial denaturation must be a consequence of the low protein concentration since, at the nucleotide to protein ratio 2.2, complete strand separation did occur (see below). In the rest of this discussion we will therefore concentrate on the complexation observed at the nucleotide to protein ratio 2.2.

*Conformation of gp32*I Complexed T4 DNA.* For the interpretation of the CD spectra of complexed poly[d(A-T)]·

poly[d(A-T)], we used the criteria I, II, and III which were introduced on the basis of experimental CD spectra of this synthetic polynucleotide. In contrast to this polynucleotide T4 DNA contains four different bases in quasi-random order. This may lead to a cancellation of certain contributions to the CD spectrum which do not cancel in the polynucleotide spectrum. It is therefore clear that the same criteria may not be used for the interpretation of the complexed T4 DNA CD spectra. However, it will be shown that by applying similar or almost the same criteria a picture of the complexation of T4 DNA is obtained which is consistent with the results obtained for the complexation of poly[d(A-T)]·poly[d(A-T)].

From the experimental CD and OD spectra it is clear that already at 1 °C a complex is formed between gp32*I and T4 DNA. In this complex the DNA has neither the double-stranded conformation nor the conformation of heat-denatured DNA since the CD spectra are different and the complexed DNA shows a hyperchromicity of 38%. Upon increase in temperature to 20 °C, a gradual further complexation takes place characterized by a slight change in CD spectrum and an increase in hyperchromicity till 54%.

To determine the conformation of the DNA in the complex at 20 °C, we first note that upon heat denaturation of double-stranded T4 DNA the CD minimum shifts from 247 nm to 249.5 nm. Upon complexation with gp32*I this minimum is even further red shifted, namely, to 253 nm. By applying a criterion similar to criterion I, we conclude from this red shift that the DNA inside the complex is single stranded. We interpret the fact that the minimum shifts to 253 nm (and not to 249.5 nm) as being due to the differential CD scattering. The conclusion that the DNA is completely denatured is confirmed by the hyperchromicity of 54%. The magnitude of the CD minimum ($\Delta\epsilon = -4.5$ at 253 nm) of the complexed T4 DNA at 19.2 °C is greater than for heat-denatured T4 DNA ($\Delta\epsilon = -2.1$ at 249.5 nm) and comparable to that of double-stranded T4 DNA at 1 °C ($\Delta\epsilon = -5.1$ at 247 nm). In analogy with criterion II this implicates that the interaction between the optical transitions in the base planes is much stronger in the gp32*I complexed single-stranded T4 DNA than in the heat-denatured T4 DNA. Apparently the interaction is of the same magnitude as in double-stranded T4 DNA. Therefore we conclude that the bases inside the complex are stacked and kept rigidly in position relative to each other.

In analogy with criterion III information about the overall conformation of the single-stranded T4 DNA molecule in the complex can be obtained from the long wavelength CD spectrum. Unfortunately the situation is not as clear as it was for poly[d(A-T)]·poly[d(A-T)]. There a characteristic minimum was found in the long wavelength CD spectrum of the complex which could be correlated with a minimum found for the double-stranded polymer at high LiCl concentration. Instead the long wavelength CD of the complexed T4 DNA is greatly reduced when compared with double-stranded T4 DNA in buffer. However, it is very well known that a similar reduction of the positive CD band of the double-stranded T4 DNA occurs at high salt concentrations (Ivanov et al., 1973). Therefore we believe that the conformation of the single-stranded T4 DNA in the complex with gp32*I at 20 °C can be most adequately described as being similar to the conformation of one strand of a double-stranded T4 DNA molecule at high salt concentrations.

Complexation above 20 °C. The reaction of the T4 DNA–gp32*I complex upon increase in temperature above 20 °C is different for the two nucleotide to protein ratios studied. At the ratio 6.0 no changes in CD spectrum are observed which indicates that the complexed T4 DNA keeps the same conformation even up until about 40 °C. At the ratio 2.2, however, a major change in CD spectrum is observed, accompanied by a slight further increase in hyperchromicity of about 3% (at 37 °C). Apparently it is the combined action of increase in temperature plus the availability of extra protein which is necessary to bring about this structural transition. As the T4 DNA in the complex is already single stranded at 20 °C, we deal here with a structural transition of the single-stranded molecule. From the decrease in magnitude of the CD minimum at 253 nm it follows that the interaction between the optical transitions is decreased relative to the interaction in the complex at 20 °C. It seems unlikely that this decrease is caused by an increase in flexibility of the complex as it is apparently due to binding of more protein. Instead a decrease due to a slight stretching of the T4 DNA strand seems more likely. A similar, but apparently smaller, stretching was found for poly[d(A-T)]·poly[d(A-T)] at the higher temperatures and protein concentration. The magnitude of the CD minimum at 253 nm of the T4 DNA gp32*I complex at 36.9 °C, $\Delta\epsilon = -3.2$, is greater than that of the heat-denatured T4 DNA at 78.8 °C ($\Delta\epsilon = -2.1$). This shows that, although decreased, the optical interaction, and therefore the stacking, it still considerably greater than in heat-denatured T4 DNA. That the conformation of the complexed T4 DNA at 36.9 °C is quite different from that of heat-denatured T4 DNA follows also from the long wavelength CD which is completely negative. A negative long wavelength CD spectrum of double-stranded DNA is only found at extremely high salt concentrations and for DNA in the conformation. Unfortunately no CD spectrum of T4 DNA in the C configuration is available for comparison. However, the shape of the CD spectrum of complexed T4 DNA measured at 36.9 °C is the same as that of calf thymus DNA lithium salt in the C-DNA configuration (Tunis-Schneider & Maestre, 1970). Therefore we believe that the single T4 DNA strand in the complex with gp32*I at temperatures above 20 °C assumes a conformation which is close to that of one of the strands of a double-stranded T4 DNA molecule in the C-DNA conformation.

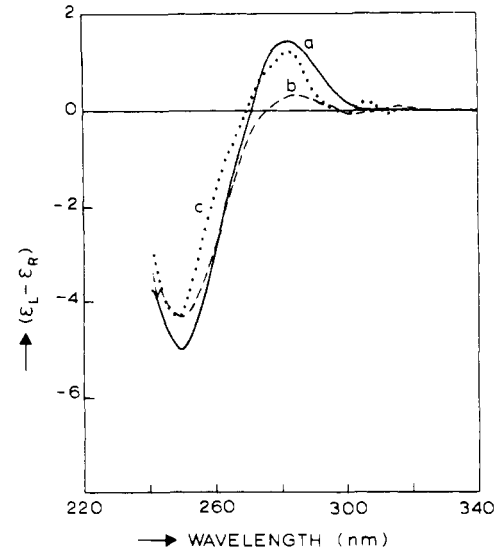

FIGURE 8: CD spectra of T4 DNA in the presence of gp32*I at a nucleotide to protein ratio of 6.0 measured at 1 °C in buffer I. (a) Directly after mixing at 4 °C. (b) After heating to 43.2 °C. (c) After heating to 43.2 °C and addition of 0.1 M $MgSO_4$.

*Renaturation of gp32*I Complexed T4 DNA.* The sample with nucleotide to protein ratio 6.0 was cooled to 1 °C after heating to 43.2 °C. The CD spectrum then remained almost equal to the one measured at 43.2 °C (Figure 8). Upon addi-

tion of 0.1 M $MgSO_4$, the spectrum did change. The CD spectrum which was then measured was in between that of native T4 DNA and the spectrum of the complex measured at 1 °C directly after mixing. Apparently the protein DNA complex dissociated partially and renaturation to almost completely double stranded molecules occurred.

Comparison with gp32 Complexation. From the data presented above and the data given in the preceding paper (Greve et al., 1978) it follows that gp32*I has a slightly different action than gp32. Firstly gp32*I shifts the melting temperature of T4 DNA by about 60 °C so that under physiological conditions the T4 DNA is strand separated. Secondly in the interaction with poly[d(A-T)]·poly[d(A-T)] complexes are already formed at 1 °C and complete strand separation occurs (at high protein concentration) at about 10 °C. This is about 15 °C lower than in the case with gp32. Thirdly the interaction with gp32*I seems to result in a single-stranded poly[d(A-T)] structure which is slightly different.

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Conformational Studies on ^{13}C-Enriched Human and Bovine Myelin Basic Protein, in Solution and Incorporated into Liposomes†

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ABSTRACT: Carbon-13 nuclear magnetic resonance (NMR) spin-lattice relaxation (T_1) and line-width studies have been performed on human and bovine myelin basic proteins in solution and incorporated into liposomes. These studies were facilitated by carbon-13 enrichment of the two methionyl residues in the protein which provided an S-methylated form and a noncovalently modified "intact" methionine carbon-13 enriched form. Values of T_1 for side chain methionine S-CH_3 carbons, in solution, found to be 0.5 to 0.6 s for the S-methylated proteins, and 0.7 to 0.8 s for the intact protein, displayed no significant variation over a range of pH or protein concentration. Line widths of corresponding spectra were essentially constant. The data were interpreted in terms of a relatively loose overall structure for the protein in which local (segmental) motion, at least in the vicinity of methionyl residues, dominated relaxation phenomena. Experiments were performed wherein ^{13}C-enriched myelin basic protein (both in the S-methylated form and the intact form) was incorporated into lipid vesicles (50:50 phosphatidylcholine:phosphatidic acid containing 30% protein by weight). Carbon-13 enriched Met S-CH_3 resonances corresponding to the protein interacting with the liposome were clearly visible and displayed line widths of about 15 Hz, as compared with about 1 Hz for the protein in free solution.

Myelin basic protein, a principal protein component of the myelin sheath, has been studied by several biophysical techniques with the goal of elucidating its structure and conformation and ultimately its function in the myelin membrane. Studies have utilized optical rotatory dispersion (ORD)[1] and circular dichroism (CD) (Eylar & Thompson, 1969; Palmer & Dawson, 1969; Chao & Einstein, 1970), intrinsic viscosity (Eylar & Thompson, 1969; Chao & Einstein, 1970; Epand, et al., 1974), low angle x-ray scattering (Epand et al., 1974), surface tension (Moscarello et al., 1974), proton magnetic resonance (Block et al., 1973; Liebes et al., 1975), and natural abundance ^{13}C NMR (Chapman & Moore, 1976). Despite the application of these diverse techniques, the detailed structure of this protein remains to be elucidated.

Although ORD/CD studies showed that no α-helical or β-structure was present in the molecule (Eylar & Thompson, 1969; Chao & Einstein, 1970; Palmer & Dawson, 1969), a specific tertiary structure was postulated on the basis of intrinsic viscosity and low angle x-ray scattering measurements (Epand et al., 1974). From the latter, the dimensions of the protein were calculated to be 15 × 150 Å, representative of a prolate ellipsoid. Evidence for the presence of a nonrandom conformation has also been presented by Chapman & Moore (1976), using natural abundance ^{13}C NMR. Their results suggested structured regions near the middle of the polypeptide chain, a sequence which includes a proline-rich region which could impart a "hair-pin" bend to the molecule (Eylar, 1970). Such a structure could leave both the N-terminal and the C-terminal ends relatively less rigid, and readily able to adopt a conformation in response to environmental constraints as might be expected to occur after interaction of this molecule with a lipid bilayer. Hence, knowledge of the structure of these ter-

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[1] Abbreviations used: NMR, nuclear magnetic resonance; MBP, myelin basic protein; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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