is another perturbing factor.

Since bovine and rat D-dimers are also susceptible (data not shown), there is an indication of a possible consensus (Table IV) of either diglycyl or histidylglutamyl residues and the presence of the cross-link. The possibility that the site-specific endopeptidase is contaminated by an exopeptidase that generates the heterogeneous ends cannot be excluded at present (Purves et al., 1986). Since the presence of the cross-links provides the specificity necessary for γ-chain cleavage by puff adder venom protease, this suggests that the fibrinogen conformation is sufficiently altered so that new epitopes might be created and raises the possibility that monoclonal antibodies could be produced with specificity for cross-linked fibrin by using puff adder venom cleaved peptides as antigen.

The manual gas-phase microsequencing technique used in this paper is very suitable for sequences of up to at least 20 residues, with a repetitive yield in this study of about 85%. The ability to detect carboxy-terminal residues was superior to an automated technique and had the advantage of demonstrating an unusual early peak, the putative cross-linked dipeptide. Carry-over from one cycle to the next is greater than with automated sequencing and cannot be appreciably reduced by extensive washing; however, it is not problematic except with repeated residues. The instrumental requirements are minimal, and this technique can be recommended for sequencing proteins blotted onto glass paper (W. F. Brandt and G. Frank, unpublished method).

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Interaction between Adenovirus DNA-Binding Protein and Single-Stranded Polynucleotides Studied by Circular Dichroism and Ultraviolet Absorption†

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ABSTRACT: The adenovirus DNA-binding protein (AdDBP) is a multifunctional protein required for viral DNA replication and control of transcription. We have studied the binding of AdDBP to single-stranded M13 DNA and to the homopolynucleotides poly(rA), poly(dA), and poly(dT) by means of circular dichroism (CD) and optical density (OD) measurements. The binding to all these polynucleotides was strong and nearly stoichiometric. Titration experiments showed that the size of the binding site is 9–11 nucleotides long for M13 DNA, poly(dA), and poly(rA). A higher value (15.0 ± 0.8) was found for poly(dT). Pronounced changes in the circular dichroism and optical density spectra were observed upon binding of AdDBP. In general, both the positive peak around 260–270 nm and the negative peak around 240–250 nm in the CD spectra decreased in intensity, and a shift of the crossover point to longer wavelengths was found. The OD spectra observed upon binding of AdDBP are remarkably similar to those obtained with prokaryotic helix-destabilizing proteins like bacteriophage T4 gene 32 protein and fd gene 5 protein. The data can best be interpreted by assuming that the AdDBP–polynucleotide complex has a regular, rigid, and extended configuration that satifies two criteria: (1) a considerable tilt of the bases in combination with (2) a small rotation per base and/or a shift of the bases closer to the helix axis.

The adenovirus DNA-binding protein (AdDBP)[1] is the major product of region E2 and is synthesized in high amounts early in infection of permissive cells (van der Vliet & Levine, 1973). Analysis of the phenotype of various temperature-sensitive and host-range mutants has indicated that the protein is multifunctional. It is essential for viral DNA replication (van der Vliet et al., 1975; Friefeld et al., 1983) and is involved in the control of early and late transcription (Carter & Blanton, 1978;

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[1] Abbreviations: AdDBP, adenovirus DNA-binding protein; CD, circular dichroism; GP32, T4 gene 32 protein; GP5, gene 5 protein; OD, optical density; dx, measure for the distance to the helix axis for a base as mentioned in Scheerhagen et al. (1986a); rotpb, rotation per base as mentioned in Scheerhagen et al. (1986a); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; ss, single stranded.

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Babich & Nevins, 1981; Klessig & Grodzicker, 1979) and possibly in virus assembly (Nicolas et al., 1982). During DNA replication, AdDBP binds to the single-stranded DNA originating from displacement synthesis, increases the rate of polymerization by the adenovirus DNA polymerase 100-fold on single-stranded templates (Field et al., 1984), and permits the DNA polymerase to translocate through duplex regions (Lindenbaum et al., 1986). During viral transcription, AdDBP modulates the stability and the rate of transcription of several early mRNAs (Nevins & Jensen-Winkler, 1980; Babich & Nevins, 1981). On the basis of studies of the mutant hr 404, located in the N-terminal part of the protein (Kruijer et al., 1981), the AdDBP may also be involved in the splicing of viral fiber mRNA (Anderson et al., 1983; Klessig & Grodzicker, 1979).

The amino acid sequence of AdDBP is composed of 529 amino acids in a single polypeptide chain M_r of 59029 (Kruijer et al., 1981). Substantial evidence exists for the presence of at least two functional domains. By limited chymotrypsin treatment a C-terminal fragment can be obtained containing amino acids 174–525 (Tsernoglou et al., 1985). This fragment, which has been crystallized (Tsernoglou et al., 1984), contains the DNA binding site of the protein and is sufficient for stimulation of DNA replication in vitro (Ariga et al., 1980; Tsernoglou et al, 1985). Mutations in this domain, like H5ts125, are impaired in early transcriptional control functions. The N-terminal domain, which is extensively phosphorylated (Klein et al., 1979; Linnē & Philipson, 1980), does not bind to DNA. Host-range mutations located in this domain are defective in late gene expression, independent of the role of the intact protein in DNA replication (Rice & Klessig, 1984). Comparison of the structures of DBP genes from four different adenovirus serotypes has shown that the conserved regions are mainly confined to the C-terminal part (Kruijer et al., 1983; Quinn & Kitchingman, 1984; Kitchingman, 1985).

So far, the interaction of AdDBP and polynucleotides has been investigated by electron microscopy, sedimentation analysis, and nitrocellulose filter binding (van der Vliet et al., 1978; Schechter et al., 1980). AdDBP binds to single-stranded DNA with low cooperativity in agreement with the concentration-dependent self-association observed in the absence of DNA. At saturation, the protein appears to cover 7 (van der Vliet et al., 1978) or 3–9 (Schechter et al., 1980) nucleotides. Binding to double-stranded DNA and synthetic polyribonucleotides has also been observed, but details of the binding constants are lacking (Fowlkes et al., 1979; Schechter et al., 1980). Electron microscopy of the complex prepared in vitro indicated that the AdDBP–single-stranded DNA complex has an extended configuration with an average length of 2.7 Å/base (van der Vliet et al., 1978). Replicative intermediates isolated from infected cells contain 20 nm thick filaments consisting of single-stranded DNA and AdDBP (Kedinger et al., 1978).

Circular dichroism (CD) and optical density (OD) measurements have been employed to obtain information about the configuration of single-stranded DNA in complex with a number of prokaryotic single-stranded DNA-binding proteins such as M13 GP5 (Anderson et al., 1975), fd GP5 (Scheerhagen et al., 1986a), *Escherichia coli* SSB (Anderson & Coleman, 1975), and GP32 (Alberts & Frey, 1970; Anderson & Coleman, 1975; Greve et al., 1978; Jensen et al., 1976; Scheerhagen et al., 1985c, 1986a). All these proteins give rise to the same kind of characteristic changes in the CD and OD spectra of the polynucleotides, suggesting that they force single-stranded DNA in a similar conformation. We have performed CD and OD measurements on the complex of AdDBP with several homopolynucleotides [poly(rA), poly(dA), poly(dT)] and with single-stranded M13 DNA to study the conformation of the polynucleotides in the complex. Titration curves were recorded to obtain information about the size of the binding site, and the observed spectra were compared with those induced by the binding of GP32 and GP5.

Materials and Methods

Buffer. In all experiments the same buffer was used: 10 mM Tris-HCl, 50 mM NaCl, 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, and 1 μg/mL L-1-(tosylamido)-2-phenylethyl chloromethyl ketone (pH 8.0 at 20 °C).

Polynucleotides and AdDBP. Poly(dA), poly(dT), and poly(rA) were purchased from P-L Biochemicals and were used without further purification. M13 DNA, a kind gift of Dr. B. J. M. Harmsen (University of Nijmegen), was purified according to Konings et al. (1973). The concentrations of the polynucleotides (on a nucleotide basis) were calculated by using the following molar extinction coefficients at 20 °C: poly(rA), ϵ_{260nm} = 9400 M^{-1} cm^{-1}; poly (dA), ϵ_{257nm} = 8600 M^{-1} cm^{-1}; poly(dT), ϵ_{264nm} = 8520 M^{-1} cm^{-1}; M13 DNA ϵ_{259nm} = 7400 M^{-1} cm^{-1}. AdDBP was isolated and purified as described (Tsernoglou et al., 1984). The purified protein was dialyzed against the buffer mentioned above and centrifuged for 10 min at 7000*g* to remove large aggregates. The absorption at 280 nm was measured to determine the molar concentration by using ϵ_{280nm} = 56 600 M^{-1} cm^{-1}. This number is calculated from $E^{1\%}_{280nm}$ = 9.6 by using a molecular weight of 59K. The former value was obtained by protein determination (Specter, 1978) using bovine serum albumin as a standard and is about the same as obtained in 2 M NaCl (van der Vliet et al., 1978). The protein solution was frozen and kept at −20 °C.

Spectral Measurements. OD spectra were recorded on a Cary 219 spectrophotometer interfaced to a HP85 computer. A bandwidth of 1 nm was used. CD spectera were recorded on a Cary 61 spectropolarimeter, which was extensively modified (Bokma et al., 1987) and interfaced to a HP85 computer. The Cary 61 was calibrated with anhydrous (+)-10-camphorsulfonic acid (Eastman Kodak) at 290.5 nm (Chen & Yang, 1977). For both CD and OD measurements a thermostated cell holder was used. OD and CD spectra were digitized at 0.1-nm intervals. All complex spectra were recorded at least twice with reproducible results. Spectra were base line corrected and smoothed with a 25-point least-squares procedure (Savitsky & Golay, 1964; Steinier et al., 1972). Titrations started with a sample containing the polynucleotide, which was weighed accurately. Protein was added and the sample was weighed again. After addition, the solution was mixed by rotating the cell gently back and forth and equilibrated for 10 min before measuring. Before a new amount of protein was added, the sample was weighed again. The spectrum of a polynucleotide in complex is defined as the spectrum of the complex, corrected for the protein contribution (measured without the polynucleotide) and for dilution (see also Discussion). The difference spectrum is defined as the spectrum of the complex minus the contributions of the polynucleotide and the protein and is corrected for dilution. CD spectra are expressed as $\epsilon_L - \epsilon_R$, the difference between the molar extinction coefficients (on a nucleotide base in the case of homopolynucleotides and DNA) for left and right circularly polarized light.

Results

Binding to Single-Stranded M13 DNA. In Figure 1 the CD spectrum of AdDBP is given. The intensity of the spectrum

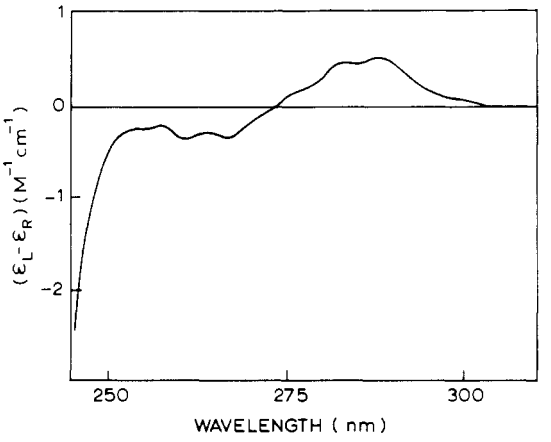

FIGURE 1: CD spectrum of AdDBP. [AdDBP] = 16.0 μM; T = 8.5 °C.

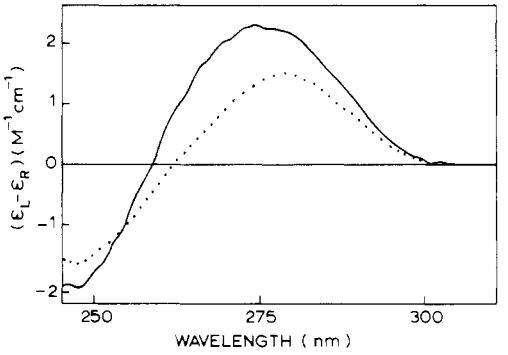

FIGURE 2: CD spectrum of M13 DNA (71 μM), free at T = 14 °C (—) and in complex with AdDBP (···), from which the protein contribution has been subtracted (see Materials and Methods). [AdDBP]/[DNA] = 0.16; T = 14 °C.

is low above 245 nm but starts to increase sharply below this wavelength. The low-intensity spectrum between 250 and 300 nm is probably due to the relatively low number of aromatic residues of AdDBP (6 Tyr, 6 Trp). To study the spectra of polynucleotides in the complex with AdDBP, only measurements above 245 nm were performed where the contribution of the protein is small.

Figure 2 shows the change in the CD spectrum of single-stranded M13 DNA due to addition of a saturating amount of AdDBP. These changes are characterized by a decrease of the intensity of the two CD peaks at 273 nm and at 248 nm, the decrease being more significant in the positive CD peak, and a shift of the crossover point to longer wavelengths.

Figure 3 shows the OD difference spectrum of single-stranded M13 DNA induced by close to saturating amounts of AdDBP. From 240 to 330 nm there is an increase in the OD of the complex relative to that of the free single-stranded M13 DNA. Because there is very little absorption at 330 nm of the DNA and the protein, the observed increase at this wavelength must reflect an increase in scattering due to the large complexes that are formed, a phenomenon similar to that reported for fd GP5 and fd DNA (Day, 1973). Nevertheless, superimposed on the scattering background, a positive absorption peak can be distinguished around 265 nm which must be due to M13 DNA and which indicates hyperchromism. Such an increase in absorption was also observed upon GP32 binding to single-stranded calf thymus DNA (Scheerhagen et al., 1986a).

Some preparations of AdDBP showed a large amount of scattering even without DNA, presumably caused by self-aggregation. Addition of M13 DNA reduced the scattering, indicating that even strong aggregation of AdDBP molecules

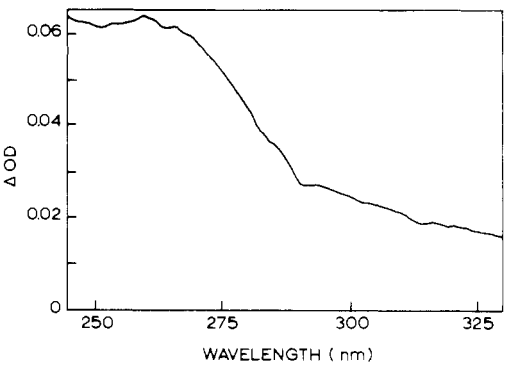

FIGURE 3: OD difference spectrum of M13 DNA with AdDBP. $\Delta OD = OD_{complex} - (OD_{DNA} + OD_{protein})$; [DNA] = 57.8 μM; [AdDBP] = 5.5 μM; T = 7.5 °C.

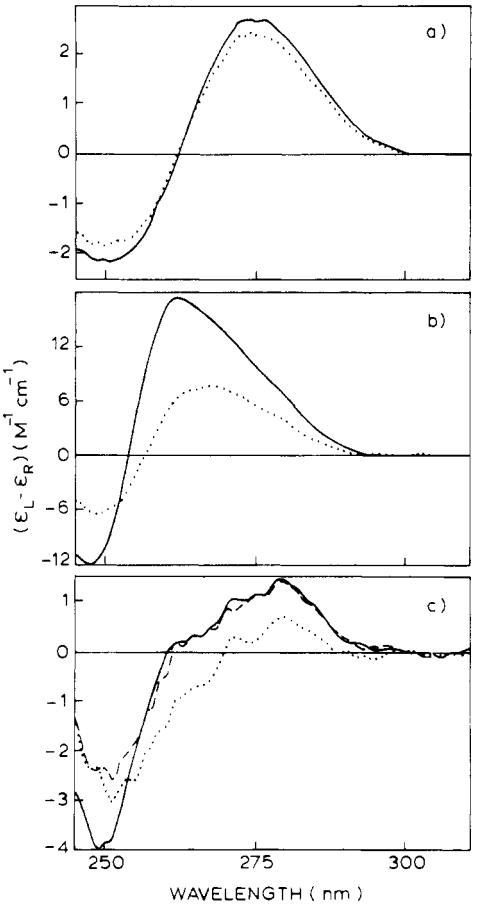

FIGURE 4: CD spectra of the homopolynucleotides, free (—) and in complex with AdDBP (···). The concentration of the polynucleotide and the concentration ratio R = [AdDBP]/[poly(X)] are as follows: (a) [poly(dT)] = 52.0 μM, R = 0.13, T = 18.0 °C; (b) [poly(rA)] = 42.4 μM, R = 0.21, T = 9.5 °C; (c) [poly(dA)] = 82.8 μM, R = 0.065 (- - -) or 0.18 (···), T = 9.5 °C.

does not necessarily inhibit their association with DNA.

Binding to Homopolymers. To obtain more specific information about the structure of polynucleotides in complex with AdDBP, the homopolynucleotides poly(rA), poly(dA), and poly(dT) were investigated. Binding of AdDBP gave rise to characteristic changes in both the CD (see Figure 4) and OD spectra (see Figure 5). For all three homopolynucleotides the CD intensity of both bands decreased upon complex formation. For poly(dT), shown in Figure 4a, the change in the CD spectrum upon binding is rather small. Poly(rA) showed the largest change in the CD spectrum (Figure 4b). The observed changes in the poly(dA) CD spectrum indicate that there may exist two different binding modes. At subsaturating

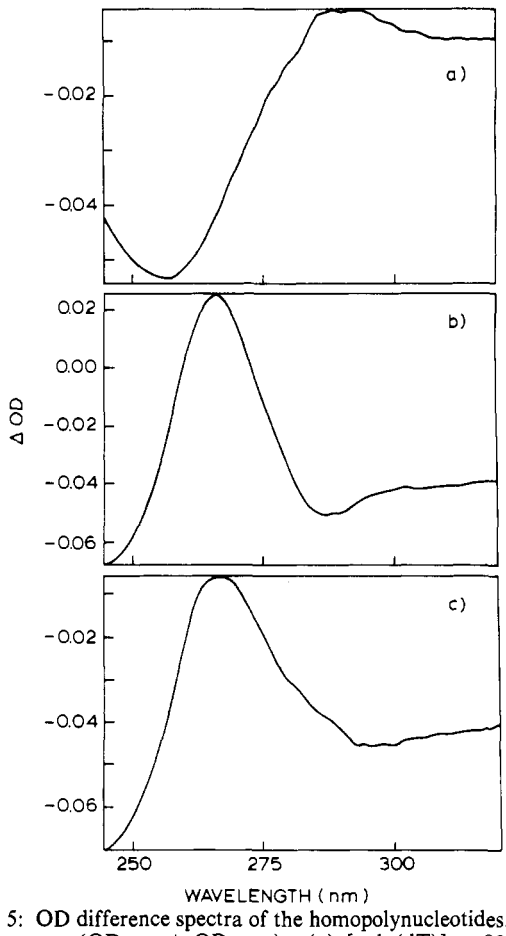

FIGURE 5: OD difference spectra of the homopolynucleotides. ΔOD = $OD_{complex} - (OD_{DNA} + OD_{protein})$. (a) [poly(dT)] = 82.9 μM, [AdDBP] = 5.1 μM, T = 20 °C; (b) [poly(rA)] = 68.3 μM, [AdDBP] = 7.2 μM, T = 20 °C; (c) [poly(dA)] = 78.6 μM, [AdDBP] = 7.4 μM, T = 20 °C.

protein levels up to [AdDBP]/[poly(dA)] = 0.065 the intensity of the negative CD band at 250 nm decreases, while the positive band is not affected. A further addition of AdDBP leads to a decrease of the positive CD band, while the negative band does not significantly change any further.

Binding of AdDBP to either of the three homopolynucleotides also resulted in pronounced OD difference spectra (Figure 5a–c) consisting of a change in absorption of the polynucleotides. Moreover, in all cases formation is accompanied by a decrease of the scattering. Poly(rA) and poly(dA) show hyperchromism (Figure 5b,c) demonstrated by the positive peak in the difference spectrum around 265 nm. For both polynucleotides this peak is much narrower than the original absorption spectrum and the maximum is at longer wavelengths.

Poly(dT) shows a marked hypochromism upon binding of AdDBP. Remarkably, both the hypochromism of poly(dT) and the hyperchromism of poly(rA) and poly(dA) were also observed upon the binding of GP32 and GP5 to these polynucleotides (Scheerhagen et al., 1985c; Scheerhagen et al., 1986a).

Size of the Binding Site. In order to determine the size of the binding site, a number of titration experiments were performed by following the changes in OD and/or CD upon the stepwise addition of protein to a polynucleotide sample. The best results were obtained by monitoring the decrease of the positive band in the CD spectrum of poly(rA). In figure 6 a series of difference spectra are shown for different ratios of [AdDBP] to [poly(rA)]. All difference spectra have a similar shape, which is indicative of only one type of binding

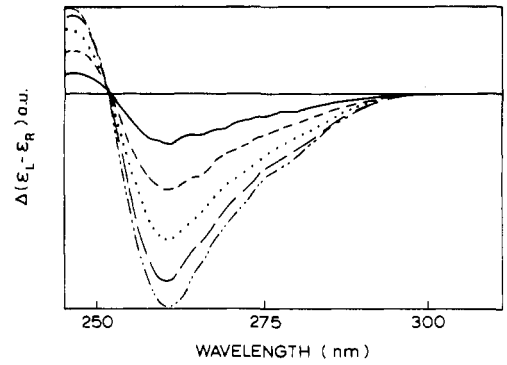

FIGURE 6: CD difference spectra of poly(rA) expressed in arbitrary units (a.u.) at increasing concentrations of AdDBP. T = 9.5 °C. The ratios R = [AdDBP]/poly(rA)] are given below. For R = 0.157 and R = 0.205 the spectra are almost identical with the spectrum for R = 0.108, and they are not given. The initial concentration of poly(rA) is 42.4 μM. (—) R = 0.019, (- - -) R = 0.039, (···) R = 0.059, (– –) R = 0.079, and (–··–) R = 0.108.

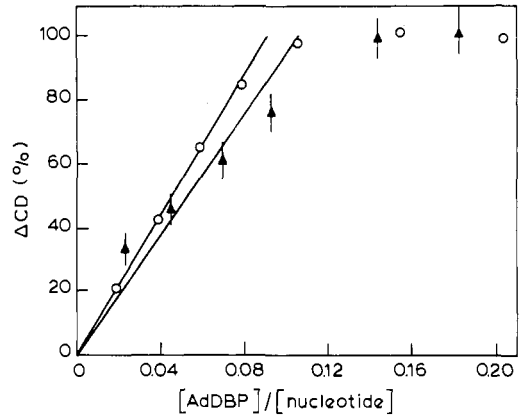

FIGURE 7: Titration curves measured with CD. ΔCD is the change in CD (%) relative to the change corresponding with saturation (for further explanation, see text). (O) Poly(rA); (▲) M13 DNA. The error bars indicate estimated standard errors. For poly(rA), they are omitted, because they are too small. [Poly(rA)] = 42.4 μM, and [M13 DNA] = 70.5 μM.

Table I: Size of the Apparent Binding Site n on Polynucleotides for AdDBP[a]

polynucleotide	n(OD)	n(CD)
poly(rA)		11.9 ± 0.4
	10.6 ± 0.3	9.6 ± 0.2
	10.6 ± 0.4	11.0 ± 0.1
poly(dA)	9.6 ± 0.6	
	10.3 ± 0.9	
M13 DNA		9.4 ± 1.2
poly(dT)	15.0 ± 0.8	

[a] n for different polynucleotides as determined by means of OD [n(OD)] or CD [n(CD)]. (For further explanation see text.) Values of n on the same line are determined with the same sample. For each titration n is determined with a corresponding standard error by means of a weighed least-squares calculation, using titration points up to 70% saturation of the polynucleotide.

process. Above a ratio [AdDBP] to [poly(rA)] of 0.10 the spectral change saturates. The corresponding titration curve is shown in Figure 7. This curve was obtained by integrating the peak of the difference spectra from 256 to 260 nm. An estimation of the standard deviation was made for every titration point. From the linear relationship between ΔCD and the degree of saturation we conclude that the binding must be nearly stoichiometric. The size of the binding site n was calculated, using a weighed least-squares method (Table I). For this calculation only titration points were taken below 70%

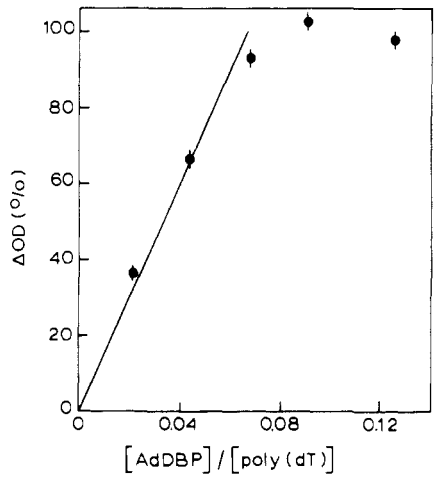

FIGURE 8: Titration curve for poly(dT) measured with OD. ΔOD is the change in the OD (%) relative to the change corresponding with saturation. The error bars indicate standard errors. [poly(dT)] = 52.0 μM.

binding. Similar n values were calculated by using the negative band of the poly(rA) CD spectrum (not shown). For OD difference spectra, the titration is somewhat more difficult to follow, because the shape of the difference spectra changes slightly during the titration, partly due to changes in the scattering. The height of the peak was taken relative to the average of the heights on opposite sides of the peak (250 and 283 nm). In this way, the contribution of scattering to the binding data is almost eliminated. For poly(rA), determination of n from the OD spectra leads to values of n that are close to those obtained with CD (see Table I). Measurements with different samples gave comparable results although there is some variation, possibly due to different qualities of the various protein samples. For OD and CD combined, the average value of n for poly(rA) is 10.7 with a standard error of 0.8, not taking into account the errors in the individually determined n values.

It was not possible to follow the binding of poly(dT) and poly(dA) at increasing [AdDBP] by means of CD, because of the small amplitude of the change for poly(dT) and poly(dA) and the occurrence of the two different kinds of spectral changes for poly(dA). For M13 DNA a CD titration could be performed (Figure 7) by averaging the CD signal at 275 nm several times during a few minutes. Due to the relatively small amplitude of the changes a large uncertainty in n was introduced. The obtained n value 9.4 ± 1.2 is very similar to that of poly(rA). The error represents the standard error in one determination of n (see Table I). For poly(dA) the binding could be followed by means of the OD difference spectra in the same way as was done for poly(rA) (not shown). For poly(dT) the height of the difference spectra at 286 nm relative to the height at 255 nm was taken as a measure for the degree of binding. The corresponding titration curve is given in Figure 8. The high value of n obtained in this way is remarkable. For M13 DNA the OD difference spectra did not allow titration, because of the large scattering background, the broad and low peaks, and the slightly changing shape (see Figure 3).

Discussion

Size of the Binding Site of AdDBP. The binding of AdDBP to various polynucleotides could be followed very well by CD and OD measurements. Although scattering due to protein aggregation is a problem in these experiments, we believe for two reasons that the final results are not significantly influenced: (1) In the OD titrations the spectra were analyzed in such a way that the contribution of scattering was largely avoided, and (2) very similar spectral and binding data were obtained, although the amount of scattering varied considerably from sample to sample. In all cases a strong binding was observed, even for poly(rA), which appeared not to bind AdDBP in a competition experiment with *φX174* DNA (van der Vliet et al., 1978). The observed complex formation with ribonucleotides may be interesting in view of a possible function in transcription.

Assuming that the binding is linearly reflected in the amount of spectral changes, the binding appeared to be almost stoichiometric in all cases. As a consequence, the binding constant K and the cooperativity factor ω could not be determined at this salt concentration. A rough estimate of the product $K\omega$ leads to a minimum value of 10^7 mol^{-1}. The size of the binding site varied between 9 and 11 in most cases, a variation probably due to different qualities of the various protein samples used. For poly(dT) a higher value for n was found. We have no explanation for this difference. Perhaps the binding process is not the same for all polynucleotides. The observation that the poly(dA) CD spectrum shows at least two different kinds of changes at different concentration ratios of AdDBP to poly(dA) may indicate that a single mode of binding is too simple.

The determined value for n is a lower bound, because of the implicit assumption that all protein molecules can bind the polynucleotide and no protein is inactivated. CD titrations with GP32 and a number of polynucleotides systematically resulted in a value for n that is 10–20% too low (Scheerhagen et al., 1986b). The value of n = 9–11 is larger than the value of n = 7 determined by sedimentation experiments (van der Vliet et al., 1978). This value of 7 must be even smaller, taking into account a molecular weight of 59K as calculated from the sequence instead of the experimentally determined value of 68K, which was used in determining n = 7. A lower value, n = 3–9, was determined by Schechter et al. (1980), using resistance to trypsin digestion as a binding criterion. A possible explanation for these discrepancies is that additional protein can still bind to the complex, when the polynucleotide is already completely covered. This would not result in a further change of the CD and OD spectra but would give lower n values than with the other approaches.

Comparison of CD and OD Spectral Changes due to AdDBP and GP32 Binding. To interpret the CD spectra of the polynucleotides in the complexes, we assume that there is no contribution of the protein to the spectra, i.e., that the CD of the protein remains the same, whether it is bound to a polynucleotide or free. This seems very reasonable as was pointed out for GP32 and GP5 (Scheerhagen et al., 1986a). However, the OD difference spectra may contain a contribution from the protein and from the interaction of the transition moments of the polynucleotide and the protein.

The observed hyperchromism for poly(rA) and poly(dA) upon binding is by far not as large as that obtained by raising the temperature to 70 °C (Scheerhagen et al., 1986a). For poly(dT) even a clear hypochromism is observed upon binding, which is absent when the temperature is raised. In addition, for all polynucleotides the CD spectra remain relatively intense. From this we conclude that an ordered sturcture must remain for the polynucleotides in the complex with AdDBP. We shall compare this structure with the one induced by the binding of GP32.

For polynucleotides in complex with GP32 a rather specific structure has been proposed (Scheerhagen et al., 1986a). Calculations show that the observed CD spectra for poly(rA)

in complex with GP32 can best be explained if the polynucleotide in the complex has a regular and rigid conformation that satisfies two criteria: (1) a considerable tilt of the bases, in combination with (2) a small rotation per base and/or a position of the bases close to the helix axis. These conclusions concerning the GP32–ssDNA complex are supported by other experimental results. Electric birefringence measurements suggest that the bases in single-stranded DNA in complex with GP32 are considerably tilted (Scheerhagen et al., 1985a), which was confirmed in recent linear dichroism spectra (van Amerongen et al., unpublished results). From electron microscopic studies (Delius et al., 1972) and later from a combination of quasi-elastic light scattering (Scheerhagen et al., 1985b) and electric birefringence measurements (Scheerhagen et al., 1985a) it appeared that single-stranded DNA is stretched by more than 50% upon binding with GP32. This stretching can only take place if the rotation per base decreases and/or the bases move closer to the helix axis. In complex with two other single-stranded DNA-binding proteins, GP5 and *E. coli* SSB, a similar secondary structure of the polynucleotides is very likely, as has been discussed by Scheerhagen et al. (1986a). To see whether such a conformation may explain the observed spectral changes in the OD and CD spectra of the polynucleotides upon binding of AdDBP, we compare the spectra with those obtained with GP32.

(1) The OD difference spectra induced by AdDBP show a strong resemblance to those induced by GP32. The sign is the same, and also the shape is very similar. The intensity of the changes in the case of AdDBP binding is about half as large for poly(rA) and poly(dA) and similar in magnitude for poly(dT) as compared with GP32. The difference spectra strongly suggest that AdDBP forces the polynucleotides in a conformation that is similar to the one induced by GP32.

(2) The CD spectra induced by AdDBP show clear differences with the ones induced by GP32. In general, the CD spectra with GP32 have a more intense negative band around 245 nm whereas in the complex with AdDBP the positive band around 270 nm is more intense. However, small conformational changes can lead to marked differences in the CD spectra. To see whether such small changes can explain the observed differences for the various proteins, we have to confine ourselves to the spectra of poly(rA) and poly(dA) since the CD calculations (Scheerhagen et al., 1986a) were done for an adenine hexamer. The poly(dA) spectrum induced by AdDBP binding has almost the same shape as the poly(rA) spectrum in the case of GP32 binding, but it is about half as intense. This may reflect a GP32-like structure, but with an even larger base–base distance. The spectrum of poly(rA) in complex with AdDBP has a positive peak, which is much more intense than in the case of GP32 binding. However, starting from a GP32-like structure [small distance to the helix axis (dx) and/or a small rotation per base (rotpb)], the positive peak will increase when dx and/or rotpb is slightly raised, leading to a spectrum similarly shaped as the one observed for AdDBP binding (M. A. Scheerhagen, personal communication). So, although the CD spectra of the complexes are different for the various proteins, they can nevertheless be explained by a similar structure of the polynucleotides.

Proposal for the Conformation of Polynucleotides in Complex with AdDBP. The secondary structure of single-stranded polynucleotides in complex with GP32 and GP5 can thus be characterized by an increase of the length of the polynucleotides and a strong tilt of the bases. This complex is probably further organized in a tertiary structure, which explains the observed differences in axial increment (contour length per base) for monodisperse single-stranded DNA in complex with GP32 (Delius et al., 1972; Scheerhagen et al., 1985a,b), *E. coli* SSB (Sigal et al., 1972), and GP5 (Torbet et al., 1981). For GP32 the structural changes are considerable: the transition moments lying in the plane of the bases make an angle with the long axis of the complex of less than 54° (H. van Amerongen, unpublished results), and the polynucleotide is stretched by at least 50%. We propose that such a stretching in combination with a tilt of the bases also underlies the observed spectra of the polynucleotides in complex with AdDBP. A stretching would be in accordance with the extended form suggested by sedimentation experiments (van der Vliet et al., 1978). Also, electron microscopic measurements indicate an extension of the phosphate–sugar backbone. An average length of 2.7 Å/base is measured by van der Vliet et al. (1978), with a maximum of 3.5 Å/base. Replicative intermediates composed of displaced single-stranded DNA complexed with AdDBP (Kedinger et al., 1978) appear to be of similar length as double-stranded adenovirus DNA. However, these complexes have a diameter of about 200 Å. This is very large, taking into account that the 39K binding part of the protein has a diameter of about 45 Å, if it is globular, taking the partial specific volume as 0.73 mL/g (van der Vliet et al., 1978). Therefore, it is very likely that the DNA–protein complex is organized in a tertiary structure. The distance between neighboring bases along the phosphate–sugar backbone will then probably be much larger than the projected distance along the contours of the complexes, seen on the electron micrographs (Kedinger et al., 1978). Future research, using hydrodynamic and linear dichroism measurements, is needed to further characterize the structure of the complex of AdDBP with single-stranded DNA.

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Registry No. Poly(dA), 25191-20-2; poly(dT), 25086-81-1; poly(rA), 24937-83-5.

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NMR–Pseudoenergy Approach to the Solution Structure of Acyl Carrier Protein†

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ABSTRACT: A method for protein structure determination from two-dimensional NMR cross-relaxation data is presented and explored by using short amino acid segments from acyl carrier protein as a test case. The method is based on a molecular mechanics program and incorporates NMR distance constraints in the form of a pseudoenergy term that accurately reflects the distance-dependent precision of NMR cross-relaxation data. When it is used in an indiscriminant fashion, the method has a tendency to produce structures representing local energy minima near starting structures, rather than structures representing a global energy minimum. However, stepwise inclusion of energy terms, beginning with a function heavily weighted by backbone distance constraints, appears to simplify the potential energy surface to a point where convergence to a common backbone structure from a variety of starting structures is possible. In the case of the segment from residues 3 to 15 in acyl carrier protein, a nearly perfect α-helix is produced starting with a linear chain, an α-helical chain, or a chain having residues with alternating linear and α-helical backbone torsional angles. In the case of the segment from residues 26 to 36 a structure having a right-handed loop is produced.

Through use of high-field proton NMR[1] and two-dimensional acquisition methods, it has recently become possible, without the aid of previous X-ray crystal structures, to attempt structure determinations of proteins in solution (Braun et al., 1981, 1983; Kaptein et al., 1985; Zuiderweg et al., 1985a; Williamson et al., 1985; Braun et al., 1986; Kline et al., 1986). While published attempts appear to be successful in some cases, there is still no universally accepted procedure for

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[1] Abbreviations: ACPSH, acyl carrier protein with the free SH group; 2D NMR, two-dimensional nuclear magnetic resonance; DQF COSY, double quantum filtered two-dimensional J-correlated NMR spectroscopy; NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE spectroscopy.

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