Although we cannot absolutely rule out the possible loss of loosely bound Zn^{2+} during the purification procedure, the presence of these additional Zn^{2+} ions seems not play an important role in the TFIIIA function.

In conclusion, our studies demonstrate that TFIIIA contains two tightly bound Zn^{2+} ions that are coordinated with both cysteine and histidine residues. The modifications of either of these amino acids change the structure of the Zn^{2+} domains and cause the loss of protein activities. These results provide some insight concerning the structure/function relationships of the intrinsic Zn^{2+} ions of TFIIIA.

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Complex Formation between the Adenovirus DNA-Binding Protein and Single-Stranded Poly(rA). Cooperativity and Salt Dependence†

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ABSTRACT: The complex formed between adenovirus DNA-binding protein (AdDBP) and poly(rA) was investigated with circular dichroism spectroscopy. The binding process was studied at a variety of salt concentrations, and the titration curves were analyzed according to the contiguous cooperative binding model given by McGhee and von Hippel [McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489]. The cooperativity factor ω of the binding process is low ($\omega \approx 20$–30) and independent of the salt concentration. This in contrast to the binding constant K for which a moderately strong salt dependence is observed: $\partial \log (K\omega)/\partial \log [NaCl] = -3.1$. The size of the binding site was consistently calculated to be about 13. We also studied the C-terminal 39-kDa fragment which is sufficient for DNA replication in vitro. Complex formation between this fragment of AdDBP and poly(rA) appeared to be characterized by spectroscopic and binding properties similar to those of the intact protein. Only, the binding constant in 50 mM NaCl is a factor of 5 lower.

The adenovirus DNA-binding protein (AdDBP) is an example of the important class of single-stranded DNA binding proteins [see, for reviews, Chase and Williams (1986) and Lohman et al. (1988)]. Apart from a role in control of early (Carter & Blanton, 1978) and late (Klessig & Grodzicker, 1979) transcription and possibly virus assembly (Nicolas et al., 1982), AdDBP is required for DNA replication both in vivo and in vitro (van der Vliet et al., 1975; Kaplan et al., 1979). The protein has a strong stimulating effect on the activity of the adenovirus DNA polymerase on single-stranded templates (Field et al., 1984), probably via a dual mechanism: (1) interaction with the single-stranded DNA, leading to a complex that enhances the rate of polymerization; (2) direct specific interaction with the polymerase. AdDBP binds to ssDNA as well as RNA (Cleghon & Klessig, 1986; Adam & Dreyfuss, 1987; Seiberg et al., 1989). In vitro binding to the attenuated RNA derived from the viral major late promotor has been observed (Seiberg et al., 1989) while also in vitro AdDBP is associated with mRNA and hnRNA (Adam & Dreyfuss, 1987). The physiological meaning of the binding

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to RNA agrees well with the role of the protein in transcription control and stability of mRNA as derived from mutant studies.

The amino acid sequence of AdDBP is composed of 529 amino acids in a single polypeptide chain and the molecular mass is about 59 kDa. Two functionally different domains can be distinguished, and the C-terminal domain (residues 174–525, 39 kDa) is involved in DNA binding (Ariga et al., 1980; Tsernoglou et al., 1985). The 39-kDa fragment has been crystallized and contains a large number of the residues that are conserved among the various adenovirus serotypes (Kruyer et al., 1981; Quinn & Kitchingman, 1984; Kitchingman, 1985). In addition, it contains all the tryptophans and tyrosines (Tsernoglou et al., 1985) responsible for the 280-nm absorption. In the in vitro replication system the 39-kDa C-terminal fragment is equally active as the integral protein, both in the replication of the parental double-stranded adenovirus DNA and in the replication of the displaced single-stranded strand (Ariga et al., 1980; Leegwater et al., 1988).

The binding of AdDBP to ssDNA has been investigated with electron microscopy, sedimentation analysis, and nitrocellulose filter binding (van der Vliet et al., 1978; Schechter et al., 1980). Recently, the binding properties of AdDBP to ssDNA and RNA were studied by using circular dichroism (CD) and absorption (OD) spectroscopy. The size of the binding site ranged between 10 and 12 for poly(rA), between 10 and 11 for poly(dA), 10 for M13 DNA, and 15 for poly(dT) (van Amerongen et al., 1987). With the exception of poly(rA), the signals were very weak and the titration curves difficult to obtain. For all the polynucleotides the OD difference spectra showed a strong resemblance to those obtained after binding of the ssDNA binding protein from T4 (GP32). For poly(rA) the CD difference spectra due to the binding of AdDBP were closely similar to those due to GP32 binding. In all cases the spectral changes were less intense. For these reasons we proposed a strong structural similarity of the complexes formed by AdDBP and GP32 with single-stranded DNA. In these structures the DNA is locally rather extended, and the bases have a strong tilt and twist (Scheerhagen et al., 1986a; van Amerongen et al., 1988).

For GP32, a series of binding studies have resulted in a detailed description of the thermodynamics of binding. The process is characterized by three parameters: the binding constant K, the cooperativity parameter ω, and the size of the binding site, n. From a titration experiment, in principle all three parameters can be obtained, although either at very high values of the product $K_{int} = K\omega$ (stoichiometric binding) or at very low values of K_{int} (no binding) accurate estimates are difficult. For the binding of GP32 to a variety of polynucleotides ω was found to be extremely large ($\omega \approx 10^3$), and K was found to vary for the various polynucleotides [$K \approx 10^5$ M^{-1} for poly(rA), $K \approx 10^6$ M^{-1} for poly(dA), and $K \approx 10^7$ M^{-1} for poly(dT) at 0.2 M NaCl (Newport et al., 1981; Kowalczykowski et al., 1980)]. The strong salt dependence of the binding process was uniquely associated to a salt dependence of K:$\partial \log (K\omega)/\partial \log [NaCl] = -7$. Surprisingly, the salt dependence was not only due to the displacement of (at most) four cations upon formation of strong ionic nucleic acid–protein interactions but, in addition, due to displacement of anions from the protein upon complex formation.

For GP32 the quantitative estimates of ω, n, and the salt dependence of binding gave rather similar results for the various deoxy- and ribonucleotides (Newport et al., 1981). Apart from estimates for the binding site size (van der Vliet et al., 1978; Schechter et al., 1980; van Amerongen et al., 1987), these parameters were not available for AdDBP. The binding is thought to be only weakly cooperative (van der Vliet et al., 1978; Schechter et al., 1980). The only polynucleotide for which we may hope to obtain quantitative data using optical–spectroscopic techniques is poly(rA). Therefore, we decided to study the binding of AdDBP to poly(rA) as a function of the salt concentration. In addition, we report the binding properties for the 39-kDa C-terminal fragment of AdDBP. Apart from direct relevance to RNA binding, we shall assume that the obtained numbers for ω, n, and $\partial \log (K\omega)/\partial \log [NaCl]$ reflect the general binding properties of AdDBP. In this respect AdDBP binding to poly(rA) seems a good choice because many of the characteristic spectral features are shared with other single-stranded DNA- and RNA-binding proteins.

Materials and Methods

All the AdDBP experiments were performed in 10 mM Tris·HCl, 0.1 mM PMSF, and 2 μg/mL TPCK at pH 8.0 at 20 °C and the indicated concentration of NaCl (buffer A). Experiments with GP32 were performed with 1 mM Na_2HPO_4 and 0.2 mM Na_2EDTA at pH 7.7 (buffer B) and the indicated concentration of NaCl. Poly(rA) was obtained from Pharmacia (Woerden, The Netherlands) and was used without further purification. AdDBP was isolated and purified as described by Tsernouglou et al. (1985). The 39-kDa fragment was prepared by digestion with chymotrypsin followed by DNA–cellulose chromatography (Leegwater et al., 1988) and was shown to be free of any remaining intact DBP by SDS gel electrophoresis. Gene 32 protein was isolated essentially as described by Hosoda and Moise (1978) with the minor modifications described by Kuil et al. (1988). Before the experiment the AdDBP and the 39-kDa fragment were dialyzed and centrifuged briefly to remove slight precipitates that might interfere during the measurement. The concentrations of poly(rA) and AdDBP were determined spectrophotometrically with the following extinction coefficients: $\epsilon_{260} = 9400$ M^{-1} cm^{-1} [poly(rA)] and $\epsilon_{280} = 56600$ M^{-1} cm^{-1} (AdDBP). The poly(rA) concentration ranged from 20 to 35 μM in all experiments described. The concentration of the 39-kDa fragment was calculated with the same molar extinction coefficient as for AdDBP since the number of tryptophan and tyrosine residues is the same.

The spectroscopic methods used in this work are described in detail (Scheerhagen et al., 1986a,b; van Amerongen et al., 1987). The binding parameters were determined according to the theory of McGhee and von Hippel (1974), which relates the saturation of a polynucleotide lattice (νn) to the values of the intrinsic binding constant $K_{int} = K\omega$, the cooperativity parameter ω, and the size of the binding site n. This theory assumes binding to an infinite lattice in a single binding mode; i.e., every protein binds in a similar fashion to the polynucleotide. CD spectroscopy was used to determine the fractional saturation of the polynucleotide with protein. A CD difference spectrum was obtained by subtracting the polynucleotide and the protein spectra weighed by their respective concentrations during the experiment. As the shape of the obtained difference spectrum does not change, one may conclude that only one binding mode is observed. To calculate the actual saturation, we assumed a maximum change in the difference spectrum and calculated for every protein to polynucleotide ratio the fractional saturation. Best fits were obtained by visual inspection. In general, a variation of the best-fit parameters with more than 20% resulted in fits that clearly deviated from the measured data points. An illustration of this is given in Figure 5. The indicated errors for the values of K, n, and ω reflect the additional inaccuracy due to errors

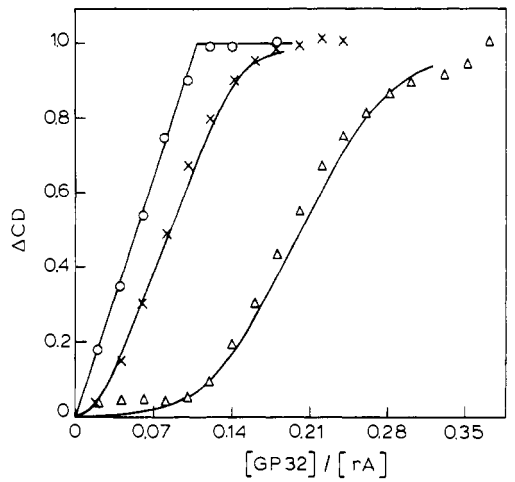

FIGURE 1: GP32 titration curves measured with CD. ΔCD is the change in CD relative to the change corresponding to saturation (νn in the McGhee–von Hippel formalism). The values of R = [protein]/[rA] are shown along the horizontal axis. The CD signal was averaged for several minutes at 260 nm and digitized. The experiments were performed in buffer B containing (O) 50, (×) 350, and (Δ) 450 mM NaCl, T = 20 °C. The drawn lines for (×) and (Δ) were calculated according McGhee and von Hippel with the parameters given in the text. The curve for (O) was drawn assuming stoichiometric binding yielding a value of 9.3 ± 0.5 nucleotides covered per protein monomer.

in the estimate of the end point of the titration.

RESULTS

Cooperativity As Determined from CD Spectroscopy. To extract information about the binding parameters K_{int}, n, and ω, we employed CD difference spectra measured at different salt concentrations and different polynucleotide to protein ratios. In order to test this method, we first studied the salt dependence of the binding of GP32 to poly(rA), a process for which data have previously been obtained (Kowalczykowski et al., 1981a,b) from protein fluorescence and polynucleotide absorption changes due to binding. In Figure 1 we show titration curves of poly(rA) with GP32 at three different salt concentrations: 50, 350, and 450 mM NaCl, respectively. The various curves reflect the salt dependence of K_{int} as described before (Kowalczykowski et al., 1981b). The binding in 50 mM NaCl is stoichiometric and allows for the calculation of the size of the binding site, n = 9.3 ± 0.5 nucleotides/protein. This is somewhat larger than the value found by Kowalczykowski et al. (1981b) using absorbance difference spectroscopy but in close agreement with the results obtained by Bobst et al. (1982), Scheerhagen et al. (1986a), Kuil et al. (1988), and recently Watanabe (1989). For 350 and 450 mM NaCl typical sigmoidal titration curves are obtained indicative of a strong cooperative binding process. Using the theory given by McGhee and von Hippel (1974), we calculate values for $K_{int} = K\omega$ of 1.6×10^6 M^{-1} at 350 mM NaCl and 3.4×10^5 M^{-1} at 450 mM NaCl. These values compare rather well with those found by Kowalczykowski et al. (1981b): 1.7×10^6 M^{-1} and 2.5×10^5 M^{-1} in 350 and 450 mM NaCl, respectively. With n = 9.3 we calculate from our titration curves that ω = 375 and 750 at 350 and 450 mM NaCl. These numbers are smaller than those given by Kowalczykowski et al. (1981b): 2×10^3 at 350 mM NaCl and 2.5×10^3 at 450 mM NaCl. However, it should be realized that it is not possible to obtain the value of ω independent from the value of n. We have used n = 9.3, and Kowalczykowski et al. used n = 7. Our best estimates for K and ω, using n = 7 instead of n = 9.3, are $K = 4 \times 10^6$ M^{-1} and $\omega = 2 \times 10^3$ for GP32 binding to poly(rA)

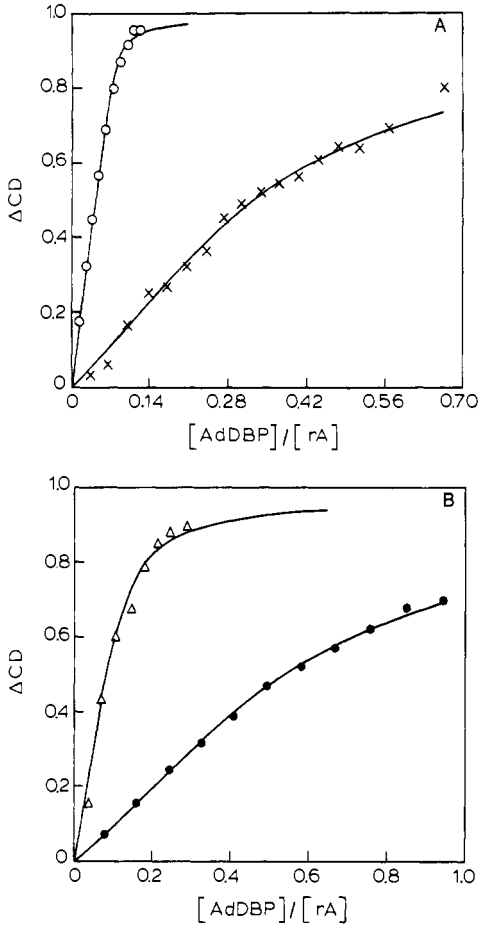

FIGURE 2: (A) AdDBP titration curves measured with CD. Axis is as in Figure 1. The lower curve was obtained in buffer A plus 210 mM NaCl, whereas the upper (steep) curve was obtained in buffer A plus 50 mM NaCl. The drawn lines were calculated according to the McGhee–von Hippel theory with the parameters given in Table I. (B) As in (A). Upper curve, 100 mM NaCl; lower curve, 250 mM NaCl.

in 350 mM NaCl. In 450 mM we obtain values of 4.4×10^5 M^{-1} and 1.5×10^3 for K and ω, respectively. The size of the binding site and the cooperativity factor ω are parameters with opposite effects in the fit of titration curves (Kowalczykowski et al., 1986), and therefore, this discrepancy should not be taken too seriously in our opinion. We note the striking similarity between our titration curves in Figure 1 and those reported earlier by Kowalczykowski (1981b) using absorption measurements. In conclusion, CD spectroscopy is a valuable technique for measuring the binding parameters.

Salt Dependence of AdDBP Binding to Poly(rA). For the AdDBP protein the binding to poly(rA) can be followed with CD spectroscopy (van Amerongen et al., 1987). The changes in the CD spectrum are similar to those observed upon the binding of GP32 but less intense. The poly(rA) CD changes were monitored carefully upon titration of AdDBP at 50, 100, 210, and 250 mM NaCl. In Figure 2A the titrations at 50 and 210 mM NaCl are given. Figure 2B shows the titrations at 100 and 250 mM NaCl. The experiment in 50 mM NaCl clearly leads to the saturation of the DNA lattice with AdDBP, and the binding occurs almost stoichiometrically, more or less in agreement with earlier work (van Amerongen et al., 1987). From Figure 2A it can be observed that saturation of poly(rA) with AdDBP is difficult to achieve at 210 mM NaCl with moderate amounts of protein. Both titrations can be analyzed with the McGhee and von Hippel theory, and the best fit parameters are given in Table I. Both curves are fit with a relatively low value for the cooperativity parameter, $\omega \approx$ 20–30,

Table I: Salt Dependence of the Binding of AdDBP and the 39-kDa Fragment to Poly(rA)

[NaCl] (mM)	K (M^{-1})	n	ω	norm
50[a]	$\approx 5 \times 10^5$	13	25	0.95
100[a]	3.8×10^4	13	25	0.92
210[a]	5.8×10^3	13	25	0.80
250[a]	3.3×10^3	13	25	0.70
50[b]	9×10^4	13	25	0.93

[a] AdDBP binding. [b] 39-kDa fragment binding.

in agreement with an earlier indication of low cooperativity (van der Vliet et al., 1978; Schechter et al., 1980). The binding constant at 50 mM NaCl, $K = 5 \times 10^5$ M^{-1}, is not extremely high, and its value has decreased significantly at 210 mM NaCl. Direct comparison of the titrations in Figure 2A with that in Figure 1 shows that even at higher salt concentrations GP32 saturates the poly(rA) lattice much easier than AdDBP, also indicative of a low cooperativity and/or a low binding constant for AdDBP.

The two additional titrations in Figure 2B in 100 and 250 mM NaCl show essentially the same behavior as the experiments described in Figure 2A. Note the large amounts of AdDBP required to achieve only a partial covering of the polynucleotide in 250 mM NaCl. The drawn curves represent fits from the McGhee and von Hippel theory with the parameters given in Table I.

Table I shows that a reasonable fit to all the titration curves can be obtained with a single value for the size of the binding site, $n = 13$, and a single value for the cooperatively parameter, $\omega = 25$. Individual titration curves could be fitted with a variety of parameter fits, but n always ranged between 11 and 15 and ω between 20 and 30. Within the resolution of these experiments no salt dependence of ω could be detected, and therefore the salt dependence of K_{int} can largely be attributed to changes in K. A similar conclusion was drawn for GP32 (Kowalczykowski et al., 1986) and for the gene V protein of bacteriophages fd and M13 (Bulsink et al., 1989). The last column of Table I gives the saturation of the DNA lattice at the final titration point, and this clearly shows that the lattice is more difficult to saturate if K_{int} is low.

The dependence of log ($K\omega$) on log [NaCl] is indicative of the number of ionic interactions involved in the binding of AdDBP to poly(rA). This follows directly from the expression derived by Record et al. (1976) and presented in a simplified form by Kowalczykowski et al. (1981b):

$$\frac{-\partial \log (K\omega)}{\partial \log [\mathrm{NaCl}]} = m'\psi + a$$

where m' is the number of ionic interactions between nucleotide phosphates and basic protein residues, ψ is the (thermodynamic) fraction of the phosphate change neutralized in the free nucleic acid by counterion condensation, and a is the number of independent anion binding sites per protein. We calculate a value of -3.1 ± 0.3 for $m'\psi$, which yields a maximum number of ionic interactions of four, using $\psi = 0.78$ (Record et al., 1976). If the displacement of anions from the protein plays a minor role in the binding of AdDBP, then the number of ion parts involved in the binding is the same as for GP32–polynucleotide binding.

Binding Properties of the C-Terminal Part of AdDBP. The DNA-binding properties of AdDBP as well as the capacity to sustain DNA replication are located in the 39-kDa conserved C-terminal part between residues 174 and 525. The 39-kDa fragment was isolated by limited chymotrypsin digestion, and its complex formation with poly(rA) was compared to that of intact DBP.

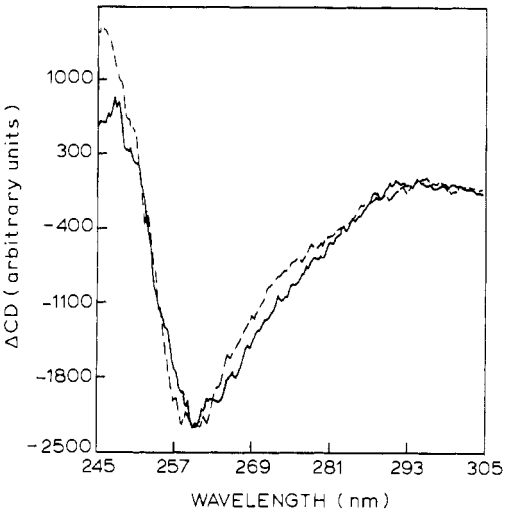

FIGURE 3: CD difference spectra of AdDBP and the C-terminal part complexed with poly(rA). The drawn curve is the spectrum of poly(rA) complexed with AdDBP in buffer A plus 50 mM NaCl. [poly(rA)] = 23.1 μM; [AdDBP] = 2.7 μM. The dashed line is the spectrum obtained when poly(rA) was titrated with the C-terminal part of the AdDBP protein in buffer A plus 50 mM NaCl ($R = 0.20$, $T = 14$ °C). [poly(rA)] = 12.7 μM; [AdDBP] = 3.8 μM.

In Figure 3 we show the difference spectra of poly(rA) complexes with AdDBP and with the 39-kDa fragment corrected to the same poly(rA) concentration. The aromatic part of the CD difference spectrum (250–300 nm) is almost identical for both complexes, indicating that at least the local DNA structures imposed by the binding of either AdDBP or the 39-kDa fragment are probably very similar. Differences between the two spectra are observed at shorter wavelengths. This may indicate a difference in protein conformation in the complex, due to either a structural change in the C-terminal fragment or a contribution of the N-terminal part of the protein. However, it should be noted that the CD spectra below 250 nm should be interpreted with some care due to the steep changes in the protein absorption in this region.

Figure 4A shows the consecutive CD difference spectra recorded upon titration of poly(rA) with the 39-kDa fragment. The changes are spectrally very similar at different protein to nucleotide ratios, indicating the existence of only a single binding mode, analogous to the same experiment with AdDBP and poly(rA) by van Amerongen et al. (1987). Figure 4B shows the OD difference spectra recorded during the same titration experiment. The spectra in Figure 4B were corrected for light scattering by use of data points in the 350–420-nm region as described by Kuil et al. (1988). The OD difference spectra are again comparable to those obtained by van Amerongen et al. (1987) for the intact AdDBP, although a direct comparison is somewhat difficult since in the latter case spectra were not corrected for scattering. It should be noted that in many of the samples a considerable amount of scattering was observed that seriously interfered with the comparison between spectral shapes. Especially, the wavelength region 240–250 nm is prone to large uncertainties in the OD difference spectra as the wavelength dependence of the scattering may change during experiments with nonstoichiometrically binding ligands. Monitoring the binding with CD spectroscopy seems less influenced by light scattering, and the binding of AdDBP with poly(rA) induces large changes in the polynucleotide CD spectrum.

A titration experiment with the 39-kDa fragment of the AdDBP was performed at 50 mM (see Materials and Methods), and the results are shown in Figure 5. Analysis of these

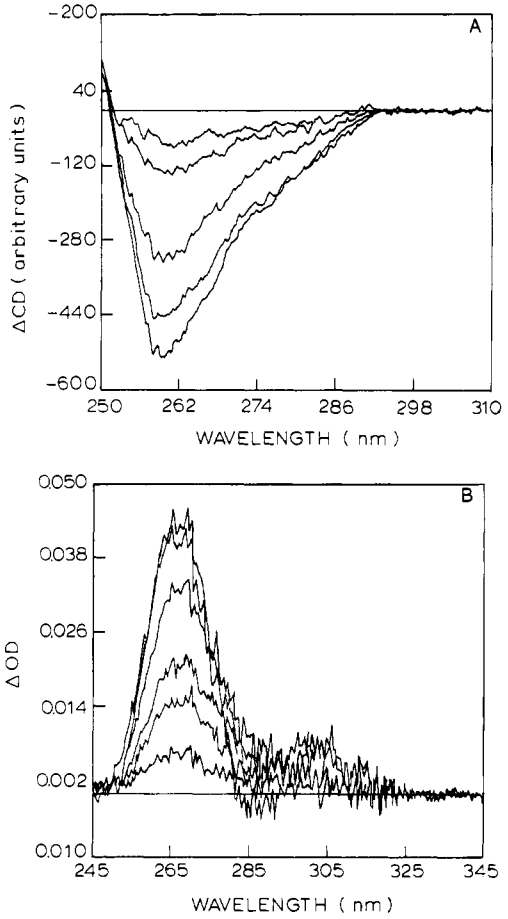

FIGURE 4: (A) CD difference spectra of the C-terminal part of the AdDBP protein with poly(rA). R = 0.016, 0.035, 0.086, 0.12, and 0.30, respectively; T = 14 °C. (B) OD difference spectra recorded during the same experiment. R = 0.016, 0.035, 0.054, 0.086, 0.174, and 0.235, respectively.

data in terms of K, n, and ω suggested that the titration curve can be described with the same values of n = 13 and ω = 25; values comparable to those of the complete protein (see Table I). A reduced affinity for poly(rA) was observed ($K \approx 9 \times 10^4$ M^{-1}), indicated by the less stoichiometric binding curve obtained. For illustration purposes, fits with $K = 7 \times 10^4$ and 11×10^4 M^{-1} have been added (lower and upper curves in Figure 5, respectively).

Discussion

The size of the binding site obtained in this work is somewhat larger than the values obtained earlier by van Amerongen et al. (1987). The main difference is that the value n = 13 consistently fits the titration experiments of poly(rA) with AdDBP at all salt concentrations. At 50 mM NaCl the deviation from stoichiometric binding is not large and difficult to detect. In fact, the value of n obtained from these experiments is rather close to that found by van Amerongen et al. (1987) for poly(dT) titration with AdDBP. If poly(dT) binding of AdDBP is relatively strong as compared to poly(rA) binding, then stoichiometric binding and thus a larger value of n can be expected. We note that also in the analysis used here the n values represent lower bounds, since any inactive protein contributes to the total protein concentration used in the McGhee and von Hippel formalism, but not to the binding.

None of the spectroscopic measurements at the various salt concentrations shows any evidence for the existence of more than one binding mode. Although the spectral changes due to AdDBP binding are very similar to those due to *Escherichia*

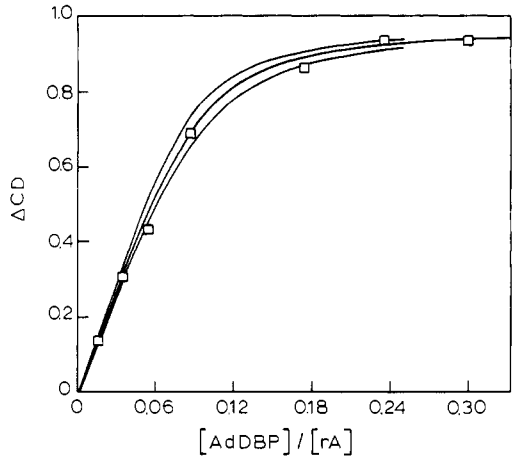

FIGURE 5: Titration of the C-terminal part of the AdDBP protein with poly(rA). Axis is as in Figures 1 and 2. The parameters used to calculate the drawn line are given on the last row of Table I, for the best fit. The higher and lower curves were calculated with a value of 11×10^4 and 7×10^4 M^{-1} for K, the same values for n and ω, being used.

coli SSB binding to poly(rA), the former can all be fitted with a single binding process, while for the latter at least two binding modes have been described (Lohman & Overman, 1985).

The cooperativity parameter is low compared to the value reported for GP32 ($\omega \approx 10^3$) but in fact rather similar to the cooperativity reported for the binding of *E. coli* SSB, ω = 50, in the n = 65 binding mode (Lohman et al., 1986). High cooperativity does not seem to be a primary requirement for a good single-stranded DNA- or RNA-binding protein. Some retroviral single-stranded DNA/RNA-binding proteins show a reasonably high affinity for poly(rεA) and poly(rA) without significant cooperativity (Karpel, 1987). However, gel retardation experiments indicate that AdDBP is able to form saturated complexes with single-stranded polynucleotides (M. H. Stuiver and P. C. van der Vliet, submitted for publication).

The salt dependence of the AdDBP binding protein constant to poly(rA) is low, $\partial \log (K\omega)/\partial \log [NaCl] = -3.1$, compared to GP32 binding to polynucleotides (Kowalczykowski et al., 1981). However, in the case of GP32 a large part of the salt dependence of $K\omega$ is due to anion binding of the protein, which is lost upon complex formation. For AdDBP, this anion binding may be absent or rather weak, and in that case, the salt dependence reflects more or less the number of ionogenic interactions that contribute to a stable complex. For AdDBP this would be at most four, but maybe less. It is not unlikely that the same number applies to AdDBP as to GP32 in view of the strong structural similarities of the complexes (van Amerongen et al., 1987; van Amerongen et al., unpublished data).

The spectral changes observed in both OD and CD spectra after binding of the 39-kDa fragment to poly(rA) are very similar to those induced by the native protein. Also, the binding stoichiometry and cooperativity parameter are very similar for the 39-kDa fragment. These observations strongly support the idea that the N-terminal part of AdDBP is involved in neither DNA binding nor the cooperative interaction among different AdDBP molecules. In addition, it seems likely that the structure of the poly(rA) in the complex with the 39-kDa fragment is rather similar to that proposed for the intact AdDBP protein. These conclusions are in agreement with the finding that the 39-kDa fragment is capable of efficiently stimulating in vitro replication, both of the original double-stranded DNA and of the displaced strand (Leegwater et al., 1988). The changes in the aromatic part of the protein CD

in the 39-kDa fragment as compared to the integral AdDBP indicate that in the 39-kDa fragment some structural changes have occurred, which do not directly affect the DNA-binding site of the 39-kDa fragment.

In conclusion, AdDBP binding to poly(rA) is characterized by a weak cooperativity (ω = 20–30) and a moderately strong, but salt-dependent binding constant. Quantitative analysis of the titration curves has shown that n = 13 is a better estimate for the size of the binding site than n = 9–11 (van Amerongen et al., 1987), although this number must still be considered a lower limit (Scheerhagen et al., 1986b). Binding of AdDBP to poly(dT) is probably very strong, yielding a close to stoichiometric binding and a "maximal" size of the binding site, n = 15 (van Amerongen et al., 1987), rather similar to the improved value of n = 13 for binding to poly(rA). Thus, our work indicates that the binding of AdDBP to poly(dT) is preferred over poly(rA), in strong analogy with other single-stranded DNA binding proteins [SSB and GP32 (Chase & Williams, 1986) and gene V protein (Bulsink et al., 1989)].

We propose, again in analogy to other single-stranded DNA binding proteins, that for AdDBP binding the values of ω, n, and $\partial \log (K\omega)/\partial \log$ [NaCl] are rather similar for the binding to other deoxy- and ribonucleotides, maybe with the exception of poly(dT). The value of ω is probably less dependent on the specific polynucleotide than on the properties of the complexed protein, and the value of n is determined by the "length" of the binding site, assuming an extended (local) structure of the polynucleotide. Although the thermodynamic fraction of the phosphate charges neutralized in the free nucleic acid by counterion condensation is somewhat variable for the various polynucleotides (Record et al., 1976), it must be expected that m', the number of ionic interactions formed between the nucleotide phosphates and basic protein residues as a consequence of the interaction, is a constant. Only the actual binding constant varies, and this probably determines any preference in the binding process.

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