Biophysical Chemistry 3 (1975) 255–262

BASIC POLYPEPTIDES AS HISTONE MODELS: CIRCULAR DICHROISM OF COMPLEXES OF MODEL POLYPEPTIDES WITH DNA

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Received 23 December 1974
Revised manuscript received 3 March 1975

Circular dichroism (CD) was used to study the complexes of DNA (in 0.15M NaCl) with two polypeptides considered as models of the histone molecules. CD spectra in the region of DNA absorption were studied with respect to the concentration used for annealing and to the molecular weight and composition of the DNA used. The properties of supernatants after centrifugation of aggregated complexes were examined. The effect of selectively bound antibiotics (actinomycin D and netropsin) on CD spectra of complexes was investigated. The induced CD of proflavine molecules bound to DNA in the various complexes was also studied. It was concluded that changes in the CD spectra of DNA in complexes with the polypeptides are due to the formation of chiral superstructures, even if some conformational changes of DNA molecules themselves may also be decisive in some cases. The superstructure is affected by the composition of DNA, the role of (G + C) rich segments being particularly important.

1. Introduction

Previous reports have described syntheses and properties of some lysine-containing polypeptides [1,2]. It has been argued that polypeptides containing lysine in amounts comparable to the amount of basic amino acids in histones might represent suitable models of these proteins. Of the compounds prepared, the sequential polymer (Ala–Lys–Pro)$_n$·HCl (referred to below as (ALP)$_n$) and the statistical copolymer (Lys$_{30}$, Ala$_{70}$)$_n$·HCl (referred to below as (L, 2A)$_n$) were selected to make a detailed study of complexes with DNA in comparison to reconstituted nucleohistones. Both polymers also resembled histones with respect to molecular weights in the range of those of histone chains (i.e. 10 000–20 000 daltons) [1,2].

The amino-acid composition of (ALP)$_n$ corresponds to the major part of the F1 histone chain. The polymer also resembles F1 histone in displaying no tendency to form ordered conformations [1]. On the other hand (L, 2A)$_n$ with nearly the same lysine content, shows under comparable conditions an α-helical and β conformation [2]. The ability to form an ordered conformation is apparently due to the absence of typical helix-breaking amino-acid residues, such as proline, but also to the presence of alanine clusters. In this respect the copolymer resembles those histones (e.g. F2a1 or F2b) which contain sequences of hydrophobic residues [3] in some parts of their chains.

The CD spectra of equilibrium complexes of these two polypeptides with DNA in physiological ionic strength (0.15M NaCl) differ dramatically [4]. The CD pattern may be compared to those of nucleohistones reconstituted from DNA and the corresponding histones. In agreement with the interpretation of CD spectra of nucleohistones [5] the view was presented that each of the model polypeptides affects the conformation of DNA in a different manner [4,6].

More detailed examination, however, suggests that the DNA CD spectra in complex aggregate systems are not defined only by DNA conformation. Possible sources of apparent circular dichroism in similar systems have recently been discussed and theoretical as well as experimental procedures of analysis have been suggested (for references see [7,8]). In the present paper no attempt is made to differentiate possible contributions of various mechanisms to the observed CD pattern. The present approach to the problem was to analyze the changes of the CD spectra observed on (1) changing some parameters of components (concentra-

tion, molecular weight and composition of DNA) which govern the aggregation behaviour of complexes, (2) addition of selectively binding ligands (actinomycin D, netropsin) which could interfere with aggregation, and (3) following induced CD spectra of bound dye which could reflect some properties of aggregates.

2. Material and methods

The polypeptides and DNA were the same preparations used before [1,2,9]. Low molecular-weight DNA was prepared by sonication on an MSE ultrasound generator after bubbling nitrogen through the solution. Measurement of sedimentation coefficients and molecular weights of DNA has been described elsewhere [1,2]. The DNA concentration was calculated from optical density at 260 nm on the basis of $A^{1\%}_{1\,cm} = 200$.

The actinomycin D used was supplied by Calbiochem and its concentration was calculated from absorbance at 425 nm. Netropsin was obtained from the Zentralinstitut für Mikrobiologie und experimentelle Therapie in Jena (by courtesy of Dr. Ch. Zimmer), proflavine hydrochloride was supplied by K and K Laboratories, USA. Their concentrations were calculated on a weight basis.

2.1. Preparation of the complexes

The solution of polypeptide hydrochloride and DNA, at a given molar ratio of lysine residues to nucleotides (L/DNA) in 2M NaCl buffered with 0.013M sodium phosphate (pH 6.8) was dialyzed against a linear molarity gradient of NaCl. The arrangement described by Carroll [10] was used with procedural details described elsewhere [1]. After flow dialysis, the complex solution was dialyzed for 15–18 h against 0.15M NaCl plus sodium phosphate.

The DNA fraction in the aggregated complex (f_{ppt}) was determined by centrifugation of the sample and measuring the optical density of the supernatant at 260 nm. Centrifugation was done in three different ways: (1) in a refrigerated laboratory centrifuge at 10 000 rev/min (about 8000 g) for 30 min, (2) in the preparative Spinco L50 centrifuge in no. 40 rotor at 20 000 rev/min (about 30 000 g) for 30 min, (3) in the same rotor and centrifuge at 27 000 rev/min (about 50 000 g), for 30 min.

2.2. Binding of antibiotics and proflavine

Binding data were obtained spectrophotometrically with the use of 5 cm light-path cells in a VSU-2 spectrophotometer (Zeiss, Jena). The titration was carried out by adding 10 µl or 20 µl aliquotes of an antibiotic or proflavine in buffered 0.15M NaCl in parallel to the solvent (0.15M NaCl + 0.013M phosphate) and to solutions of DNA and DNA–polypeptide complexes in the same solvent ($1.1–1.4 \times 10^{-4}$M in nucleotide bases). To correct for the light scattering contribution the same solutions of DNA or respective complexes were used as blanks.

The average number r of the ligand molecules bound per nucleotide and the free ligand concentration C_f were calculated [11] from the difference in absorbancies of the respective solution and the solvent containing the same total amount of the ligand [12]. Absorbancies at 425, 320 and 440 nm were read for actinomycin, netropsin and proflavine, respectively. The values of molar extinction coefficients of free and bound ligands were taken from the literature [11–13].

2.3. Circular-dichroism measurements

The circular-dichroism (CD) spectra were measured with a Roussel Jouan Dichrograph, CD 185, at 22–24°C, in 0.5, 1 and 5 cm cells. Circular dichroism was expressed in terms of specific ellipticity $[\psi]$ (deg·cm^2·dg^{-1}) and was not corrected for refraction index of the solvent. The $[\psi]$ values refer to the total DNA concentration, unless stated otherwise, and can be converted to $[\theta]$ by simple multiplication with 3.3.

3. Results

3.1. Effect of concentration and molecular weight of DNA on CD spectra

Previous reports [1,2,4] have dealt with the CD spectra of $(ALP)_n$–DNA and $(L, 2A)_n$–DNA complexes. In both cases, the absolute value of the specific ellipticity of the DNA absorption band near 260 nm increased as a function of L/DNA ratio reaching very high values. In

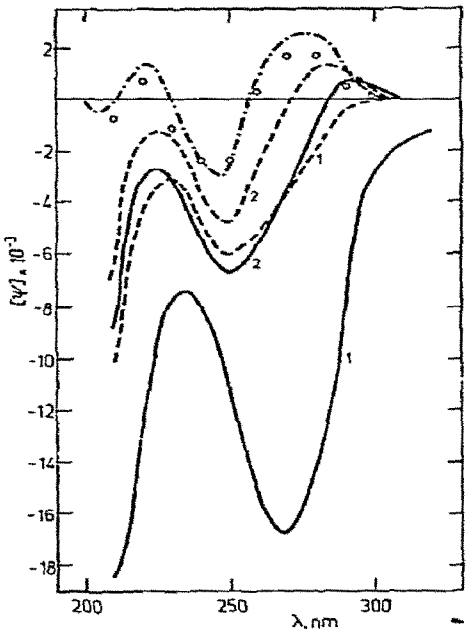

Fig. 1. CD spectra of complexes $(ALP)_n$–DNA, L/DNA = 0.5. –·–·– DNA; ––––– $(ALP)_n$–DNA, M_{DNA} = 13 × 10^6; ——— $(ALP)_n$–DNA, M_{DNA} = 6 × 10^5. Curves 1 c_{DNA} = 60 μg/ml, curves 2 c_{DNA} = 6 μg/ml. ○○○ Supernatant of the $(ALP)_n$–DNA complex, c_{DNA} = 6 μg/ml, after centrifugation C (see sect. 2).

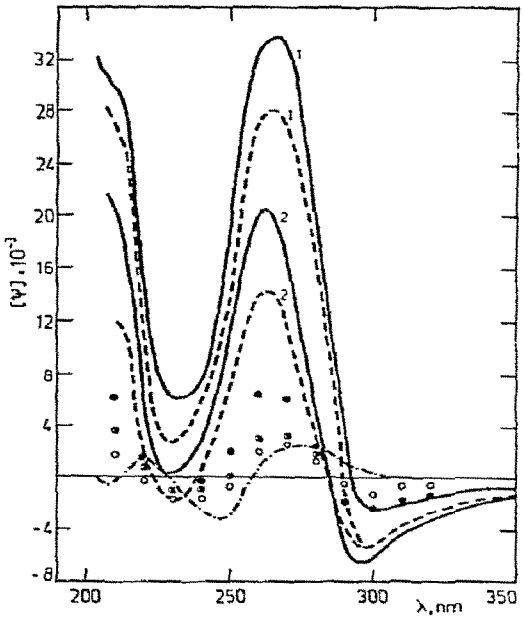

Fig. 2. CD spectra of complexes $(L,2A)_n$–DNA, L/DNA = 0.5. –·–·– DNA; ––––– $(L,2A)_n$–DNA, M_{DNA} = 13 × 10^6; ——— $(L,2A)_n$–DNA, M_{DNA} = 6 × 10^5. Curves 1 c_{DNA} = 60 μg/ml, curves 2 c_{DNA} = 6 μg/ml. Supernatants of the complex $(L,2A)_n$–DNA, c_{DNA} = 6 μg/ml, after centrifugation A (●●●), B (◑◑◑), and C (○○○) (see sect. 2).

this spectral region the CD curves of the two complexes are approximately mirror images, the $(L,2A)_n$–DNA complex having in addition a band at about 300 nm.

In order to investigate the effect of aggregation the dependence of the CD pattern on the initial complex concentration (i.e., the concentration of components during annealing) and on the molecular weight of DNA used was analysed. From the results in figs. 1 and 2 it can be seen that both parameters influenced the CD spectra of complexes. A decrease in complex concentration by one order of ten resulted in a substantial decrease of the band intensity, more so with $(ALP)_n$–DNA than with $(L,2A)_n$–DNA. On lowering the molecular weight of DNA used for annealing, an increase of the band ellipticity of the complexes was found in all cases, but different ellipticity changes were observed depending on the starting concentration, especially with $(ALP)_n$–DNA. At the higher starting concentration (60 μg DNA/ml, curves 1) a decrease in the molecular weight of DNA resulted in an increase of intensity of the 260 nm band by about 70% with $(ALP)_n$–DNA but by only 20% with the $(L,2A)_n$–DNA. At the lower complex concentration (6 μg DNA/ml, curves 2) the effect of DNA degradation was smaller, and of similar magnitude with both complex types.

The dependence of aggregate size on starting concentration is shown in table 1. From solution of the complexes prepared at the higher starting concentration (60 μg DNA/ml) about 50% DNA can be removed in the form of aggregates using low-speed centrifugation. If complexes obtained at a lower concentration are used (6 μg DNA/ml) the same centrifugation will remove only 5–7% DNA. However, even in this case as much as 50% DNA can be removed from the solution by sedimentation at higher speeds (centrifugation 1 and 2). Fig. 1 shows further that in the case of $(ALP)_n$–DNA the CD spectrum of supernatant C, which may be assumed to contain practically no

Table 1
DNA fraction removed with the aggregated complex in the course of differential centrifugation; L/DNA = 0.5.

Centrifugation			$(ALP)_n$–DNA		$(L,2A)_n$–DNA	
no.	m(g)	t(min)	6 μg/ml	60 μg/ml	6 μg/ml	60 μg/ml
1	8000	30	0.05	0.48	0.07	0.42
2	30 000	30	0.45	–	0.35	–
3	50 000	30	0.54	–	0.61	–

aggregate, differed only very little from the CD spectrum of DNA. On the other hand, fig. 2 shows that with $(L,2A)_n$–DNA the intensity of the positive band in the supernatant CD spectrum decreased with increasing centrifugation field, but with no change in band position. The spectrum of supernatant 3 differed in this case clearly from that of a DNA solution in the exact position of bands, and by the presence of a long wave-length negative band. This indicates the presence of a nonaggregated 'soluble' complex with a CD spectrum different from that of DNA.

3.2. Effect of DNA composition on CD spectrum

Fig. 3 shows the dependence of CD spectra on the composition of DNA used in the $(ALP)_n$–DNA complex formation. Band intensity increased with the (G + C) content, but the general character of the spectrum remained the same. A similar, somewhat less pronounced, dependence was found also with the $(L,2A)_n$–DNA complex (fig. 4). The molecular weights of the DNA preparations used in those experiments were low, in a range in which molecular weight has little effect on CD spectrum of the complexes [1], except for the Streptomyces chrysomallus DNA, with a very high (G + C) content. Fig. 3 shows the CD spectra of $(ALP)_n$–DNA with two DNA preparations of this origin with different molecular weights. The spectrum of the complex containing high molecular-weight DNA fits well into the ascending dependence of the DNA band ellipticity on (G + C) content. The CD spectrum of the complex of the low molecular-weight DNA has its maximum red-shifted and is altered particularly in the range of the short wave-length band. It would appear that increasing particle size has a considerable effect on CD spectra [12]. Figs. 3 and 4 show that a high molecular weight

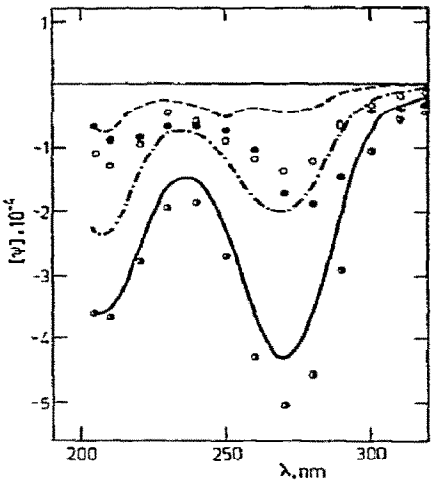

Fig. 3. CD spectra of $(ALP)_n$–DNA complexes as a function of DNA composition, L/DNA = 0.5, c_{DNA} = 60 μg/ml. ——— crab poly d(A–T)·poly d(A–T), 3% (G + C), $M_{DNA} = 0.9 \times 10^6$; ○○○ Staphylococcus aureus DNA, sonicated, 30% (G + C), $M_{DNA} = 1.1 \times 10^6$; – – – – calf thymus DNA, 42% (G + C), $M_{DNA} = 6.8 \times 10^6$; ——— calf thymus satellite DNA, 55–60% (G + C), $M_{DNA} = 2.7 \times 10^6$; ●●● DNA Streptomyces crysomallus, 70% (G + C), $M_{DNA} = 11.5 \times 10^6$; ●●● DNA Streptomyces chrysomallus, sonicated, 70% (G + C), $M_{DNA} = 0.6 \times 10^6$.

does not suppress the formation of structures characterised by high band intensities if the DNA used has a high (G + C) content. Fig. 4 also shows the gradual disappearance of the negative band at 300 nm with increasing (G + C) content.

3.3. Interaction with antibiotics

Actinomycin D (At) and netropsin (Nt) bind strongly to DNA and display a marked binding specificity for (G·C) and (A·T) respectively [12–15]. From the Scatchard plots of the binding data (fig. 5) for both antibiotics it follows that the binding of DNA proceeds unabated even in complexes with polypeptides, i.e. polypeptides and antibiotics do not compete for DNA binding sites. The apparent number of bases per binding site $(B_{app})^{-1}$ is under present conditions about 40 for actinomycin and 33 for netropsin and does not change with polypeptide binding at least within the limits of experimental error. A more detailed study of binding properties of complexes will be published [16].

The binding of netropsin altered the CD spectrum of

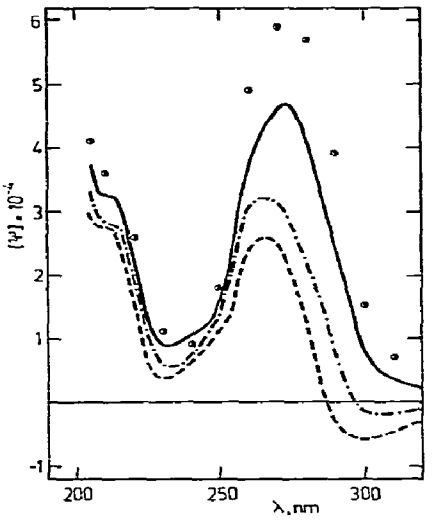

Fig. 4. CD spectra of $(L,2A)_n$–DNA complexes as a function of DNA composition, L/DNA = 0.5, c_{DNA} = 40–60 μg/ml; DNA as in fig. 3.

DNA and gave rise to the induced CD of netropsin itself in the 300–350 nm region (fig. 6, dot-and-dash curve). Furthermore, fig. 6 shows the spectra of both types of complexes in the course of titration with netropsin. Some modification of the spectra occurred mainly in the region of the induced CD of netropsin. In the region of DNA absorption bands the character of the spectra did not change with an increasing Nt/DNA ratio up to 0.75. Even then the pattern of the two complexes differed substantially from each other and also from that of the Nt–DNA complex. It follows that binding of netropsin to (A + T)-rich sites of DNA does not substantially alter the specific aggregation properties of the complexes.

In fig. 7 an analogous dependence of the CD pattern of complexes on increasing ratio of actinomycin to DNA (At/DNA) is shown. Although the maximum concentration of actinomycin D used was much lower than that of netropsin, the changes observed in the CD spectra of the complexes in the range of DNA absorption were more significant. Fig. 7 shows that at an At/DNA molar ratio of 0.20 the CD spectrum of At–$(ALP)_n$–DNA resembled that of At–DNA under similar conditions in general character and in band intensity. On the other hand, the CD spectrum At–$(L,2A)_n$–DNA retained its typical character even

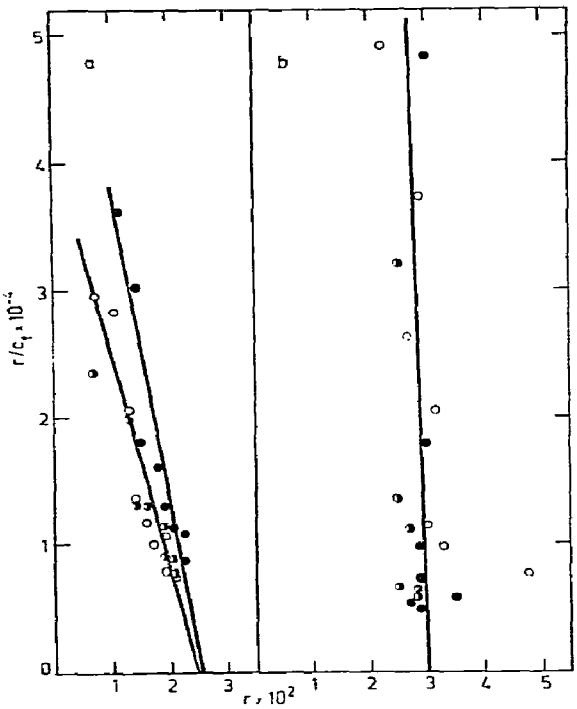

Fig. 5. Scatchard plots for the binding of (a) actinomycin D, (b) netropsin to DNA and DNA–polypeptide complexes. ○ DNA, ● $(ALP)_n$–DNA, ◑ $(L,2A)_n$–DNA.

at At/DNA = 0.20, although with decreased intensity (fig. 7). The results suggest that binding of actinomycin D to the (G + C)-rich sites of DNA alters aggregation properties more than binding of netropsin, in particular for $(ALP)_n$–DNA, and that At is much more effective at much lower concentrations.

3.4. Induced CD of bound proflavine

The binding of proflavine to DNA and polypeptide–DNA complexes is of a different type than that of the antibiotics. The binding of proflavine is not sensitive to DNA composition [11] and the binding of polypeptides to DNA substantially lowers the binding of proflavine, probably due to shielding of some of the DNA binding sites by polypeptides [16].

Fig. 8 shows the CD spectra of proflavine (Pf) bound to DNA and to polypeptide–DNA complexes in the long wave-length absorption band which does not interfere with the CD of DNA (specific ellipticity was related to the concentration of the bound dye). Only a

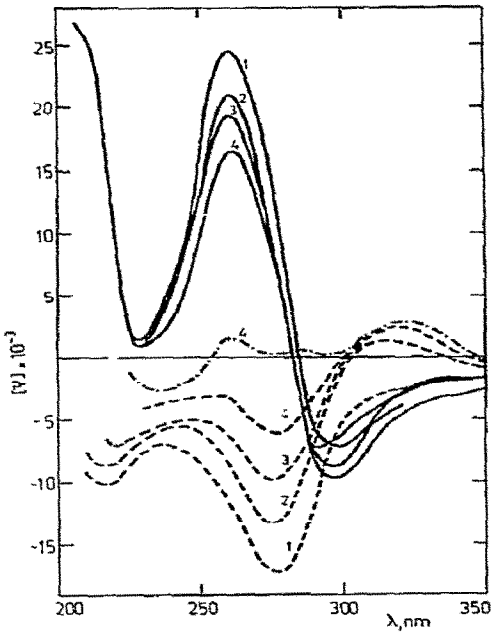

Fig. 6. CD spectra of complexes titrated with netropsin (Nt). $M_{DNA} = 6 \times 10^6$. –·–·– Nt-DNA, ––––– Nt–$(ALP)_n$–DNA, L/DNA = 0.5; ——— Nt–$(L,2A)_n$–DNA, L/DNA = 0.5; 1: Nt/DNA = 0; 2: Nt/DNA = 0.05; 3: Nt/DNA = 0.25; 4: Nt/DNA = 0.73.

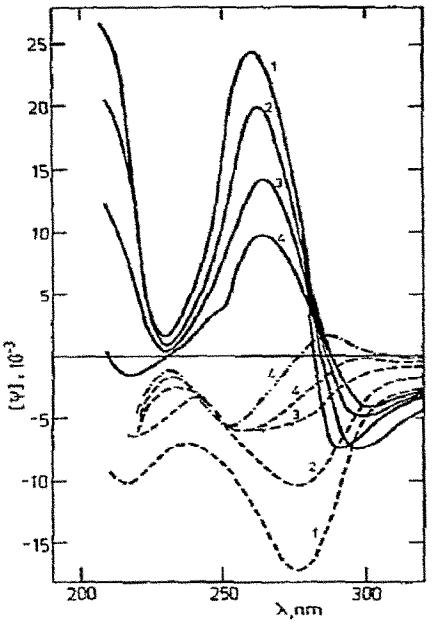

Fig. 7. CD spectra of complexes titrated with actinomycin D (At). $M_{DNA} = 6 \times 10^6$. –·–·– At-DNA; ––––– At–$(ALP)_n$–DNA, L/DNA = 0.5; ——— At–$(L,2A)_n$–DNA, L/DNA = 0.5; 1: At/DNA = 0; 2: At/DNA = 0.01; 3: At/DNA = 0.06; 4: At/DNA = 0.20.

small quantity of Pf was added (Pf/DNA = 0.04) to allow us the assumption that its binding does not substantially alter the structure of the complexes. The induced CD spectrum of proflavine bound to DNA has a conservative character [17] and a low intensity. The induced CD spectra of proflavine bound to $(ALP)_n$–DNA_{son} (prepared from sonicated low molecular-weight DNA) showed, on the contrary, a very intense negative band at about the maximum of proflavine absorption. The induced CD spectrum of proflavine bound to $(ALP)_n$–DNA (prepared from DNA of high molecular weight) showed a negative band of much lower intensity and a weak positive band. Comparing these CD spectra with those of the respective complexes in the region of DNA absorption (fig. 1) (at a low Pf/DNA ratio this part of the spectrum is little affected by proflavine) one can clearly observe the similarity of the induced CD of proflavine and of the CD spectrum of complexed DNA, both in band intensity and sign. Induced CD spectra of proflavine bound to complexes of both polypeptides, prepared using high molecular-weight DNA, showed a mirror-image relationship similar to that found in the DNA absorption region (figs. 1 and 2).

4. Discussion

Aggregated complexes of model polypeptides with DNA in 0.15M NaCl are characterised by CD spectra of a nonconservative type, with bands in the DNA absorption region displaying extraordinarily high ellipticity values. The band intensity directly depends (1) on the starting concentration of the components, and (2) on the aggregate size, which proves that it is associated with the presence and nature of the aggregated phase in the complexes of both polypeptides. Since similar high-intensity CD effects have been found in certain ordered DNA systems (Ψ DNA [18,19]), an ordered structure of DNA–polypeptide aggregates is a plausible

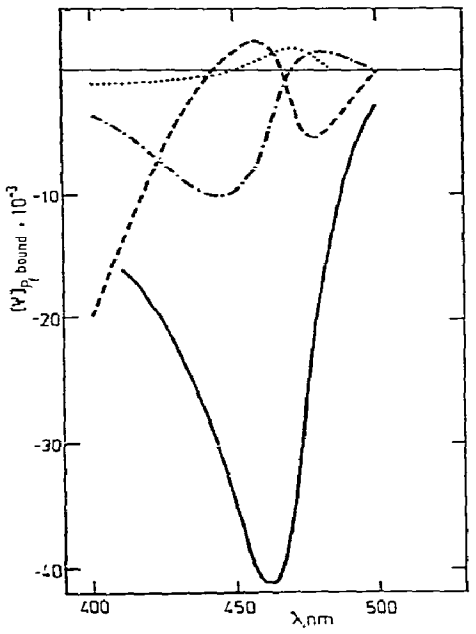

Fig. 8. Induced CD spectra of proflavine (Pf) bound to DNA and to the complexes; $[\psi]$ refers to bound Pf; Pf/DNA = 0.04, L/DNA = 0.5. ···· Pf-DNA; –·–·– Pf–$(ALP)_n$–DNA, $M_{DNA} = 13 \times 10^6$; ——— Pf–$(ALP)_n$–DNA_{son}, $M_{DNA} = 6 \times 10^5$; – – – – Pf–$(L,2A)_n$–DNA, $M_{DNA} = 13 \times 10^6$.

assumption. The inverse dependence of CD band intensity on DNA molecular size can be considered as an additional argument for an ordered structure of aggregates as already suggested in the analogous case of histone F1–DNA complexes [20]. Since this superstructure is able to induce a very high optical activity, it may be assumed to be chiral. In this respect it can be compared to the structure of cholesteric phase of liquid crystals [8]. However, the observed system as a whole is obviously isotropic, the optical axes of the quasi-crystalline aggregates being randomly oriented with respect to the incident light beam. Very little is known about the chiroptical properties of such systems [8].

Nevertheless the concept of a chiral superstructure of aggregates permits a ready explanation of further experimental findings, such as the high intensity of bands in the region of an induced effect of bound proflavine [21]. The opposite signs of the CD bands of the two types of complexes could be accounted for by an opposite (enantiomer) chirality of the superstructure.

The predominant effect of aggregate structure does not exclude the possibility that certain changes of CD spectra of the DNA in the complexes are due to changes in the DNA conformation. In the case of $(ALP)_n$–DNA experimental results indicate that the CD pattern both in the DNA absorption region and in the region of induced CD depend almost entirely on the aggregation properties of the complexes, i.e. on superstructure formation.

In $(L,2A)_n$–DNA complexes the aggregation properties also affect the intensity of the CD bands but the CD pattern remains different from that of the DNA, even in the 'soluble' supernatant complex, practically free of aggregates. Furthermore, the intensity of the induced CD effect of bound proflavine is much lower than the intensity of bands in the region of DNA absorption. This can all be explained by assuming that the intense non-conservative CD spectrum in the region of DNA absorption is due only partly to superstructure chirality. A conformational change (tilting of bases) may result in a lower degree of compensation of positive and negative contributions of interacting bases in the polymer, similarly as in the A form of DNA or in double-stranded RNA [22].

An interesting problem is the dependence of the CD spectra of complexes on DNA composition. This experimental finding, together with suppression of the CD effect by actinomycin D and the influence of methylation of the guanine residue on the CD spectra [23] suggest an important role of regions rich in (G·C) pairs in superstructure formation. A similar selectivity has been found with histone F1–DNA complexes [20]. The origin of this selectivity is not clear at present.

The study of optical properties of DNA–polypeptide complexes emphasized the importance of a certain type of aggregation interactions resulting in formation of ordered superstructures. The ability of DNA to form condensed structures with high-intensity CD bands has also been shown in DNA films [24], in solutions containing high concentrations of neutral polymers [18, 19] or polyethylene glycol [25] and is therefore not a unique feature of DNA–polypeptide complexes. In the latter case, however, two different types of superstructure were observed in dependence on the conformation of bound polypeptide, and the degree of ordering was shown to depend on DNA composition. Thus a

certain type of specificity of interactions leading to superstructure formation is indicated, which can possibly play an important role in chromatin and chromosomes. At present there is no evidence for the existence of superstructures with optical properties of the above model complexes in biological material, but in chromatin they can be largely obscured due to the complexity of the system. Furthermore, the studies on soluble chromatin have usually been carried out in a low ionic strength, under conditions where most of the aggregation interactions seem to disappear [4,6].

Acknowledgement

The authors are indebted to Mrs. J. Jelínková for skillful technical assistance.

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