Interaction of Clupeine with Deoxyribonucleic Acid. II. Optical Rotatory Dispersion Studies*

Sadako Inoue and Toshio Ando

ABSTRACT: Optical rotatory dispersion spectra were measured for various complexes, prepared by the mixing method, between sonicated herring sperm deoxyribonucleic acid and basic peptide including clupeine and polyamino acids. The optical rotatory dispersion spectral pattern of DNA–clupeine at the level of Arg:P close to unity was similar to that of deoxyribonucleic acid–$(Arg)_{20}$ at Arg:P = approximately 0.5. The general pattern of the changes from the spectrum of deoxyribonucleic acid under standard conditions, observed for these complexes, was rather similar to that observed for heat-denatured deoxyribonucleic acid, suggesting the presence of conformational changes in the deoxyribonucleic acid helix in these complexes. However, anomalous changes observed in the optical rotatory dispersion spectra of deoxyribonucleic acid–$(Arg)_{20}$ and deoxyribonucleic acid–$(Lys)_{20}$ at the cation:P ratios higher than 0.7 could not be explained by a simple conformational change of the deoxyribonucleic acid helix. These anomalous optical rotatory dispersion curves appeared to arise from light scattering present in the solutions of complexes, but no apparent correlation was found between the extent of light scattering and the optical rotatory dispersion change for the complexes examined.

The fact that the optical rotatory dispersion spectra of deoxyribonucleic acid–clupeine are different from those of deoxyribonucleic acid–$(Arg)_{20}$ at the level of Arg:P close to unity suggests the effect of neutral amino acid residues in clupeine molecules in the structure formation and/or properties of the complexes.

In the preceding paper, the melting properties of DNA–clupeine complexes were compared with those of DNA–oligoarginine, DNA–polyarginine, DNA–polylysine, and DNA–polyornithine. It has been shown that the binding of clupeine and the basic polyamino acids (the degree of polymerization $\simeq$20) to DNA is irreversible at low ionic strength and results in a marked stabilization of the DNA helix against thermal denaturation. Differences were found in the stability and in the cooperativity of the melting transitions between each of these complexes. In this paper, optical rotatory dispersion spectra of these complexes were measured in order to obtain information on conformational changes of DNA in the complexes and structural differences between the complexes.

Experimental Procedures

Materials. Preparation and characterization of herring sperm DNA and its sonicated specimen were described in the preceding paper (Inoue and Ando, 1970). dDNA[1] was obtained by heating the solution of the sonicated DNA in the standard buffer for 15 min in a boiling-water bath and quickly chilling it in ice. Clupeine and other model peptides were also of the same source as described previously. The concentration of peptides was determined by the amino acid analysis as described in the preceding paper and expressed in terms of the mole residue of basic amino acid (Arg or Lys) per liter.

The concentration of DNA was determined by phosphorus analysis and expressed as moles of P per liter.

Preparation of Complexes. Unless otherwise specified, complexes were prepared by direct mixing of the sonicated DNA and the peptide as described in the preceding paper. The standard buffer was 0.01 M NaCl–0.001 M Tris-Cl (pH 7.0). Glass-distilled deionized water was used throughout. The final concentration of DNA was usually 1.5×10^{-4} M and the absorbance of solutions was about 1.0 at 260 mμ.

Optical Rotatory Dispersion Measurements. Optical rotatory dispersion measurements were made on a JASCO Model ORD/UV-5 spectropolarimeter with a 1-cm quartz cell at 20°. Blanks were determined just before and after each measurement and all measurements were repeated at least twice to ensure the reproducibility. Rotations of DNA and complexes were expressed in terms of residue rotation, $[\phi] = 100\alpha/m$, where m is the molar concentration on the basis of nucleotide phosphorus and α the observed rotation in degrees. Rotations of peptides were also expressed in terms of residue rotation where molar concentration is on the basis of the basic amino acid residue.

Results

Characterization of Optical Rotatory Dispersion Spectra of Complexes. Figure 1 shows optical rotatory dispersion curves of DNA–clupeine, DNA–$(Arg)_{20}$, and DNA–$(Lys)_{20}$ prepared in the standard buffer at two representative cation:P ratios for each of the three complexes. Clupeine, like polyarginine and polylysine, is in random conformation in the solvent used and has considerable magnitude of negative rotation only below 240 mμ (Suzuki and Ando, 1968). Figure 1 shows that optical rotatory dispersion curves of the complexes are

* From the Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo, Japan. *Received April 29, 1969.* This work is supported in part by a grant from the Ministry of Education of Japan. A preliminary account has been reported (Inoue and Ando, 1968).

[1] Abbreviation used is: dDNA, heat-denatured DNA (see text for the preparation).

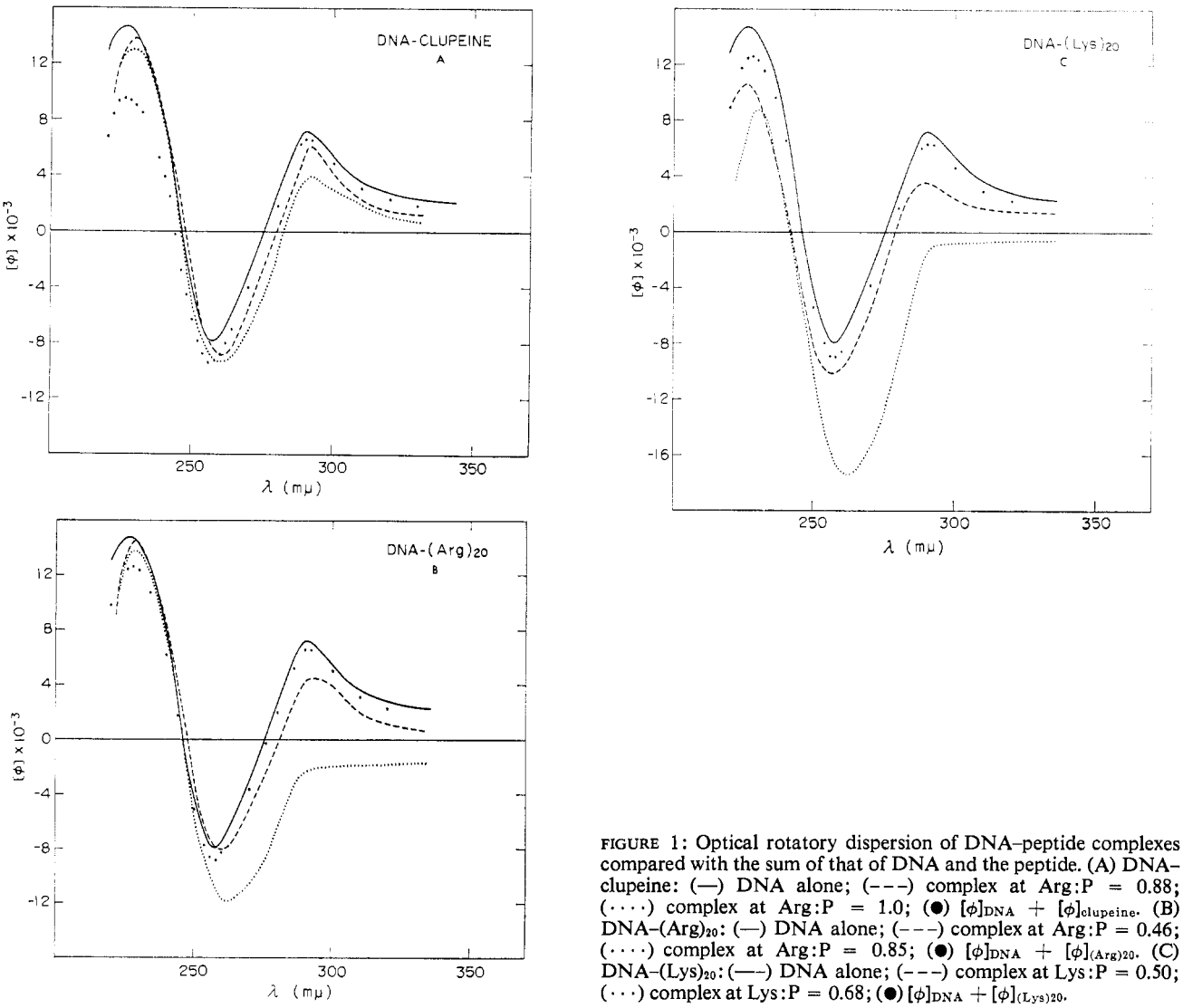

FIGURE 1: Optical rotatory dispersion of DNA–peptide complexes compared with the sum of that of DNA and the peptide. (A) DNA–clupeine: (—) DNA alone; (– – –) complex at Arg:P = 0.88; (· · · ·) complex at Arg:P = 1.0; (●) $[\phi]_{DNA} + [\phi]_{clupeine}$. (B) DNA–$(Arg)_{20}$: (—) DNA alone; (– – –) complex at Arg:P = 0.46; (· · · ·) complex at Arg:P = 0.85; (●) $[\phi]_{DNA} + [\phi]_{(Arg)20}$. (C) DNA–$(Lys)_{20}$: (—) DNA alone; (– – –) complex at Lys:P = 0.50; (· · ·) complex at Lys:P = 0.68; (●) $[\phi]_{DNA} + [\phi]_{(Lys)20}$.

TABLE I: Optical Rotatory Dispersion of DNA and DNA–Peptide Complexes.

	Cation:P	λ_p	$[\phi]_p$	λ_t	$[\phi]_t$	λ_p	$[\phi]_p$
DNA		290	7200	257	−7800	228	14600
DNA in 2 M NaCl		290	4900	257	−6000	228	15600
dDNA		292	5400	262	−9600	228	9000
DNA–clupein[a]	1.0	292	4000	262	−9400	230	13000
DNA–clupein[a]	0.88	292	6000	262	−8800	230	13800
DNA–$(Arg)_{20}$	0.85			262	−11900	229	13800
DNA–$(Arg)_{20}$	0.46	292	4400	260	−8100	229	14500
DNA–$(Lys)_{20}$	0.68			262	−17400	230	8800
DNA–$(Lys)_{20}$	0.50	290	3500	257	−10100	228	10600
DNA–$(Lys)_{100}$	0.78			269	−34800	233	3100
DNA–$(Orn)_{19}$	0.89			270	−29000	233	4500
dDNA–clupein[a]	0.88	294	3700	262	−10300	230	8300
dDNA–$(Arg)_{20}$	0.91	294	3700	262	−10400	230	8400
dDNA–$(Lys)_{100}$	0.97	294	4300	262	−10200	230	8200

[a] An equimolar mixture of three clupeine components was used. Similar spectra were obtained when any one component of clupeine was used.

TABLE II: Light-Scattering Parameters for Complexes.

Complex	Cation:P	A_{260}[a]	A_{340}	a
DNA–clupeine	1.0	1.906	0.440	2.9
	0.88	1.294	0.097	2.8
DNA–$(Arg)_{20}$	0.85	1.404	0.124	3.2
	0.46	1.095	0.028	4.0
DNA–$(Lys)_{20}$	0.68	1.168	0.051	3.0
	0.50	1.050	0.015	4.0
DNA–$(Orn)_{19}$	0.89	1.313	0.115	3.3
dDNA–clupeine	0.88	1.476	0.088	2.3
dDNA–$(Arg)_{20}$	0.91	1.770	0.180	3.2
dDNA–$(Lys)_{100}$	0.97	1.480	0.104	2.8

[a] The values of A_{260} for DNA and dDNA at a concentration equal to that found in the solutions of complexes were 1.050 and 1.310, respectively.

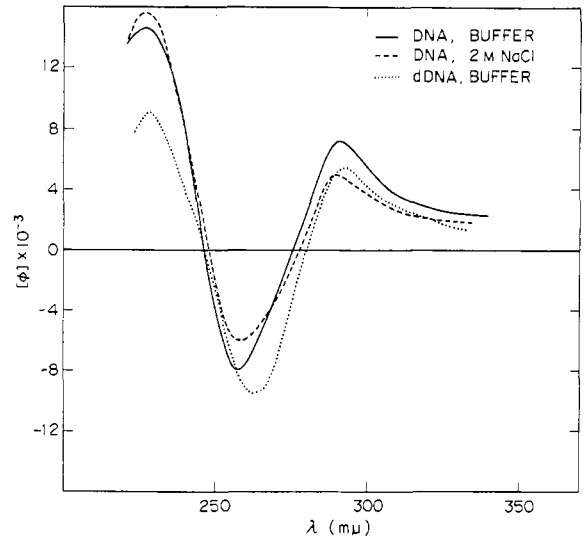

FIGURE 2: Optical rotatory dispersion spectra of DNA in the standard buffer and in 2 M NaCl, and that of dDNA in the standard buffer.

apparently different from the arithmetical sum of the curves of DNA and the peptides. These optical rotatory dispersion curves are characteristic of the nature of the DNA–peptide complexes. Thus in the optical rotatory dispersion curves of the DNA–clupeine complexes, the peak near 290 mμ is decreased in magnitude and shifted by 2 mμ toward longer wavelength in comparison with the corresponding peak in the arithmetical sum. The trough at 260 mμ also shows a red shift of about 5 mμ.

The optical rotatory dispersion curve of DNA–$(Arg)_{20}$ at an Arg:P ratio of 0.46 is rather similar to the curves of DNA–clupeine complexes at Arg:P ratios above 0.88. However, the DNA–$(Arg)_{20}$ complex at an Arg:P ratio of 0.85 gives a quite different curve from them. The peak near 290 mμ disappears and the magnitude of negative rotation of the trough near 260 mμ is greatly increased. The peak near 230 mμ is little altered from the corresponding peak in the arithmetical sum.

Optical rotatory dispersion spectra of the DNA–$(Lys)_{20}$ complexes show further alteration from the arithmetical sum. At a Lys:P ratio of 0.50, both of the two peaks are considerably decreased and the trough is increased in magnitude. The position of these extrema is almost unchanged. At a Lys:P ratio of 0.68, the peak near 290 mμ disappears and the magnitude of the trough is greatly increased. The optical rotatory dispersion characteristic of DNA–$(Orn)_{19}$ complexes was quite similar to that of DNA–$(Lys)_{20}$. The optical rotatory dispersion extrema for DNA and DNA–peptide complexes are tabulated in Table I.

Comparison with Optical Rotatory Dispersion Spectra of DNA in Various States. An optical rotatory dispersion spectrum of DNA in a high salt solution and that of the heat-denatured DNA is shown in Figure 2 and the extrema are given in Table I. Values of the melting temperature for the DNA–clupeine and other model complexes prepared under conditions similar to those used in the optical rotatory dispersion study were comparable with that for DNA in 2 M NaCl, indicating that DNA molecules in these complexes are in an ionic environment similar to that in 2 M NaCl. In 2 M NaCl, both the 290-mμ peak and the 257-mμ trough of the optical rotatory dispersion spectrum for DNA are smaller in magnitude as compared with those measured in the standard buffer. The magnitude of the 228-mμ peak is slightly increased. The position of these extrema is not changed. From these results it is rather difficult to correlate the optical rotatory dispersion of the complexes with that of DNA in high salt solutions. The optical rotatory dispersion spectrum of the heat-denatured DNA shows some similarity with those of the DNA–clupeine and DNA–$(Arg)_{20}$ complexes at small Arg:P ratios. General shifts of the peaks and the trough toward longer wavelength, the lowering of the peaks, and deepening of the trough are characteristic changes for the heat-denatured DNA.

Effects of Light Scattering. Since the solutions of the complexes are more or less turbid, light scattering should be considered as a possible origin for the alteration of their optical rotatory dispersion spectra. In Table II, values of absorbance at 340 mμ are given as an estimate of light scattering in the solutions of the complexes at a constant concentration of DNA (1.5×10^{-4} M). The size of the light scattering particles was estimated from the slope a of the log A *vs.* log λ plots in the region above 340 mμ where the apparent absorbance is due to scattering and proportional to λ^{-a} (Rayleigh light scattering). The values of a are also listed in Table II. Apparently, no direct correlation is seen between the extent of light scattering and the optical rotatory dispersion change. Thus the DNA–clupeine complexes show turbidity larger than that of the other complexes, but their optical rotatory dispersion spectral patterns are rather similar to that of DNA and do not show distinct changes as seen for the DNA–$(Arg)_{20}$ and DNA–$(Lys)_{20}$ complexes. On the contrary, the DNA–$(Lys)_{20}$ complexes show the smallest turbidity and the largest change of the optical rotatory dispersion curve from the arithmetical sum. The values of a for various complexes suggest that the size of the particles is smaller for the DNA–$(Arg)_{20}$ and DNA–$(Lys)_{20}$ complexes than for the DNA–clupeine complexes.

Another evidence that the optical rotatory dispersion spectral changes observed for the complexes are not simply explained

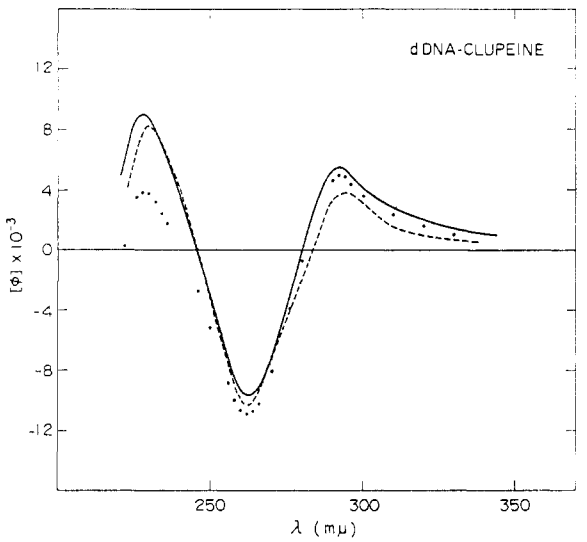

FIGURE 3: Optical rotatory dispersion of a dDNA–clupeine complex compared with the sum of that of DNA and clupeine: (—) dDNA alone; (– – –) complex at Arg:P = 0.88; (●) $[\phi]_{dDNA} + [\phi]_{clupeine}$.

by the effect of light scattering was given by examining the optical rotatory dispersion of dDNA–peptide complexes. A typical optical rotatory dispersion curve of the dDNA–clupeine complex at an Arg:P ratio of 0.88 is given in Figure 3 together with the curves for dDNA and the arithmetical sum of the rotation of dDNA and clupeine. The curves of the dDNA–$(Arg)_{20}$ and dDNA–$(Lys)_{100}$ complexes were similar to that of the dDNA–clupeine complex and the extrema for these curves are given in Table I. Data relevant to light scattering of the dDNA–peptide complexes are listed in Table II. These results show that although the extent of light scattering for the dDNA–peptide complexes are similar to that for the corresponding native DNA–peptide complexes, the optical rotatory dispersion spectra of the dDNA–peptide complexes undergo relatively small change from that of dDNA.

Discussion

The present study shows that the optical rotatory dispersion spectra of DNA–clupeine, DNA–$(Arg)_{20}$, and DNA–$(Lys)_{20}$ are different from the sum of rotation of the individual component of the complexes. The optical rotatory dispersion spectra of the DNA–clupeine complexes at Arg:P ratios of 0.88 and 1.0 are similar to that of the DNA–$(Arg)_{20}$ complex at an Arg:P ratio of 0.46. These spectra are rather similar to that of heat-denatured DNA, with respect to the changes in the 290 mμ peak and the 260-mμ trough. There is little similarity between the optical rotatory dispersion curves of these complexes and that of DNA in 2 M NaCl. The results suggest that the optical rotatory dispersion spectra of the complexes reflect some change in the helicity of DNA rather than the change in its ionic environment. The DNA–$(Arg)_{20}$ complex at an Arg:P ratio of 0.85 and the DNA–$(Lys)_{20}$ complex at a Lys:P ratio of 0.68 show distinct optical rotatory dispersion curves. These optical rotatory dispersion spectra cannot be explained by an artifact introduced by light scattering in the solutions.

We have too little information which correlates the conformational changes of DNA and the optical rotatory dispersion spectra in order to interpret the results of the present study. The difference observed for the DNA–clupeine and DNA–$(Arg)_{20}$ complexes at high Arg:P ratios is of interest, since it seems to be showing the difference arising from the unique amino acid sequence of clupeine. Further study is in progress by using the complexes formed between basic oligoamino acids and DNA or its model nucleotides. Incidentally, it is interesting to note that the optical rotatory dispersion spectra similar to those for the DNA–peptide complexes were observed by Maestre and Tinoco (1967) for T-series bacteriophages.

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