4193-4200.
Webb, P., and Fresco, J. R. (1973), *J. Mol. Biol. 74,* 387-402.
Wong, Y. P., Kearns, D. R., Shulman, R. G., Yamane, T., Chang, S., Chrikjian, J. G., and Fresco, J. R. (1973), *J. Mol. Biol. 74,* 403-406.
Yang, S. K., Söll, D. G., and Crothers, D. M. (1972), *Biochemistry 11,* 2311-2320.

Infrared Linear Dichroism Investigations of Deoxyribonucleic Acid Complexes with Poly(L-arginine) and Poly(L-lysine)†

J. Liquier, M. Pinot-Lafaix, E. Taillandier, and J. Brahms*

ABSTRACT: Complexes between DNAs from various sources and poly(L-lysine) and poly(L-arginine) were studied by means of infrared linear dichroism. The measurements of dichroic ratios allowed us to determine the orientation of the phosphate group of DNA in the complexes with basic polypeptides. At high relative humidities (higher than 90%, B form), the bisector of the ∠OPO in the complexes forms an angle with respect to the helical axis which has a value lower by about 4° than in the corresponding DNA sample. This change of orientation of the phosphate group of DNA indicates a modification of the B form upon binding of polylysine or polyarginine. The structural transitions B → A and B → C measured as a function of relative humidities were not affected by formation of complexes with both basic polypeptides. Similar results were obtained for complexes prepared by direct mixing or by salt gradient dialysis. The presence of A and C forms was observed in complexes of DNA with poly(L-lysine) and poly(L-arginine) at lower relative humidity. Thus, the conformational flexibility of DNA in complexes with polylysine and polyarginine is not changed despite a substantial increase in the T_m (melting temperature). These results are considered as a model for the understanding of interactions between DNA and histones and particularly of the binding of the N-terminal fragment, lysine or arginine rich.

Deoxyribonucleic acid exists in the nuclei of higher organisms in the form of complex structures associated with proteins. The histones are basic proteins which are found in nuclei tightly bound to DNA and which appear to stabilize the condensed structure of eukaryotic DNA (Pardon and Wilkins, 1972). Detailed knowledge of the structural properties of these complexes might provide a basis for the understanding of the mechanism of function of eukaryotic nuclei; in particular, one may be able to understand the regulation of transcription (Paul, 1972; Sutton, 1972; Elgin et al., 1971). It is evident that the complexity of these DNA-protein complexes and their interactions with DNA have rendered the development of our knowledge difficult. Investigation of complexes of DNA and synthetic nucleic acids with basic homopolypeptides poly(L-lysine) and poly(L-arginine) provides a greater simplicity and has been used by several groups of investigators employing different methods (Raukas, 1965; Tsuboi et al., 1966; Leng and Felsenfeld, 1966; Olins et al., 1967, 1968; Cohen and Kidson, 1968; Shapiro et al., 1969; Davidson and Fasman, 1969; Haynes et al., 1970; Caroll, 1972; Chang et al., 1973; Zama and Ishimura, 1973). Thermal denaturation studies showed that the formation of the complex with basic homopolypeptides leads to a substantial increase in the melting temperature which was interpreted as reflecting a marked stabilization of the double-helical structure of DNA (Olins et al., 1967, 1968; Inoue and Ando, 1970). The optical activity of DNA is drastically changed when it is complexed with these basic homopolypeptides. Thus, circular dichroism spectra of DNA covered by poly(L-lysine) are of inverted shape and nonconservative character; that is, a broad negative band is observed in the region of 280-240 nm, the intensity of which is greatly enhanced with respect to that of DNA (Haynes et al., 1970; Caroll, 1972).

Similar spectral changes were observed in DNA complexed with the histone f1 (Fasman et al., 1970; Adler et al., 1971). In contrast, complexes of arginine-rich histones f2a1 and also f2b with DNA exhibit circular dichroic (CD) spectra with increased amplitude of the 275-nm positive band (Shih and Fasman, 1971, 1972; Adler et al., 1974). The former spectral changes were considered as indicative of B → C transitions and the latter of B → A. Interpretations of spectral changes are very difficult since measurements of optical activity in these complexes may not only reflect the contribution of secondary structure. In fact, electron microscopy provides evidence that DNA-polylysine complexes and also f1-DNA and f2a1-DNA complexes form doughnut-shaped or globular particles with some internal periodicity (Haynes et al., 1970; Slayter et al., 1972). It is thus necessary to obtain structural information by another direct method, such as infrared linear dichroism (Bradbury et al., 1962). It was shown previously by Pilet and Brahms (1972, 1973) and Brahms et al. (1973) that precise structural parameters and structural transitions can be obtained from infrared linear dichroism studies of DNA. Unfortunately, previous X-ray diffraction investigations on DNA-poly(L-lysine) and DNA-poly(L-arginine) complexes cannot be considered here since they were performed under conditions

† From the Institut de Biologie moléculaire de la Faculte des Sciences de Paris, Paris 5ème, France, and the Uer de Médecine et Biologie Humaine, Université Paris XIII, 93000 Bobigny, France. *Received February 13, 1975.*

* Address correspondence to this author at the Institut de Biologie Moléculaire.

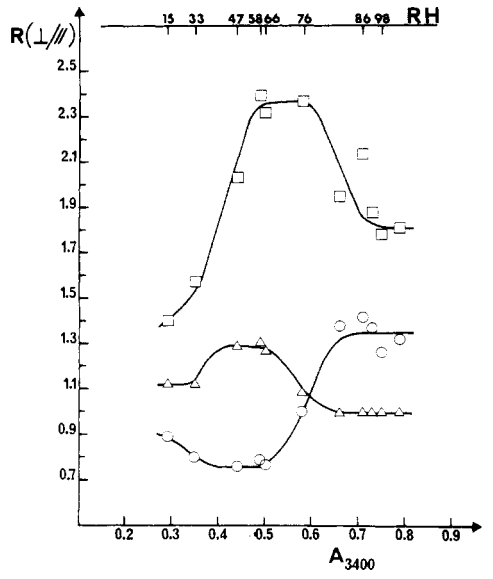

FIGURE 1: Dichroic ratio $R(\perp/\parallel)$ of the DNA (*Micrococcus lysodeikticus*)-polyarginine complex at different humidities; arginine to phosphate molar ratio, 0.36/1; (□) band at about 1710 cm^{-1}; (△) band at about 1230 cm^{-1}; (○) band at about 1090 cm^{-1}; sodium chloride content of the sample, 3–5% (w/w). Complex prepared by direct mixing method.

of undefined salt content (Suwalsky and Traub, 1972).

We report here structural parameters and conformational changes in DNA-poly(L-lysine) and DNA-poly(L-arginine) complexes prepared by different methods, mainly by a direct mixing method and also by reconstitution experiments under rigorously controlled salt conditions. Our results compared with those obtained with DNA indicate that the presence of polylysine or polyarginine bound to DNA changes the orientation of DNA phosphate groups of a modified B-type form (B*) at high humidities, but does not influence structural changes to A or C forms at lower humidities.

This conformational flexibility of DNA complexes with basic polypeptides may serve as a model for the understanding of interaction of DNA with proteins and histones. All five histone fractions are characterized by uneven charge distribution (De Lange and Smith, 1974). Lysine-rich or arginine-rich fragments situated in the NH_2-terminal region are considered to be the binding site to DNA (Bradbury et al., 1972; De Lange and Smith, 1974). A tentative model is proposed according to which the suppression of structural changes in DNA complexes with histones should be primarily caused by the C-terminal fragment rich in hydrophobic amino acids whereas the lysine- and arginine-rich segment may not influence conformational transitions B → A or B → C normally occurring in free DNA.

Experimental Section

Materials. Poly(L-lysine) hydrobromide of mol wt 80,000 and 140,000 was prepared in the laboratory of Dr. Spach in C.R.M. (Strasbourg) and also was purchased from Miles-Yeda Co. Poly(L-arginine) hydrochloride, mol wt 40,000, was purchased from Miles-Yeda Co. Salmon sperm DNA was a Worthington product. Deoxyribonucleic acid from *Micrococcus lysodeikticus* was obtained from Miles. Other samples of *M. lysodeikticus* DNA (purified by chromatography on a hydroxylapatite column) were prepared in the laboratory of and were a generous gift of Drs. A. and G. Bernardi; *Clostridium perfringens* DNA was purchased from Sigma.

Preparation of Complexes. The complexes were obtained by two different methods. (1) Direct Mixing Method. This method was mostly employed throughout this work. The DNA–polypeptide mixture was dialyzed overnight or for 48 hr at 4° against the appropriate NaCl solution.

(2) Salt Gradient Dialysis Method (Huang et al., 1964). The sample was mixed and allowed to stand for several hours in 2 *M* NaCl. The dialysis was successively carried out against 2, 0.9, 0.3, and 0.15 *M* NaCl for 3–4 hr each. The final step consisted of dialysis against 10–1.3 × 10 m*M* NaCl solution at pH 7.5 overnight at 4° against two to three changes of solution.

To avoid the formation of turbid complexes, the polypeptide solutions used contained 3–4 mg/ml, and the DNA concentration was about 20 mg/ml. The complexes of polypeptides with DNA contained 0.20, 0.36, 0.44, and 0.66 input ratios of amino acids per nucleotide residues. The final NaCl content of the film was about 4–6%.

Infrared Measurements. Infrared measurements of oriented films were performed as described previously (see Pilet and Brahms, 1972, 1973). The orientation and birefringence of the film were checked using a polarizing microscope. The infrared spectra were recorded on a Beckman I.R.9 spectrophotometer (between 700 and 4000 cm^{-1}) equipped with a wire grid polarizer with a AgBr support.

The infrared (ir) dichroic ratios $R_{\perp/\parallel} = A_\perp/A_\parallel$ were determined from measurements of the spectra of oriented films with electric field of the incident light parallel and perpendicular to the orientation axis. We have also measured the spectra of a sample oriented at 45° with regard to the slit.

The water content of the sample was monitored by measurement of the H_2O or D_2O absorption band at 3400 or 2520 cm^{-1}, respectively.

The relative humidity of the films was achieved by placing saturated solutions of different salts in an infrared cell specially constructed for this purpose (see Pilet and Brahms, 1973). The dichroic ratios were calculated by correcting for water absorption which is particularly intense in the 1650-cm^{-1} region and by measuring absorbancy of deuterated samples. However, D_2O cannot be used for the determination of $R_{1230\ cm^{-1}}$ having a large band in this frequency region; thus, both deuterated and normally hydrated samples were investigated.

The dichroic ratio allows one to calculate the angle θ which the transition moment forms with the DNA axis:

$$R_{\perp/\parallel} = (\sin^2\theta + g)/(2\cos^2\theta + g)$$

where g is the parameter which characterizes the semicrystalline state of the oriented sample. The parameter g can be related to the fraction of perfectly oriented chains:

$$f = 1/(1 + \tfrac{3}{2}g)$$

g is evaluated by using the value of $R_\perp/R_\parallel$ at 1710 cm^{-1}. $\theta_{1710} = 90°$ in the B form and 80° in the A form (see Pilet and Brahms, 1973). The uncertainties in the measurements of the dichroic ratio were estimated from the measurements of R according to:

$$R_{1710} = (A_\perp - A_{H_2O})/(A_\parallel - A_{H_2O})$$

which allowed one to derive easily an appropriate expression for $\Delta R/R$. For different DNA–polypeptide complexes

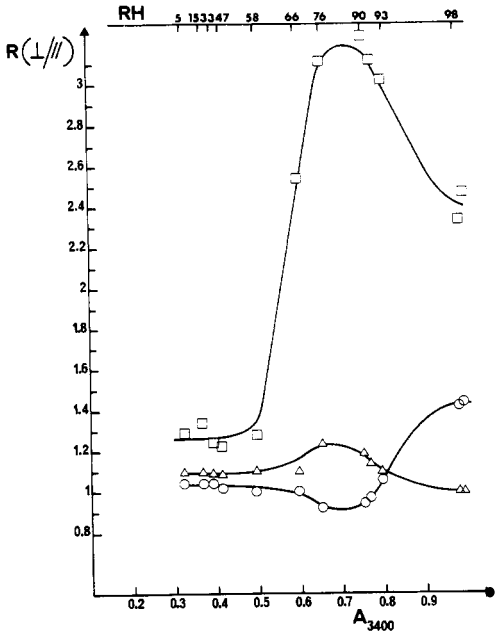

FIGURE 2: Dichroic ratio $R(\perp/\parallel)$ of DNA (*Micrococcus lysodeikticus*)-poly(L-arginine) complex at different humidities; arginine to phosphate molar ratio, 0.66/1; (□) band at about 1710 cm^{-1}; (△) band at about 1230 cm^{-1}; (○) band at about 1090 cm^{-1}; sodium chloride content of the sample, 3-5% (w/w). Complex prepared by direct mixing method.

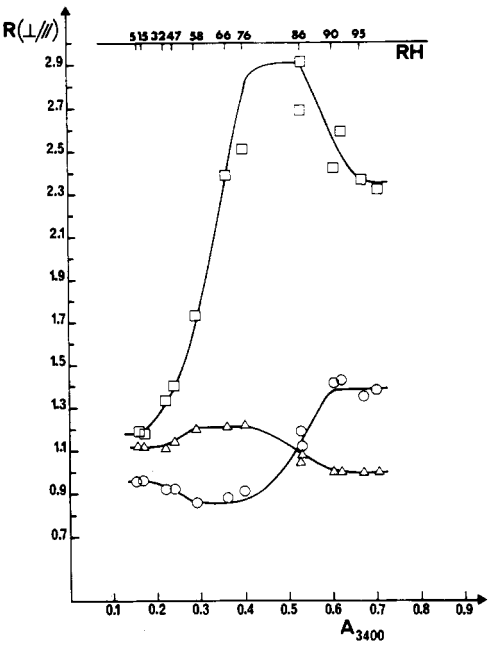

FIGURE 3: Dichroic ratio $R(\perp/\parallel)$ of DNA (salmon sperm)-poly(L-lysine) complex at different humidities; lysine to phosphate molar ratio, 0.66/1; (□) band at about 1710 cm^{-1}; (△) band at about 1230 cm^{-1}; (○) band at about 1090 cm^{-1}. Complex prepared by salt gradient dialysis.

the values of $\Delta R/R$ varied an average of about 6%, which gave an estimate of uncertainties θ and $\Delta\theta$ of about $\pm 1\%$.

At the frequencies of interest in this investigation (1710, 1230, and 1090 cm^{-1}), polypeptide chains make no appreciable contribution to the infrared absorption spectra, at the concentration used.

Ultraviolet "Melting Curves". Ultraviolet thermal denaturation curves were performed in order to test the formation of the complex (see Olins et al., 1967; Li et al., 1974). The melting curves were measured on a Shimadzu spectrophotometer specially adapted for this purpose. The ultraviolet absorption spectra were recorded on a Cary 17 spectrophotometer with a thermostated cell holder. The formation of the DNA-polypeptide complex was observed by comparing the melting with that of free DNA under identical salt conditions. From previous studies it is known that the DNA-poly(L-lysine) complex melts at temperatures above 100° (Li et al., 1974).

Results

Structural Transitions of DNA Complexes. Figures 1, 2, and 3 show plots of the dichroic ratio ($R_{\perp/\parallel}$) of oriented complexes of different DNAs with poly(L-lysine) or poly(L-arginine) measured at various degrees of relative humidity. Figures 1 and 2 show the results observed from complexes prepared by direct mixing, while Figure 3 represents the results for a complex prepared by salt gradient dialysis. In all complexes of DNA-poly(L-lysine) and DNA-(poly(L-arginine) studied, the pattern of changes of the infrared dichroic ratio R as a function of relative humidity is very similar to that of free DNA (see also Pilet and Brahms, 1973). Structural transitions of oriented complexes of DNA with poly(L-lysine) and poly(L-arginine) were investigated using DNA samples of different origins. Various forms of DNA were observed in the complexes studied as a function of relative humidity. These conformations were determined applying the method of DNA infrared linear dichroism as described by Pilet and Brahms (1972, 1973) and Brahms et al. (1973). Comparative studies were made investigating each complex in parallel with an identical DNA sample prepared under similar conditions as a reference. The salt content was rigorously controlled. The following three bands are plotted in Figures 1, 2, and 3. (1) The band at 1713 cm^{-1} which is assigned to the C=O and C=N double bond stretching vibrations is characteristic of the base pairing (Tsuboi, 1970). (2 and 3) The bands in the regions of 1230 cm^{-1} and at about 1090 cm^{-1} are assigned to antisymmetric and symmetric stretching vibrations of the phosphate group, respectively (Shimanouchi et al., 1964). The corresponding transition moments are oriented following the O(2)-O(3) line and the bisector of the OPO angle.

Three regions are observed in Figures 1-3: (a) at elevated values of relative humidity (98-90%) the dichroic ratio $R_{\perp/\parallel}$ yields a first plateau; (b) at intermediate relative humidity (76-45%) a second plateau appears; (c) at very low relative humidity disorientation of the sample occurs. The first two plateaus correspond to two different semicrystalline forms and were characterized by the orientation of the bases and phosphate groups (Pilet and Brahms, 1972, 1973).

The high humidity B form or B family form is characterized by values of R at 1710 cm^{-1} much larger than 1, whereas R at 1230 cm^{-1} is almost equal to unity and R at 1090 cm^{-1} is larger than 1. The A form is recognized by a parallel dichroism at 1090 cm^{-1} (R smaller than unity) and a weak perpendicular dichroism at 1230 cm^{-1}, whereas dichroism for 1710 cm^{-1} is strongly perpendicular. The C form differs from the other forms and particularly from the B form by a parallel dichroism at 1230 cm^{-1} (Brahms et al., 1973), which indicates a change of the orientation of the transition moment directed following the O(2)-O(3) line of the phosphate group.

Table I: Comparison of Structural Transitions of Different DNAs and of Their Complexes with Poly(L-arginine) and Poly(L-lysine).[a]

Sample	Input Ratio	Base Content	DNA Forms: High Rel. Humidity (Above 90%)	DNA Forms: Low Rel. Humidity (at About 76%)
M. lyso DNA (Na^+)				
M. lyso DNA (Na^+), PLA	1/0.36	72% G + C	B	A
M. lyso DNA (Na^+), PLL	1/0.36			
M. lyso DNA (Na^+), PLA	1/0.66			
M. lyso DNA (Na^+), PLL	1/0.66			
SSp DNA (Na^+)				
SSp DNA (Na^+), PLA	1/0.40			
SSp DNA (Na^+), PLL	1/0.40	40% G + C	B	A
SSp DNA (Na^+), PLA	1/0.66			
SSp DNA (Na^+), PLL	1/0.66			
Cl. p DNA (Na^+)				
Cl. p DNA (Na^+), PLA	1/0.66	32% G + C	B	B
Cl. p DNA (Na^+), PLL	1/0.66			
SSp DNA (Na^+), low salt				
SSp DNA (Na^+), PLA, low salt	1/0.20	40% G + C	C	A
SSp DNA (Na^+), PLL, low salt	1/0.20			
SSp DNA (Li^+), PLA	1/0.24	40% G + C	B	C
SSp DNA (Li^+), PLL	1/0.54			

[a] Abbreviations used in the table: SSp, salmon sperm; *M. lyso, Micrococcus lysodeikticus; Cl. p, Clostridium perfringens;* PLL, poly(L-lysine); PLA, poly(L-arginine).

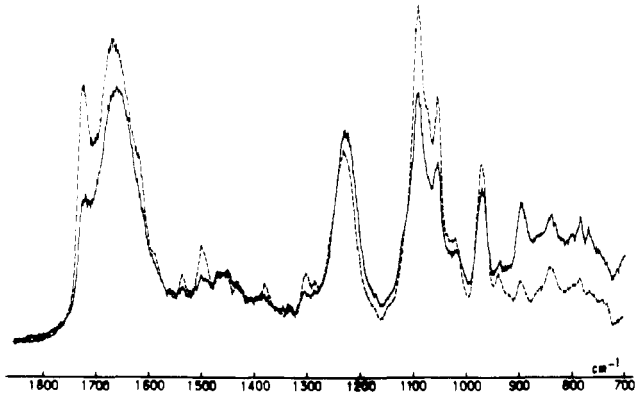

FIGURE 4: Infrared spectrum of oriented DNA (salmon sperm)-poly(L-lysine) complex at low sodium chloride content; the salt content of the film was 1-2% (w/w) of a 0.2/1 lysine to phosphate molar ratio; (—) electric vector of the polarized light parallel to the DNA axis; (- - -) electric vector of the light perpendicular to the DNA axis; relative humidity, 98% H_2O. Spectrum characteristic of the C form.

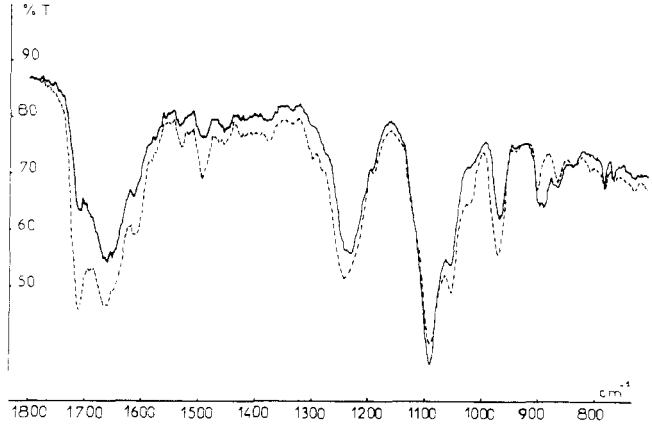

FIGURE 5: Infrared spectrum of oriented DNA (salmon sperm)-poly(L-lysine) complex; 0.66/1 lysine to phosphate molar ratio; (—) electric vector of the polarized light parallel to the DNA axis; (- - -) electric vector of the light perpendicular to the DNA axis; relative humidity, 66%. Spectrum characteristic of the A form.

Table I summarizes the results of comparative investigations on several DNA complexes with different input ratios of polypeptides to DNA. The investigations were extended to different sodium chloride contents of the film in the limit between 4 and 7% (w/w). Qualitatively the transition from the B to the A form was observed for DNA (Na^+) in complexes with different amounts of poly(L-lysine) or poly(L-arginine) similar to that observed for free DNA (Figures 4-6). It is to be emphasized that the investigations of DNA-basic polypeptide complexes were extended to different sodium chloride concentrations.

The limits of NaCl concentrations used within which structural changes occur were previously established for DNA by X-ray diffraction studies (Cooper and Hamilton, 1966) and by infrared studies (Pilet and Brahms, 1973). In fact, the DNA structural transition such as B → A is strongly dependent on the amount of the salt and occurs only within a limited amount of sodium chloride (4-6%, w/w) content. In all cases a similar transition was found between DNA-polylysine or -polyarginine complexes and free DNA (Figures 5 and 6).

DNAs of different origins and different G + C content were used for the formation of complexes (salmon sperm DNA, *M. lysodeikticus* DNA), but it was not possible to detect any structural differences or other manifestation of specificity for poly(L-lysine) or poly(L-arginine). The similarity of structural changes between free DNA and DNA-basic polypeptide complexes was also tested on A + T rich DNAs (*Cl. perfringens*) under identical salt conditions. The same conformations were observed for both complexes DNA-poly(L-lysine) and DNA-poly(L-arginine) and for DNA (*Cl. perfringens*).

The appearance of the C form or C-type form in these complexes was also investigated. The lithium salt of DNA-polylysine and DNA-polyarginine complexes exhibits the B → C structural transition as a function of relative humidity

Table II: Conformational Parameters of the Phosphate Group in Different DNA and in Their Complexes with Poly(L-arginine) and Poly(L-lysine).[d]

Form	Sample	Input Ratio	θ_{1230}[a] (deg)	θ_{1090}[b] (deg)	f
High humidity	SSp DNA (Na^+)		56 ± 1	67 ± 1	0.33
(rel. humidity above 90%),	SSp DNA (Na^+), PLA	1/0.40	55 ± 1	64 ± 1	0.50
B^+ form	SSp DNA (Na^+), PLL	1/0.40	55 ± 1°30′	64 ± 1°30′	0.38
	M. lyso DNA (Na^+)		55 ± 1°30′	69 ± 2°30′	0.40
	M. lyso DNA (Na^+), PLA	I/0.36	55 ± 2	66 ± 2°30′	0.33
	M. lyso DNA (Na^+), PLL	1/0.36	56 ± 2	66 ± 2°30′	0.46
	M. lyso DNA (Na^+), PLA	1/0.66	55 ± 2	65 ± 2°30′	0.46
	M. lyso DNA (Na^+), PLL	1/0.66	55 ± 2	65 ± 2°30′	0.43
	C. p DNA (Na^+), PLL	1/0.66	55 ± 2	64 ± 2°30′	0.52
Low humidity	SSp DNA (Na^+)		60 ± 1	50 ± 1	0.50
(rel. humidity 76%),	SSp DNA (Na^+), PLA	1/0.40	61 ± 1	50 ± 1	0.60
A form	SSp DNA (Na^+), PLL	1/0.40	61 ± 1	50 ± 1	0.50
	M. lyso DNA (Na^+), PLA	1/0.36	62 ± 1°30′	48 ± 1	0.50
	M. lyso DNA (Na^+), PLL	1/0.36	61 ± 2°30′	52 ± 1	0.55
C form	SSp DNA (Li^+)[c]	*c*	49 ± 3	67	0.63
	SSp DNA (Li^+), PLA	1/0.24	52 ± 1	70 ± 1°30′	0.51
	SSp DNA (Li^+), PLL	1/0.54	52 ± 1	70 ± 3	0.61
	SSp DNA (Na^+), PLA	1/0.20	48 ± 1°30′	63 ± 2	0.51
	SSp DNA (Na^+), PLL	1/0.20	48 ± 1°30′	65 ± 1	0.43

[a] θ_{1230}, orientation of the O2–O3 line to the helix axis. [b] θ_{1090}, orientation of the ∠OPO bisector to the helix axis. [c] Brahms et al., 1973. [d] See Table I for abbreviations used.

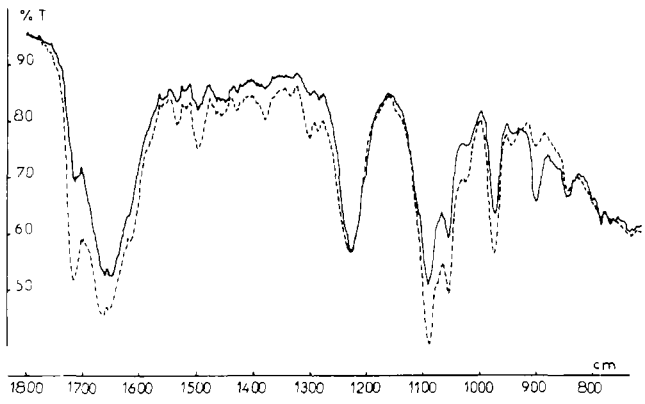

FIGURE 6: Same sample as Figure 5, relative humidity, 98%. Spectrum characteristic of the B form.

in agreement with previous X-ray diffraction data (Marvin et al., 1961) and ir characterization of DNA alone (Brahms et al., 1973). The C form was observed at lower humidities (at about 66% relative humidity) in DNA complexes with basic polypeptides as well as in DNA alone (Table I). On the other hand it was found that the C-like form appears at high relative humidity in the presence of sodium chloride when the salt content is very low, i.e. below 2% w/w (Brahms et al., 1973). Similarly in complexes of DNA (Na^+) with poly(L-lysine) and poly(L-arginine), DNA adopted the C form at high relative humidity (above 90%) and low salt content (Figure 4).

From these qualitative comparisons, one may conclude that the formation of complexes with poly(L-lysine) or poly(L-arginine) does not change the structural transitions of DNA at different humidities. Similar results were obtained independently of the method of preparation of complexes either by direct mixing or by reconstituted DNA–basic polypeptide complexes obtained by salt gradient dialysis (Huang et al., 1964).

Structural Parameters of DNA–Poly(L-lysine) and DNA–Poly(L-arginine) Complexes. In Table II the calculated angles which the DNA [PO_2] group forms with the helical axis in different complexes and under different humidity and salt conditions are shown. The orientation of the bisector of the ∠OPO angle θ_{1090} and the O(2)–O(3) line θ_{1230} with respect to the helical axis is compared with a noncomplexed DNA measured under identical conditions of humidity, salt content, etc. The values of angles were calculated taking into account the *maximum* uncertainties, i.e. the error due to the orientation factor (see Experimental Section) and the error due to the measurements of the corresponding phosphate bands. Thus, the measured angles allow one to reach the conclusion about the orientation of the DNA phosphate groups and about their differences when comparing DNA with DNA–polypeptide complexes. At high humidity (i.e., at relative humidity 92–100%), the orientation of the ∠OPO bisector is lower in DNA–poly(L-lysine) and DNA–poly(L-arginine) complexes than in corresponding DNA. The change of the orientations of the ∠OPO angle of about 4° with respect to the helical axis is observed in all DNAs studied regardless of their G + C content (Table I). The orientation of the ∠OPO bisector may vary slightly in different native DNAs at high relative humidity depending on their origin and G + C content, i.e. from 67 to 68° in salmon sperm and calf-thymus DNAs to above 69–70° with respect to the helical axis in *M. lysodeikticus* DNA (see also Pilet and Brahms, 1973). A change of the direction of the ∠OPO bisector to a value about 4° lower was systematically observed in all DNAs complexed with basic polypeptides.

Thus, in the complexes DNA–poly(L-lysine) and DNA–poly(L-arginine) the orientation of the ∠OPO bisector angle is 64–66° with respect to the DNA axis. We denote this high humidity modified B form as the B* form. At lower humidity, i.e. at about 76–66% relative humidity, the characteristic orientations of the ∠OPO bisector (θ_{1090}) in the A form or of the O(2)–O(3) line (θ_{1230}) in the C form were not significantly different in the complexes DNA–poly(L-lysine) and DNA–poly(L-arginine) than in DNA alone.

Discussion

One of the most important effects observed in this investigation is the complete reproducibility of the structural transitions (B → A and B → C forms) which occurs in free DNA and in complexes of DNA with basic polypeptides at various humidities. Thus, in spite of the strong electrostatic interactions between negatively charged DNA phosphate groups and positively charged basic polypeptides in the complexes; which are reflected in a very pronounced increase of melting temperature (T_m) (of about 35°), the conformational flexibility of DNA is sufficient to allow structural transitions at different humidities. The present investigation may serve as a model for the understanding of the role of binding of proteins, which are more or less rich in basic amino acids, to DNA. Particularly, all five fractions of histones are characterized by the asymmetry of charge distributions (Iwai et al., 1970; De Lange and Smith, 1974; Bradbury and Rattle, 1972). Lysine-rich or arginine-rich segments represent compact fractions, or even half of the molecule, located in the NH_2-terminal region and are considered to be the binding site to DNA. The second part of the molecule has an amino acid composition like many enzymes with many hydrophobic and hydroxyl residues (De Lange and Smith, 1974; Bradbury and Rattle, 1972; Adler et al., 1974). The suppression of B → A or B → C transitions recently observed in complexes of DNA with histone f2a1 (J. Liquier, E. Taillandier, and J. Brahms, manuscript in preparation) and also protamine (Herskovits and Brahms, 1975) must be thus due to the presence of hydrophobic residues which are found with particularly high frequency in the COOH-terminal region. This COOH-terminal region was postulated to be capable of interhistone interactions (Bradbury et al., 1972; De Lange and Smith, 1974). One may thus propose that if the binding to DNA is the main function of basic polypeptide chain, the second half with the COOH end containing hydrophobic residues may protect DNA from changes in hydration and cause the suppression of its normal structural transitions.

The second main result of the present investigation provides information about the intriguing question of structural alterations in DNA as a result of binding of the basic polypeptides poly(L-lysine) and poly(L-arginine) to DNA, obtained by a direct method of ir linear dichroism. The results of the present investigation on DNA indicate that binding of poly(L-lysine) or poly(L-arginine) slightly modifies the DNA native B conformation, a point which is reflected in the changes of orientation of the phosphate group at high humidity. Particularly affected is the bisector of the ∠OPO angle, the orientation of which with respect to the helical axis is decreased by 4°, i.e. changes from 69–67° to 65–64°. Similar results were obtained upon binding of polylysine or polyarginine (Table II). This leads to an important conclusion obtained by direct measurements, which indicate a similar modification of the native B form to a B-type form which we denote as the B* form. Thus, at high humidity the binding of both basic polypeptides neutralizes the charged phosphate groups and changes their orientation. The mechanism of binding may be more complex and may involve some exclusion of water molecules accompanied by reorganization of water layers around DNA. It is worthwhile to notice that the same structural modification is observed for different DNAs regardless of base composition, i.e. for *M. lysodeikticus* DNA, *Cl. perfringens* DNA, and salmon sperm DNA.

Our results indicate that phosphate group structural modifications are similar for both polylysine and polyarginine complexes. Many previous studies utilizing optical methods such as optical rotatory dispersion (ORD) and circular dichroism indicate the existence of pronounced spectral changes in the DNA–polylysine complexes (Shapiro et al., 1969; Haynes et al., 1970; Caroll, 1972) which are qualitatively very similar to those observed in the formation of histone f1–DNA complexes (Fasman et al., 1970; Shih and Fasman, 1971; Adler et al., 1971). The interaction of poly(L-arginine) (Zama and Ishimura, 1972) and also of the arginine-rich histone f2a1 and f2b fractions with DNA leads to different CD spectra (Shih and Fasman, 1972; Adler et al., 1974) which were interpreted as reflecting different conformational changes, i.e. the B → C in the former case and B → A in the latter. Furthermore, the altered CD and ORD of chromatin are considered as due to the association of DNA with chromosomal proteins (Shih and Fasman, 1970; Henson and Walker, 1970; Wagner and Spelsberg, 1971). The observed modified B form at high humidity or the B*-type form appear to be similar for both complexes, DNA–polylysine or DNA–polyarginine. According to present results, no difference in structure or structural stability can be observed between polyarginine or polylysine complexes with DNA when investigated by ir linear dichroism as a function of relative humidity.

The observed B* form of DNA–basic polypeptide complexes at high humidity has probably a more general biological significance since it was also recently observed in our laboratory in DNA–protamine complexes (Herskovits and Brahms, 1974). It is possible that this modified B*-type form is the form of DNA in chromatin. Our current investigation of complexes of DNA–histone fraction IV indicates the existence of a similar B* form at high humidity (J. Liquier, E. Taillandier, and J. Brahms, manuscript in preparation). Furthermore, our characterization of the B* form at high relative humidity seems to be in qualitative agreement with the modified B form of DNA proposed for chromatin on the basis of observation obtained by X-ray scattering in solutions and gels (Bram, 1972).

One may thus conclude that the observed changes in the orientation of the phosphate groups upon binding of poly(L-lysine) or poly(L-arginine) essentially involve a similar modification of the B form at high humidity, whereas at the lower relative humidity the structural transition to the A form of DNA (Na^+) or to the C form of DNA (Li^+) is essentially not altered. These results may serve as a model for the understanding of interactions of DNA with histones.

Added in Proof

After this article was submitted for publication we learned that our results were confirmed by X-ray diffraction studies on DNA–poly(L-lysine) complexes by Dr. J. Subirana (private communication).

References

Adler, A., Ross, D. G., Chen, K., Staffords, P. A., Woiszwillo, M. J., and Fasman, G. D. (1974), *Biochemistry 13,* 616.

Adler, A., Schaffhausen, B., Langan, T. A., and Fasman, G. D. (1971), *Biochemistry 10,* 909.

Bradbury, E. M., Cary, P. D., Crane-Robinson, C., Riches, P. L., and Johns, E. W. (1972), *Eur. J. Biochem. 26,* 482.

Bradbury, E. M., Price, W. C., and Wilkinson, G. R. (1962), *J. Mol. Biol. 4,* 39.
Bradbury, E. M., and Rattle, H. W. E. (1972), *Eur. J. Biochem. 27,* 270.
Brahms, J., Pilet, J., Tran, T. P. L., and Hill, L. R. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3352.
Bram, S. (1972), *Biochimie 54,* 1005.
Caroll, D. (1972), *Biochemistry 11,* 421.
Chang, C., Weisskopf, M., and Li, M. J. (1973), *Biochemistry 12,* 3028.
Cohen, P., and Kidson, C. (1968), *J. Mol. Biol. 35,* 241.
Cooper, P. J., and Hamilton, L. D. (1966), *J. Mol. Biol. 16,* 562.
Davidson, B., and Fasman, G. D. (1969), *Biochemistry 8,* 4116.
De Lange, R. J., and Smith, E. L. (1974), *Ciba Found. Symp. Struct. Funct. Chromatine.*
De Santis, P., Forni, E., and Rizzo, R. (1974), *Biopolymers 13,* 313.
Elgin, S. C. R., Froehner, S. C., Smart, J. E., and Bonner, J. (1971), *Adv. Cell Mol. Biol. 1,* 1.
Fasman, G. D., Schaffhausen, B., Goldsmith, L., and Adler, A. (1970), *Biochemistry 9,* 2814.
Feughelman, M., Langridge, R., Seeds, W. E., Stokes, A. R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F., Barcklay, K. R., and Hamilton, L. D. (1955), *Nature (London) 175,* 834.
Haynes, M., Garrett, R. A., and Gratzer, W. B. (1970), *Biochemistry 9,* 4410.
Henson, P., and Walker, I. O. (1970), *Eur. J. Biochem. 16,* 524.
Herskovits, T. T., and Brahms, J. (1975), manuscript submitted for publication.
Huang, R. C. C., Bonner, J., and Murray, K. (1964), *J. Mol. Biol. 8,* 54.
Inoue, S., and Ando, T. (1970), *Biochemistry 9,* 388.
Iwai, K., Ishikawa, K., and Hayashi, H. (1970), *Nature (London) 226,* 1056.
Leng, M., and Felsenfeld, G. (1966), *Proc. Natl. Acad. Sci. U.S.A. 56,* 1325.
Li, H. J., Chang, C., Weisskopf, M., Brand, B., and Rotter, A. (1974), *Biopolymers 13,* 649.
Marvin, D. A., Spencer, M., Wilkins, M. H. F., and Hamilton, L. D. (1961), *J. Mol. Biol. 3,* 547.
Olins, D. E., Olins, A. L., and von Hippel, P. H. (1967), *J. Mol. Biol. 24,* 151.
Olins, D. E., Olins, A. L., and von Hippel, P. H. (1968), *J. Mol. Biol. 33,* 265.
Pardon, J. F., and Wilkins, M. H. F. (1972), *J. Mol. Biol. 68,* 115.
Paul, J. (1972), *Nature (London) 238,* 444.
Pilet, J., and Brahms, J. (1972), *Nature (London), New Biol. 239,* 99.
Pilet, J., and Brahms, J. (1973), *Biopolymers 12,* 387.
Raukas, E. (1965), *Biochemistry (Engl. Transl.) 30,* 964.
Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969), *Biochemistry 8,* 3219.
Shih, T. Y., and Fasman, G. D. (1970), *J. Mol. Biol. 52,* 125.
Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10,* 1675.
Shih, T. Y., and Fasman, G. D. (1972), *Biochemistry 11,* 398.
Shimanouchi, T., Tsuboi, M., and Kyogoku, Y. (1964), *Adv. Chem. Phys. 7,* 435.
Slayter, H. S., Shih, T. Y., Adler, A. J., and Fasman, G. D. (1972), *Biochemistry 11,* 3044.
Sutton, W. D. (1972), *Nature (London), New Biol. 237,* 70.
Suwalsky, M., and Traub, W. (1972), *Biopolymers 11,* 2223.
Tsuboi, M. (1970), *Appl. Spectrosc. Rev. 1,* 45.
Tsuboi, M., Matsuo, K., and Ts'o, P. O. P. (1966), *J. Mol. Biol. 15,* 256.
Wagner, Th., and Spelsberg, Th. (1971), *Biochemistry 10,* 2599.
Wilhelm, X., Champagne, M., and Daune, M. (1970), *Eur. J. Biochem. 15,* 321.
Wilkins, M. H. F. (1956), *Cold Spring Harbor Symp. Quant. Biol. 21,* 75.
Zama, M., and Ishimura, S. (1971), *Biochem. Biophys. Res. Commun. 44,* 936.
Zama, M., and Ishimura, S. (1973), *Biochim. Biophys. Acta 294,* 214.