Conformations and Structural Transitions in Polydeoxynucleotides†

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ABSTRACT: Polydeoxynucleotides of different base sequence, the alternating poly[d(A-T)] · poly[d(A-T)], crab satellite DNA, on the one hand, and double-stranded homopolymer complexes poly[d(A)] · poly[d(T)], poly[d(I)] · poly[d(C)], on the other, display significant differences in their conformation and conformational transitions. Infrared linear dichroism investigations indicate that the alternating poly[d(A-T)] · poly[d(A-T)], enzymatically synthesized, adopts at lower humidity a well-expressed A* form in which stability is relatively small, i.e., restricted to limited relative humidity. This A form is characterized by the orientation of the bisector of the phosphate OPO group at 34° with respect to the helical axis, which is slightly lower than that of DNA. In contrast, for the homopolynucleotide double-stranded complex poly(dA) · poly(dT) and also for poly(dI) · poly(dC), the B → A conformational change is not observed. Instead poly(dA) · poly(dT) exists at lower humidity in a stable modified B form. Thus the present results indicate that homo(dA) · homo(dT) double-stranded sequences prevent the B → A structural transition. All AT-containing polydeoxynucleotides and crab satellite DNA adopt at high humidity a modified B form characterized by the orientation of the bisector of the phosphate group OPO at 64° with respect to the helical axis which is significantly lower than 68–74° observed in DNAs. The base pairing geometry in poly(dA) · poly(dT), poly[d(A-T)] · poly[d(A-T)], and also in poly(dI) · poly(dC) is apparently a Watson and Crick type. Thus the observed differences in conformation are not due to different base pairing scheme. It is suggested that in DNAs of high AT content the presence of homo(dT) · homo(dA) sequences and the relatively low stability of the A form of d(A-T) alternating sequences may inhibit the change to the A form. A possible role of these sequences in DNA recognition by protein is suggested.

Previous infrared (ir) studies of 15 different DNAs suggest that DNA conformational transitions depend, to some degree, on base sequence as well as base composition (Brahms et al., 1973; Pilet and Brahms, 1972 and 1973). We have observed that although the B form, or B family forms (Bram and Tougard, 1972), are adopted by all DNAs at high humidity, the B → A conformational transition is often prohibited in DNAs with a large AT content. Instead these DNAs usually undergo a transition to the C-like form as the humidity is decreased (Brahms et al., 1973). In addition, DNA with a moderate GC content, e.g., *Bacillis subtilis* DNA (44% GC) but with long oligo (dA), oligo (dT) sequences (Yamagashi and Takahashi, 1971; Rudner et al., 1972), does not adopt the A conformation at low humidity. This latter point suggests that the base sequence has an influence on DNA conformational transition. Some additional support for this hypothesis can be found in Langridge's (1969) preliminary X-ray diffraction studies of homopolypurine · homopolypyrimidine and alternating poly[d(purine-pyrimidine)] · poly[d(purine-pyrimidine)] sequences at high relative humidities (r.h.), and also in solution X-ray scattering studies (Bram, 1971).

The present report presents the results of an infrared dichroism study of several polydeoxynucleotides of known sequence which are good models of DNA molecule and of local segments. The experiments, performed under stringently controlled salt conditions, provide strong support for the hypothesis that nucleotide sequence plays an important role in determining conformational properties of DNA.

Material and Methods

Synthesis of Polydeoxynucleotides. Polydeoxynucleotides were enzymatically synthesized using DNA-polymerase I extracted either from *Escherichia coli,* according to Schachman et al. (1960), or from *Micrococcus luteus,* according to Wells and coworkers (Burd and Wells,1970; Harwood et al., 1970). All polydeoxynucleotides were prepared by incubating the enzyme with the appropriate XTPs and a template, and the reaction was followed spectrophotometrically. Polydeoxynucleotides were deproteinized either by the phenol or chloroform–isoamyl alcohol procedures. Synthesis of high molecular weight polydeoxynucleotide samples was facilitated by the use of the Wells' polymerase prepared from *M. luteus,* which has a relatively small nuclease activity in contrast to the enzyme prepared from *E. coli.* The sedimentation coefficients of our samples ranged from $s_{20,w}$ = 13 for poly(dA) · poly(dT) to 17–19 for poly[d(A-T)] · poly[d(A-T)]. The purity of synthesized samples was tested by ir, uv absorption spectroscopy, and circular dichroism (Wells et al., 1970). Our poly(dA) · poly(dT) samples enzymatically synthesized were double stranded. Satellite DNA was extracted from crab "*Cancer pagurus*" and purified by chromatography on hydroxylapatite following the method of Brzezinski et al. (1969).

Preparation of the Samples. All infrared linear dichroism measurements were made on high molecular weight polydeoxynucleotides. The samples were oriented by unidirectional stroking on a calcium fluoride or silver chloride plate as described previously (Pilet and Brahms, 1972, 1973). This procedure was sometimes carried out at 4–7° in the cold room, e.g., for poly(dI) · poly(dC), which improved

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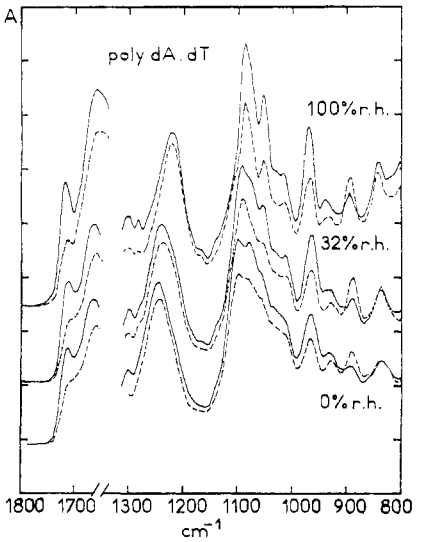

FIGURE 1: Infrared spectra of oriented poly(dA) · poly(dT) (sodium chloride content 3-4%), measured with polarized light at 15-18° in a thermostated cell. (—) Electric vector of the light perpendicular to the polynucleotide chain, (- - -) electric vector parallel to the chains. Top, high humidity form (form B*) measured at 100% r.h.; middle, low humidity form (form D_{IR}*) measured at 32% r.h.; bottom, same (D_{IR}* form) measured at 0% r.h.

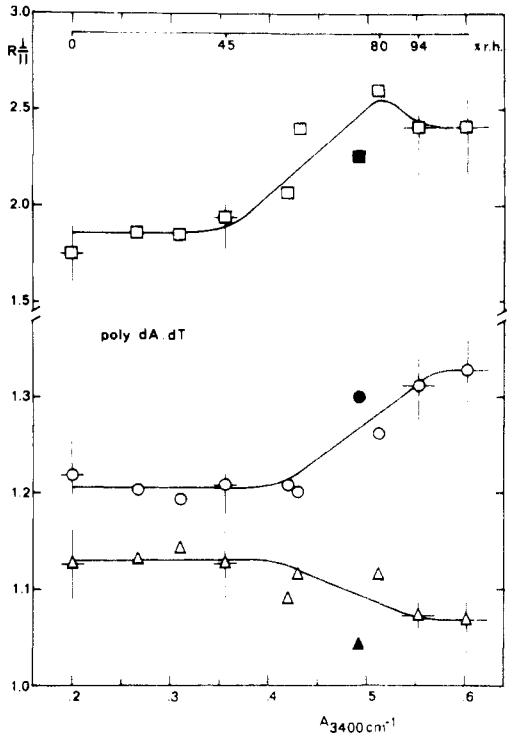

FIGURE 2: The dichroic ratio $R(\perp/\parallel)$ of poly(dA) · poly(dT) (sodium chloride content 3-4%), as a function of water content of the film, estimated from the absorbancy at 3400 cm^{-1}. (□) Band at 1710 cm^{-1}; (△) band at 1230 cm^{-1}; (○) band at 1090 cm^{-1}. The base line was measured at about 1850 cm^{-1} and corrected for the contribution of water on the basis of its absorbancy at 3400 cm^{-1}.

the result. The salt content of the sample was controlled by the previously described method (Pilet and Brahms, 1973; Pitha, 1971; Rupprecht and Forslind, 1969) of equilibrating an oriented polydeoxynucleotide film in a 73% ethanol–aqueous solution containing 0.1 *M* NaCl during several days or weeks. The salt content of the polydeoxynucleotides ranged from 3 to 4% NaCl (w/w). The above procedure has the advantage of eliminating the strongly infrared absorbing EDTA or citrate used in the course of synthesis. However, sometimes it was necessary to add a drop of doubly distilled water to the film and reorient the sample.

Infrared Measurements. The infrared linear dichroism method as applied to the study of oriented DNA films at various degrees of relative humidity has been previously described in detail (Pilet and Brahms, 1973). The dichroic ratio $R = A\perp/A\parallel$ was obtained from measurements of the spectra of oriented samples with electric *E* vector of the light polarized perpendicular and parallel to the orientation axis. The dichroic ratio R $(\perp/\parallel)$ allows one to calculate the angle θ, which the transition moment forms with the polynucleotide axis:

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

where g is the parameter which characterizes the semicrystalline state of the oriented sample and which can be related to the fraction f of perfectly oriented chains by $f = 1/(1 + \frac{3}{2}g)$. The present studies were performed at temperatures between 15 and 18° in thermostated infrared cells.

We found that even at 4° the infrared spectra of poly(dI) · poly(dC) were essentially the same as those at 18° except for some increase in band resolution at the lower temperature.

Results

Poly(dA) · Poly(dT). Figure 1 shows the polarized infrared spectra of an oriented sample of poly(dA) · poly(dT) at different relative humidities. The spectra presented in Figure 1 cover the region of base absorption at about 1700 cm^{-1} and of phosphate absorption at about 1230 and 1090 cm^{-1} (Tsuboi, 1964, 1970).

Figure 2 represents the changes in the dichroic ratio $(R(\perp/\parallel) = A\perp/A\parallel)$ of these bands as a function of hydration of the film. The hydration of the film is determined from absorbance measurements of absorbancy at 3400 cm^{-1} which is due to the water contained in the film. The curves shown in Figure 2 reveal two plateaus, each corresponding to a different DNA semicrystalline form. These two plateaus are separated by a broad transition zone which indicates the noncooperative character of the process.

The structural and infrared parameters, characteristic of these two forms, are summarized in Table I. The form observed at high relative humidities (i.e., at r.h. above 95%) is of the B type, as evidenced by a dichroic ratio, strongly perpendicular at 1090 cm^{-1} and weakly perpendicular at 1230 cm^{-1} (see Figure 1 and Pilet and Brahms, 1972, 1973). Quantitatively the angles (θ) characteristic of the orientation of PO_2^- group with respect to the helical axis are as follows: (1) The θ_{1230} angle corresponding to the direction of the (0 - - - 0) line is 57° (± 2°) which represents the usual value of the B form (Pilet and Brahms, 1972, 1973). (2) The θ_{1080} angle characteristic of the orientation of the bisector of OPO angle is 63° (± 2°) which is significantly lower than the values obtained for the B form of DNA. The latter usually yields a θ_{1090} from 68 to 74° (Pilet and Brahms, 1972, 1973). Because of this difference we denote this form B*.

The form existing at low humidity (i.e., at r.h. below 45%) is characterized by a strong perpendicular dichroism at about 1230 cm^{-1} (the orientation of the sample has slightly decreased). This low humidity form also belongs to B family. This is indicated by the θ_{1230} angle (0 - 0 line)

Table I: Infrared and Conformational Parameters of Different Forms of Poly(dA)·Poly(dT) (3–4% NaCl).

Band at about 1710 cm^{-1}			Band at about 1230 cm^{-1}			Band at about 1090 cm^{-1}		
cm^{-1}	$R(\perp/\parallel)$	f (%)	cm^{-1}	$R(\perp/\parallel)$	θ (deg)	cm^{-1}	$R(\perp/\parallel)$	θ (deg)
			High Humidity Form B* (r.h. >95%)					
1718	2.4 ± 0.2	48 ± 4	1223	1.07 ± 0.03	57 ± 2	1087	1.33 ± 0.03	63 ± 2
			Low Humidity Form D* (r.h. <48%)					
1714	1.85 ± 0.15	36 ± 4	1242–1246	1.13 ± 0.03	60 ± 2	1092–1100	1.20 ± 0.03	62 ± 2

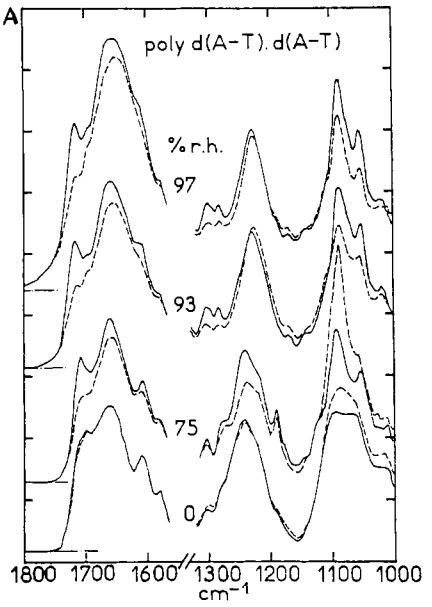

FIGURE 3: Infrared spectra of oriented poly[d(A-T)] · poly[d(A-T)], NaCl 3–4%, measured with polarized light. From top to bottom: B type form measured at 97% r.h.; contribution of C-like form at 93% r.h.; A* form measured at 75% r.h.; disordered form measured at 0% r.h. The symbols and the conditions of measurements are the same as those of Figure 1.

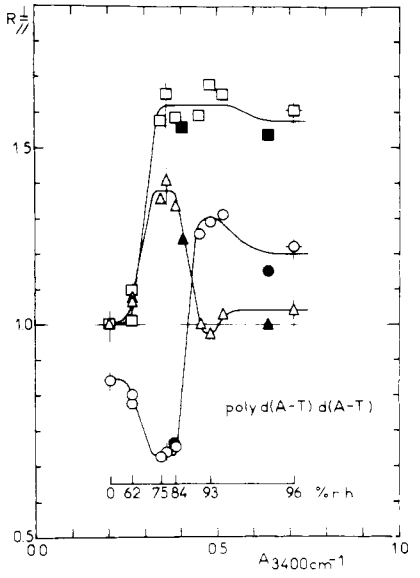

FIGURE 4: The dichroic ratio $R(\perp/\parallel)$ of poly[d(A-T)] · poly[d(A-T)] as a function of water content of the film. Open points represent the measurements at decreasing humidities and full points at increasing humidities which were done as a test of reversibility. All symbols and conditions of measurements as in Figure 2.

which changes from 57 to 60° and by the non-cooperative character of the transition between these two forms (see Figure 1). This form is different from the C form of DNA which, in contrast, is characterized by a parallel dichroism at about 1230 cm^{-1} (Brahms et al., 1973). This low humidity form may be analogous to the D form sometimes observed in the X-ray diffraction studies of poly[d(A-T)] · poly[d(A-T)] under similar conditions by Davies and Baldwin (1963). We denote this form D_{IR}*. It is remarkable that this low humidity DNA form is stable down to 0% r.h. At this humidity the frequencies of the antisymmetric and symmetric phosphate vibrations are ν_{as} (PO_2) = 1246 cm^{-1} and ν_s (PO_2) = 1100 cm^{-1}. The presence of the band at 1714 cm^{-1} is indicative of a base-paired structure (Tsuboi, 1970). The question of base pairing will be considered in more detail below. The observed stability of this helical structure during the course of dehydration is remarkable. It can be considered as indicating a much greater stability of base-paired homopolynucleotide chains d(A) · d(T) when compared to the alternating poly[d(A-T)] · poly[d(A-T)] (see below and Inman and Baldwin, 1964).

The observations described above have been reproduced on several other poly(dA) · poly(dT) samples and no transition to the A form or A family of forms has been observed.

Poly[d(A-T)] · poly[d(A-T)]. Figure 3 presents the polarized ir spectra of poly[d(A-T)] · poly[d(A-T)] at several relative humidities. The changes in the dichroic ratio of the three bands characteristic of base and phosphate vibration are plotted as a function of the relative humidity of the film in Figure 4.

These curves are very different from those of poly(dA) · poly(dT) (Figure 2) obtained under identical conditions. During the dehydration of poly[d(A-T)] · poly[d(A-T)] films one observes the following changes. (1) At high humidities, i.e., at r.h. above 95%, a B-type form is observed with strong perpendicular dichroism at 1090 cm^{-1} and weak perpendicular dichroism at 1230 cm^{-1} (Pilet and Brahms, 1972, 1973). The structural parameters calculated for this form, shown in Table II, are consistent with those observed for the B form of AT rich viral DNA (Champeil and Brahms, 1974) and are closely related to those of the B* form observed for poly(dA) · poly(dT) under identical conditions. One should notice that θ_{1090} angle (64°) is certainly smaller than the θ_{1090} of 74° found for GC rich DNA (Pilet and Brahms, 1973). (2) At intermediate humidities, about 93% and below, the curve of Figure 4 shows a minimum in the dichroic ratio at about 1230 cm^{-1}. The possibility of the simultaneous existence of both the B and C forms is suggested by the presence of this minimum of R. (3) The transition to the lower humidity form is very cooperative as judged by the steep slope of the curve at humidities between 85 and 75%. The lower humidity form is of the A type (Arnott, 1970) characterized by a strong parallel dichroism at about 1090 cm^{-1} and a strong perpendicular one at 1230

Table II: Infrared and Conformational Parameters of Different Forms of Poly[d(A–T)]·Poly[d(A–T)] (3–4% NaCl Content).

Band at about 1710 cm^{-1}			Band at about 1230 cm^{-1}			Band at about 1090 cm^{-1}		
cm^{-1}	$R(\perp/\parallel)$	f (%)	cm^{-1}	$R(\perp/\parallel)$	θ (deg)	cm^{-1}	$R(\perp/\parallel)$	θ (deg)
			High Humidity Form of B type (r.h. >95%)					
1718	1.6 ± 0.2	29 ± 7	1227	1.04 ± 0.07	57 ± 4	1088	1.2 ± 0.1	64 ± 7
			Form A* (85% > r.h. > 75%)					
1708	1.6 ± 0.2	31 ± 7	1242	1.38 ± 0.07	70 ± 7	1088	0.63 ± 0.02	34 ± 7

Table III: Infrared and Conformational Parameters of Different Forms of Crab Light DNA (3–4% NaCl).

Band at about 1710 cm^{-1}			Band at about 1230 cm^{-1}			Band at about 1090 cm^{-1}		
cm^{-1}	$R(\perp/\parallel)$	f (%)	cm^{-1}	$R(\perp/\parallel)$	θ (deg)	cm^{-1}	$R(\perp/\parallel)$	θ (deg)
			Form of Type B (r.h. >95%)					
1712	1.37 ± 0.08	20 ± 4	1225	1.02 ± 0.06	56 ± 3	1086	1.15 ± 0.06	65 ± 6
			D* Like Form[a] (r.h. ~30%)					
1708	1.13 ± 0.06	8 ± 4	1234	1.06 ± 0.06	~65	1088	1.07 ± 0.06	~65

[a] D* form is not pure at this r.h., since DNA contains more than 50% of the disordered form.

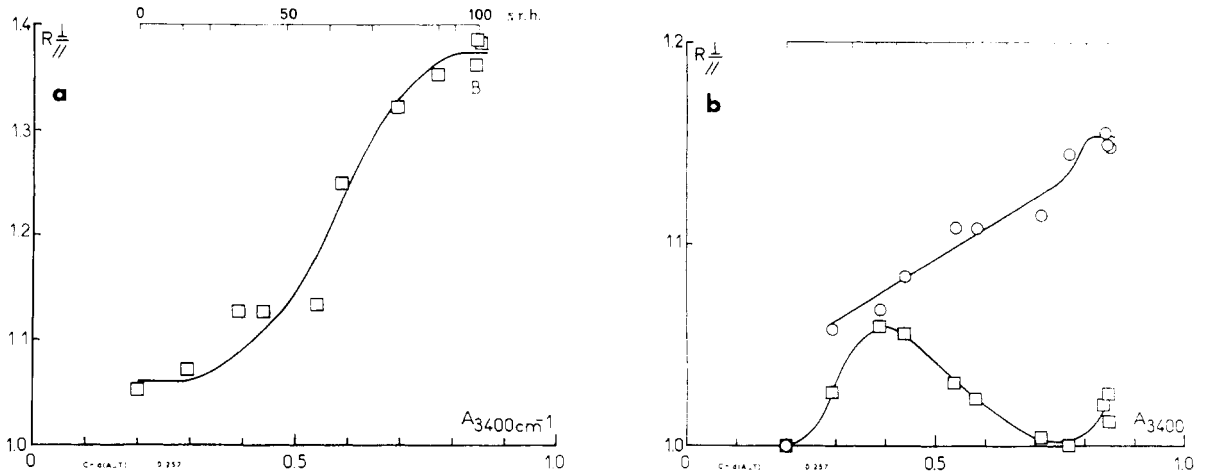

FIGURE 5: The dichroic ratio $R(\perp/\parallel)$ of crab light DNA, containing 3–4% NaCl, as a function of water content of the film. (a) Band at 1710 cm^{-1} (□), (b) band at 1230 cm^{-1} (□) and at 1090 cm^{-1} (○). Conditions of measurements as in Figure 2.

cm^{-1} (Pilet and Brahms, 1972, 1973). The parameters calculated for the orientation of the phosphate group indicate a departure from the A form usually seen with DNAs of moderate GC content. This is clearly seen from the value of the angle which the bisector of the OPO angle forms with the helical axis, θ_{1090} = 34° (± 7°), which is lower than the value of 45° (± 3°) found for the classical A form of *Micrococcus lysodeikticus* DNA (Pilet and Brahms, 1972, 1973). For this reason we call this modified A form, A*. However, the value of θ_{1090} = 34° for the A* form is in relatively close agreement with the A form value of RNA, θ_{1090} = 40° (Brahms et al., 1969; Sato et al., 1966; Pilet and Brahms, 1974, in preparation). The stability of this A form is restricted to a limited region of r.h. when compared to other GC rich DNA (see Pilet and Brahms, 1972, 1973) which have much more extended plateau to lower r.h. This form reappears reversibly when increasing relative humidity. (4) A transition to a disordered form is observed at humidities below 65%. This transition occurs at a higher r.h. than the corresponding transition for GC containing DNAs. The process is perfectly reversible and upon rehydration no hysteresis is observed.

Light Satellite Crab DNA. It is interesting to compare the conformational transitions of synthetic A-T polydeoxynucleotides with those of the light satellite DNA extracted from *Crab cancer pagurus*. The latter DNA is essentially poly[d(A-T)] · poly[d(A-T)] with 3–5% GC and about 7% homoadenylate and homothymidylate nonalternating sequences (Ehrlich et al., 1973; Simon et al., 1970). At high humidities (r.h. >95%) the values of the dichroic ratios are very similar to those of the synthetic AT polymers and the calculated structural parameters, shown in Table III, are those of a B type form. It is difficult to decide if this form is a classical B form or a B* like form, similar to that of poly(dA) · poly(dT) and poly[d(A-T)] · poly[d(A-T)] with which the values of Table III are in better agreement.

Decreasing the humidity below 95% results in a progressive decrease in the orientation of the film. In addition base pairing also decreases as judged by changes in the dichroic ratio of the band at 1710 cm^{-1} (Figure 5a). The change observed in the phosphate bands between 95 and 80%, i.e., the decrease of the dichroic ratio, at 1230 cm^{-1} (although the dichroism at 1090 cm^{-1} remains perpendicular), is suggesting a C form contribution.

Table IV: Infrared Frequencies of Base Pairing.[a]

Crab Light DNA 3–4% NaCl 100% r.h. D_2O ν_{max} (cm^{-1})	Poly[(d(A-T)]·Poly[(d(A-T)] 3–4% NaCl 100% r.h. D_2O ν_{max} (cm^{-1})	Poly[(d(A-T)]·Poly[(d(A-T)] 3–4% NaCl 75% r.h. D_2O ν_{max} (cm^{-1})	Poly(dA)·Poly(dT) 3–4% NaCl ν_{max} (cm^{-1})	dAMP in D_2O (cm^{-1})	dTMP in D_2O (cm^{-1})
1692 (s)	1695 (s)	1693 (s, br)	1693 (s, br)		1690 (s, br)
1665 (s)	1664 (s)	1664 (s)	1670 (s)		1663 (s)
1644 (sh)	1643 (sh)	1643 (sh)	1644 (sh)		1632 (s)
1621 (s)	1620 (s)	1620 (s)	1624 (s)	1627 (s)	
1576 (w)	1575 (w)	1575 (w)	1575 (w)	1578 (w)	
1483 (m)	1483 (m)	1480 (m)	1484 (m)	1484 (m)	1483 (m)
1378 (w)	1379 (w)	1376 (v.w.)	1379 (w)		
1306 (m)	1304 (m)	1304 (m)	1304 (m)		

Poly(A)·2Poly(U) + Poly(A) D_2O[a]	Poly(A-U)·Poly(A-U) D_2O[c]	Poly(A)·Poly(U) D_2O[b]	Poly(A)[b] in D_2O	Poly(U)[b] in D_2O
1696 (s)	1690 (m)	1691 (s)		1692 (m)
1677 (m, sh), 1657 (s)	1665 (s)	1672 (s)		1657 (s)
1628 (s)	1646 (m), 1618 (m)	1631 (s)	1628 (s)	
1569 (w)	1567 (w)	1569 (w)	1575 (w)	

[a] (s) strong; (m) medium; (w) weak; (v.w.) very weak; (sh) shoulder; (br) broad. [b] Miles and Frazier, 1964. [c] Morikawa et al., 1973.

At intermediate humidities, i.e., between 80 and 30%, the dichroism at 1230 cm^{-1} increases strongly whereas the dichroism at 1090 cm^{-1} remains perpendicular. This indicates formation of the D_{IR}* form similar to that previously observed for poly(dA) · poly(dT) under identical conditions. The parameters calculated for this form, shown in Table III, must be considered qualitative because of the small degree of sample orientation, particularly at the lower humidities. At humidities below 95%, the base pairing is far from complete. All structural changes discussed above have been reproduced on several samples and no transition to the A form has been observed.

Poly(dI) · Poly(dC). At high humidities, i.e., at 100%, one observes the presence of a B type form characterized by a perpendicular dichroism at about 1090 cm^{-1} and almost no dichroic band at 1230 cm^{-1} (Figure 6). This form is conserved until 75% r.h. but as the humidity is lowered further the orientation decreases and finally disappears. In general, samples having low degrees of orientation (f <10%) can only be described qualitatively, but the data still allow a comparison with the AT containing polynucleotides. At 100% r.h., one observes a B type form, characterized by a perpendicular dichroism at 1090 cm^{-1} and almost no dichroism at 1230 cm^{-1} (Figure 6). This form is conserved till 76% r.h., but the organization of the sample disappears at lower humidities. No A form or A family form is observed. The transition observed for poly(dI) · poly(dC) are very similar to the conformational transitions observed for the AT containing polydeoxynucleotides, particularly poly(dA) · poly(dT).

Base-Pairing Configurations. We have studied the base-pairing configurations of poly[d(A-T)] · poly[d(A-T)], poly(dA) · poly(dT), and poly (dI) · poly(dC).

Poly[d(A-T)] · poly[d(A-T)] is known from X-ray diffraction studies to adopt A and B configurations essentially isomorphous to those of DNAs having moderate GC contents (Davies and Baldwin, 1963). One may conclude that the Watson and Crick base pairing is obeyed.

The spectrum of poly(dA) · poly(dT) in a D_2O atmosphere is slightly different from that of poly[d(A-T)] · poly[d(A-T)] in the 1600–1700 cm^{-1} region (Table IV). The strong d(A-T) · d(A-T) band at 1664 cm^{-1} is displaced to 1670 cm^{-1} in dA · dT and the band at 1620 cm^{-1} shifts to 1624 cm^{-1}. Differences of the same nature but even more pronounced have been observed between the spectra of the analogous polyribonucleotides poly(A-U) · poly(A-U) and poly(A) · poly(U) in D_2O (Morikawa et al., 1973). The strong (A-U) · (A-U) band at 1665 cm^{-1} shifts to 1672 cm^{-1} in A · U and the band at 1618 cm^{-1} merges with the 1646 band to form one band at 1631 cm^{-1} in A · U. It has been shown that both (A-U) · (A-U) and poly(A) · poly(U) have Watson and Crick base pairing. The spectral differences we have described above can be attributed to differences in stacking between A-A, U-U, and A-U and consequently to differences in vibrational coupling. Similar reasoning suggests that the spectrum observed for poly(dA) · poly(dT) is thus perfectly compatible with Watson-Crick base pairing of adenine and thymine.

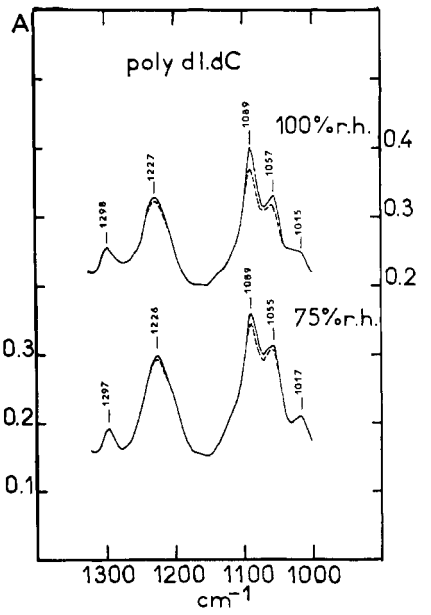

FIGURE 6: Infrared spectra of oriented poly(dI) · poly(dC), NaCl 3–4%, measured with polarized light. (—) B type form measured at 100% r.h. (top) and at 75% r.h. (bottom). Symbols and conditions as in Figure 1.

Table V: Infrared Spectral Characteristics of I and C Containing Polynucleotides in the Base Absorption Region.

Poly(dI)·Poly(dC)	Poly(I)·Poly(C)		Poly(I)	Poly(C)
ν_{max} (cm^{-1})[a]	ν_{max} (cm^{-1})[b]	ν_{max} (cm^{-1})[b]	ν_{max} (cm^{-1})[c]	ν_{max} (cm^{-1})[c]
1690 (sh)	1690 (s)	1697 (s)	1677 (s)	
1652 (s)	1652 (s)	1648 (s)		1653 (s)
1630 (sh)	1630 (sh)	1630 (sh)		1617 (m)
1546 (v.w.)	1546 (s)			
1526 (s)	1530 (m)			
1510 (s)	1505 (s)			

[a] Film 4% NaCl w/w exposed to 100% r.h. D_2O (studied in this work). [b] Film exposed to 75% r.h. D_2O. [c] D_2O solution, 5×10^{-2} *M* NaCl–10^{-2} *M* cacodylate (Miles and Frazier, 1964).

The formation of three stranded structures of the type poly(dT) · poly(dA) · poly(dT) should also be considered since the analogous ribopolymer undergoes such a reaction under some conditions. Therefore we have compared the IR spectrum of our sample poly(dA) · poly(dT) with the spectra of double-stranded poly(A) · poly(U) and triple-stranded poly(U) · poly(A) · poly(U). These two polyribonucleotide structures exhibit large spectral differences (Miles and Frazier, 1964). The most important difference is the shift of the main band from 1657 cm^{-1} in poly(U) · poly(A) · poly(U) to 1672 cm^{-1} in poly(A) · poly(U) (Miles and Frazier, 1964). Our sample of poly(dA) · poly(dT) exhibits a band at 1670 cm^{-1} (Table IV) which closely matches the frequency of the band of poly(A) · poly(U).

It is also important to notice that the conformational stability of poly(dA) · poly(dT) as a function of temperature and ionic strength is much greater than that of poly(A) · poly(U). In solutions at 20° poly(dA) · poly(dT) is stable at ionic strengths up to 0.1 *M* Na^+ whereas the ribopolymer forms a triple-stranded structure at concentrations above 10 m*M* Na^+ (Riley et al., 1966).

We thus conclude that poly(dA) · poly(dT) has the Watson and Crick base pairing, similar to poly[d(A-T)] · poly[d(A-T)], and that the existence of small spectral shifts discussed above reflects differences in the vibrational coupling between stacked bases in homopolynucleotide sequences as opposed to alternating sequences (Morikawa et al., 1973).

For the reversed Hogsteen base pairing, one could expect a completely different spectrum similar to the situation observed for the adenosine-thymine riboside crystal (Sakore et al., 1969). Consequently reversed Hogsteen base pairing is excluded.

We have also studied the base pairing geometry of poly(dI) · poly(dC). Table V shows the comparison of characteristic ir absorption frequencies of poly(dI) · poly(dC) with poly(I) · poly(C), poly(I) and poly(C) in the region of base absorption. This comparison leads to the following observations. (1) The maximum absorption frequencies of poly(dI) · poly(dC) are different from those of poly(I) or poly(C) which suggests that base pairing does exist. (2) The spectrum of poly(dI) · poly(dC) exhibits the features of less base pairing, as one can observe from the decrease of intensity of the 1690- and 1546-cm^{-1} bands. These two bands are characteristic of base paired I and C following the Watson–Crick base pairing (Tsuboi et al., 1968; Miles, 1961). Thus the spectra of poly(dI) · poly(dC) do not yield any indications of different base pairing. The relatively weak base pairing of poly(dI) · poly(dC) is in good agreement with its very low thermal stability, the lowest of all polydeoxynucleotide (Inman and Baldwin, 1964).

Discussion

Importance of Base Sequence. The results of this investigation indicate significant differences between the conformation and conformational transitions of complementary homopolymer DNAs and alternating sequence DNAs. These differences provide direct evidence that nucleic acid base sequence is an important determinant of nucleic acid conformational properties. To summarize, the following infrared evidence indicates the effect of sequence on DNA conformation and DNA conformational transitions. (1) The conformational transitions which poly[d(A-T)] · poly[d(A-T)] undergoes as the r.h. is decreased are completely different from those that poly d(A) · d(T) undergoes. The latter DNA does not undergo the B → A transition. The alternating sequence DNA, poly[d(A-T)] · poly[d(A-T)], changes from B form at high humidity to A form at lower humidities which has a limited range of stability. (2) In contrast, the complementary homopolymer poly(dA) · poly(dT) helix does not adopt the A form. At high humidities the B form is the stable form (above 95%) and after a long transition without appearence of the A form, a new form, D_{IR}*, appears which is stable from 45% r.h. to 0%. Even at 0% r.h., the D_{IR}* form has well-expressed base pairing indicating remarkable stability. (3) Poly(dI) · poly(dC) seems also incapable of undergoing the B → A transition.

These data strongly suggest that the presence of homo(dA) · homo(dT) sequences is not compatible with the B → A transition since the A form does not appear at lower humidities for either of the complementary homopolymers studied.

The inability of these double helices composed of complementary homopolymers to undergo the B → A conformational transition is in good agreement with the preliminary X-ray diffraction observations. On the basis of our data, one may suggest that DNAs containing significant amounts of homo(dA) · homo(dT) sequences will have conformational transitions that are altered with respect to those occurring in DNAs containing alternating or statistically random base sequences. In particular, the presence of localized homo(dA) · homo(dT) regions would be expected to perturb the B → A transition and perhaps even prevent the formation of the A conformation. This appears to be the case for several viral and bacterial AT-rich DNAs and also for the *B. subtilis* DNA which is known to contain long oligo(dA) and oligo(dT) segments (Rudner et al., 1972). In fact it was shown previously that these DNAs are undergoing a transition from the B- to a C-like form at lower r.h. and under identical salt conditions (Brahms et al., 1973; Champeil and Brahms, 1974).

The observed absence of the B → A transition in satellite DNA from crab (Figure 5) is explained by the presence of nonalternating A and T sequences in the amount of about 7% (Ehrlich et al., 1973; Skinner, 1967; Simon et al., 1970) and by the limited stability of the A form of alternating A-T sequences.

The demonstration that the B → A transition is inhibited in homopolynucleotide sequences such as poly(dA) · poly(dT) and poly(dI) · poly(dC) may have important functional consequences, e.g., for the control of transcription. However it does not seem necessary to invoke the hypothesis of the B → A transition as a general prerequisite

for transcription (Arnott et al., 1968) which was proposed on the basis of analogy with the A form of DNA–RNA hybrid (Milman et al., 1967). The mechanism of control of the transcription process might be more subtle, and may be primarily related to the structural peculiarity of these homopolymer sequences that are unable to undergo B → A transition and which may serve as promotor regions. In agreement with the proposal of Szybalski et al. (1966) long oligopyrimidine sequences comprise part of the promotor regions and may serve as signal for the initiation of transcription. In such regions a transition from B to C (or B → D_{IR}*), i.e., to a deformed B form may rather occur upon binding of protein (Brahms et al., 1973). Further structural investigations are necessary to provide a more solid basis for the understanding of the mechanism of transcription.

Alteration of Form with Base Composition. It is remarkable that all AT containing polydeoxynucleotides adopt at high humidity conformation of the B* type. It is generally accepted that the forms B and A do not depend on base composition (Hamilton et al., 1959). Our results at high r.h. indicate a slight rotation of the phosphate group for all AT polydeoxynucleotides (Tables I–III), when compared to DNA of medium GC content (Pilet and Brahms, 1972). Particularly the angle which the bisector of the OPO angle forms with the helical axis is about 64° whereas that of DNA B form is of about 68–70°. This change of phosphate orientation is systematically observed under constant conditions of salt content. The main factor responsible for the departure from the B form of DNA seems to be the base composition. This alteration of phosphate orientation may be considered as evidence of a modified B form which is in agreement with an ir investigation on some viral DNA (Champeil and Brahms, 1974).

The A form of poly[d(A-T)] · poly[d(A-T)] is well expressed at lower humidities but also slightly changed and of limited stability range when compared to the A form of DNA of medium GC content (Pilet and Brahms, 1973).

In conclusion, the results of polydeoxynucleotide investigations indicate that the base composition is an important factor responsible for modification of the B form, whereas the base sequence may determine the occurrence of B → A structural transition; this B → A change is suppressed in non-alternating homopolynucleotide A,T sequences. The nonoccurrence of B → A transition observed in AT rich DNA is thus explained by the absence of the A form in homo(dA) and homo(dT) sequences and by the relatively small stability of the A form in poly[d(A-T)].

Added in Proof

After this article was submitted for publication two articles by Arnott et al. appeared (Arnott and Selsing, 1974; Arnott, et al., 1974). Their X-ray diffraction results on poly[d(A-T)] · poly[d(A-T)] and poly[d(A)] · poly[d(T)] are in agreement with the results obtained in the present ir study. In particular, Arnott et al. found, as we have, that poly[d(A)] · poly[d(T)] does not adopt the A form of DNA. In addition, they also found the high humidity conformation of both these polydeoxynucleotides to be distinct from the classical B and C forms of DNA. In agreement, we find the phosphate group orientation in the high humidity forms of these two polymers is different from that of classical B form of DNA. Our finding that all these polynucleotides are Watson–Crick base paired is also in agreement with these X-ray data. In addition, we have found a new stable form of poly[d(A)] · poly[d(T)] called D_{IR}* at low humidity.

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References

Arnott, S. (1970), *Prog. Biophys. 21,* 265.
Arnott, S., Chandrasekaran, R., Hukins, D. W. L., Smith, P. J. C., and Watts, L. (1974), *J. Mol. Biol. 88,* 523–533.
Arnott, S., Fuller, W., Hodgson, A., and Prutton, I. (1968), *Nature (London) 220,* 561.
Arnott, S., and Selsing, E., (1974), *J. Mol. Biol. 88,* 509–521.
Brahms, J., Maurizot, J. C., and Pilet, J. (1969), *Biochim. Biophys. Acta 186,* 110.
Brahms, J., Pilet, J., Tran-Thi, P. L., and Hill, R. L. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3352.
Bram, S. (1971), *Nature (London), New Biol. 233,* 161.
Bram, S., and Tougard, P. (1972), *Nature (London), New Biol. 239,* 128.
Brzezinski, A., Szafranski, P., Johnson, P. H., and Laskowski, M., Sr. (1969), *Biochemistry 8,* 1228.
Burd, J. F., and Wells, R. D. (1970), *J. Mol. Biol. 53,* 435.
Champeil, Ph., and Brahms, J. (1974), *Eur. J. Biochem. 45,* 253.
Davies, D. R., and Baldwin, R. L. (1963), *J. Mol. Biol. 6,* 251.
Ehrlich, S. D., Thiery, J. P., and Bernardi, G. (1973), *Biochim. Biophys. Acta 312,* 633.
Hamilton, D. M., Barclay, R. K., Wilkins, M. H. F., Brown, G. L., Wilson, H. R., Marwin, D. A., Ephrussi-Taylor, A., and Simmons, N. S. (1959), *J. Biophys. Biochem. Cytol. 5,* 397.
Harwood, J. J., Schendel, P. F., and Wells, R. D. (1970), *J. Biol. Chem. 245,* 5614.
Inman, R. B., and Baldwin, R. L. (1964), *J. Mol. Biol. 8,* 452.
Langridge, R. (1969), *J. Cell. Physiol. 74,* 1.
Miles, H. T. (1961), *Proc. Natl. Acad. Sci. U.S.A. 47,* 791.
Miles, H. T., and Frazier, J. (1964), *Biochem. Biophys. Res. Commun. 14,* 21.
Milman, G., Chamberlain, M., and Langridge, R. (1967), *Proc. Natl. Acad. Sci. U.S.A. 57,* 1804.
Morikawa, K., Tsuboi, M., Takahashi, S., Kyogoku, Y., Mitsui, Y., Itaka, Y., and Thomas, G. R., Jr. (1973), *Biopolymers 12,* 799.
Pilet, J., and Brahms, J. (1972), *Nature (London), New Biol. 236,* 99.
Pilet, J., and Brahms, J. (1973), *Biopolymers 12,* 387.
Pitha, J. (1971), *Biochim. Biophys. Acta 232,* 607.
Riley, M., Malingn B., and Chamberlin, M. J. (1966), *J. Mol. Biol. 20,* 359.
Rudner, R., Ledoux, M., and Mazels, A. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2745.
Rupprecht, A., and Forslind, B. (1969), *Biochim. Biophys. Acta 186,* 110.
Sakore, T. D., Tavale, S. S., and Sobell, H. M. (1969), *J. Mol. Biol. 43,* 361.
Sato, T., Kyogoku, Y., Higuchi, S., Mitsui, Y., Iitaka, Y., Tsuboi, M., and Miura, K. (1966), *J. Mol. Biol. 16,* 180.

Schachman, H. K., Adler, J., Radding, C. M., Lehman, I. R., and Kornberg, A. (1960), *J. Biol. Mol. 235,* 3242.
Simon, M., Ohki, I., Chang, H. C., Lohr, K., and Laskowski, M., Sr. (1970), *Biochim. Biophys. Acta 224,* 253.
Skinner, D. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 103.
Szybalski, W., Kubinski, H., and Sheldrick, P. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31,* 129.
Tsuboi, M. (1964a), *Biopolym. Symp. 1,* 527.
Tsuboi, M. (1964b), *J. Polymer. Sci. 7,* 125.
Tsuboi, M. (1970), *Appl. Spectrosc. Rev. 1,* 45.
Tsuboi, M., Matsuo, K., and Nakanishi, M. (1968), *Biopolymers 6,* 123.
Wells, R. D., Larson, J. E., Grant, R. C., Shortle, B. E., and Cantor, C. R. (1970), *J. Mol. Biol. 54,* 465.
Yamagashi, H., and Takahashi, I. (1971), *J. Mol. Biol. 57,* 369.

Conformations and Interactions of Histone H2A (F2A2, ALK)†

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ABSTRACT: Conformational changes in histone H2A (ALK, F2A2, IIb1) as a function of ionic strength and pH have been followed using high resolution nuclear magnetic resonance (NMR), circular dichroism (CD), and infrared (ir). While change in pH from 3 to 7 (no added salt) causes little structural change, added salt induces the formation of both α helix (28% maximum) and intermolecular associates in the region of the molecule between 25 and 113. No β structure was observed at high salt. By the use of different salts it was shown that the structural changes were due largely to nonspecific counterion screening by the added anion. Comparison of observed with simulated NMR spectra has led to the proposal that an ionic strength dependent equilibrium exists between largely unstructured coil molecules and fully structured and aggregated molecules. NMR spectra of H2A obtained in the presence of DNA showed that both the N- and C-terminal regions bind to DNA, i.e., not the portion of the chain that is involved in interhistone interactions.

The sequence determinations of histones[1] H4 (F2A1), H2A (F2A2), H2B (F2B), and H3 (F3) and the partial sequence and compositions of peptides of H1 (F1) listed in Croft (1973) have all shown that histones have a marked asymmetry in the distribution of amino acids along the polypeptide chains. All histone sequences contain well-defined regions which are rich in basic residues and the helix destabilizing residues proline, serine, and glycine. The regions complementary to these basic segments contain a high proportion of the helix favoring apolar residues, aromatic, acidic residues, and relatively few basic residues. These factors have led to the suggestion that the basic segments are the primary sites for interactions with DNA while the apolar segments contain the potential for secondary conformations and act as sites for histone–histone or for other chromosomal–protein interactions. Evidence for these interactions has been obtained from high-resolution nuclear magnetic resonance (NMR) studies of histones H1, H4, and H2B (Boublik et al., 1970a,b; Bradbury and Rattle, 1972).

The nonuniformity in the distribution of residues in the calf thymus H2A sequence (Yeoman et al., 1972; Sautière et al., 1974) is shown in Table I which gives the composition of each quarter of the molecule. The amino quarter 1–32 is very basic with a net positive charge of +10, contains 10 helix-destabilizing residues (glycine + serine + proline), and only three of the apolar residues, leucine, valine, and isoleucine. (The division of residues into the three categories helix breaker, indifferent, and helix forming is given, for example, in Lewis and Scheraga, 1971.) In contrast the second quarter 33–65 contains only three basic residues which together with four glutamic acid residues gives a net negative charge of −1; it contains four helix destabilizing residues and 11 apolar residues. The third quarter has a similar character to the second though the net charge is +3. The final quarter is mixed, having an apolar nature from 98 to 116 (eight apolar and no basic residues), while from 117 to 129 it is very basic with six lysines and three helix destabilizing residues. A more detailed analysis of sequence-conformation relations is to use the predictive methods developed by Scheraga and his coworkers. These have been applied to histones (Lewis and Bradbury, 1974) and in the case of calf H2A (Figure 1) at neutral pH the plot of the probability that residue i is helical against the residue number i contains a strong maximum between residues 47 and 67 with a weaker maximum in the region of residue 84. The

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[1] The new histone nomenclature used here was accepted by the participants at the CIBA Foundation Symposium on the Structure and Function of Chromatin, April 3–5, 1974. This new nomenclature which has been proposed to the appropriate international nomenclature committee is as follows for each histone, where the previous names are given in parentheses: H1 (F1, I, KAP); H2A (F2A2, IIb1, ALK); H2B (F2B, IIb2, KSA); H3 (F3, III, ARK), H4 (F2A1, IV, GRK), and H5 (F2C, V, KAS).