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Circular Dichroic Probes for Aspects of Chromatin Structure: Aromatic Polycationic Ionen Probes

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Summary. Two ionens (II and X) formed complexes with DNA and chromatin with extrinsic CD bands and reduced intrinsic bands. The salt and urea sensitive, AT-specific probe (II) gave $\Delta\varepsilon$ >100 L-(residue II)$^{-1}$-cm^{-1} with DNA and $\Delta\varepsilon$=0-14 with chromatin; II reduced the intrinsic bands from $\Delta\varepsilon\simeq$0.7 to $\Delta\varepsilon\simeq$0.5. Ionen X gave $\Delta\varepsilon_{345}$=30 with DNA, and $\Delta\varepsilon_{345}$=15-20 with chromatin. X reduced the intrinsic band to ~1.6. X show less base specificity. Extrinsic $\Delta\varepsilon_\lambda$ of X increased linearly to r (residue/phosphate) = 0.5 for DNA and only 0.3 in chromatin. DNA in chromatin may have ~10% of the II and 50-60% of the X binding sites and those in an altered conformation.

Evidence has been advanced that the histones are associated with the major groove in the DNA of various preparations of chromatin.[1,2] Among the various physical methods CD spectra of both chromatin and DNA complexed with modified polyamines indicated the presence of free minor groove regions in the chromatin DNA.[3,4] We report here new sensitive CD probes which (1) give intense extrinsic bands, (2) appear to be sensitive to base composition, (3) appear to be differentiate between minor and major grooves, and (4) are sensitive to DNA secondary structure. These polycationic ionen probes (ionens II and X for structures, see inserts of figures) yield both qualitative and quantitative information. We had reported that some polycationic ionen polymers with aromatic chromophores give intense extrinsic bands in DNA complexes.[5]

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Experimental

The ionens II and X were prepared by the method of Rembaum and coworkers.[6] Separation of oligomers was effected on Sephadex CM-50 with a salt gradient. Calf thymus DNA was purchased from Sigma, A_{260}/A_{230} = 2.3. The deoxynucleotide polymers were obtained from Sigma, Miles, and from Boehringer-Mannheim.

The water-soluble, high viscosity, extended type of rabbit thymus chromatin was prepared by standard techniques.[1] The salt soluble, low viscosity, compact type rabbit thymus chromatin was prepared from rabbit thymus nuclei via an endogenous nuclease according to the method of Krueger and coworkers.[7] It was a polynucleosomal type of complex with a DNA molecular weight of 2 to 10 X 10^6 daltons.

CD measurements were done on a Cary 60 spectropolarimeter with the Model 6002 CD attachment. The salt soluble chromatin measurements were done in 0.04 M KCl and the water soluble in deionized water.

The $\Delta\varepsilon_{232.5}$ values were calculated after substracting out the expected contribution of the DNA or chromatin at 232.5 nm by interpolating the observed control profile through the region of the extrinsic band.

Results

Both ionens II and X gave extrinsic CD bands when complexed with DNA. Both II and X are themselves achiral. Ionen II gave an intense ($\Delta\varepsilon_{232.5}$ > 120 L-(residue II)$^{-1}$ cm^{-1} with purified oligomers) sharp band at λ = 232.5 nm [Fig. 1 (-Δ-Δ-) and Table I]. Ionen X complexed with DNA gave a broader envelope of peaks at 300-360 nm ($\Delta\varepsilon_{345}$=30 L-(residue X)$^{-1}$ -cm^{-1}) with no extrinsic contribution at λ = 232.5 (Fig. 2 (-Δ-Δ-) and Table 1). The extrinsic $\Delta\varepsilon$ values were a linear function of r in both cases.

Ionen II ($\bar{n} \simeq 10$) produced a reduced extrinsic signal ($\Delta\varepsilon_{232.5}$) with either type of chromatin preparations. We show here (Fig. 3) the largest signal observed in any of our chromatin preparations; the $\Delta\varepsilon_{232.5}$ L-(residue II)$^{-1}$ -cm^{-1} values were 9.2% (r = 0.076) and 14.4% (r = 0.256)

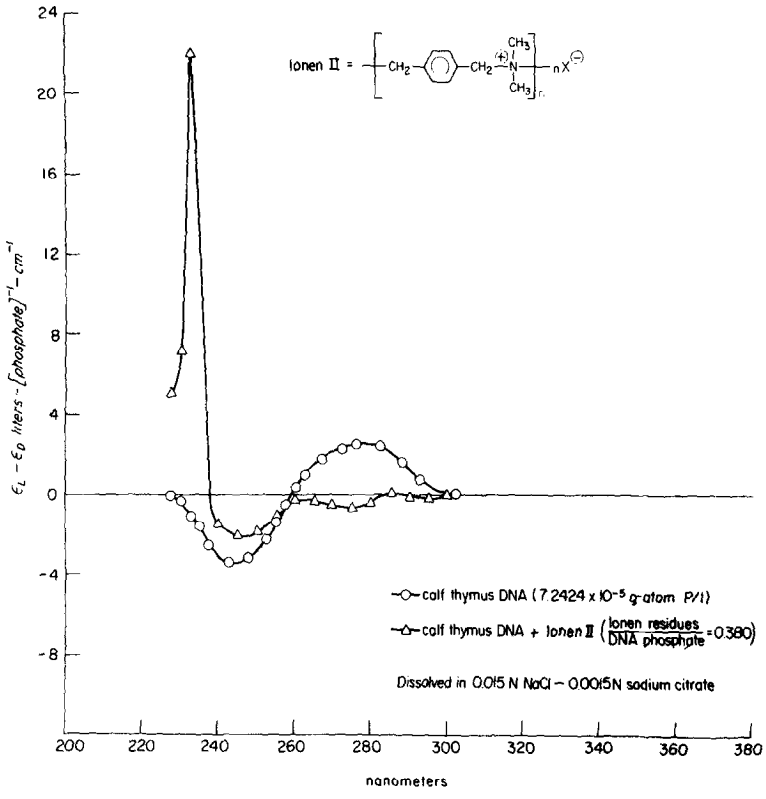

Figure 1. Circular Dichroic (CD) Spectra of DNA and of Ionen Polymer II - DNA Complex. See inset for structure and conditions.

that of the DNA-II complex at the same r values. Two effects were consistently seen with both types of chromatin preparations: (1) lowering of the positive band at >260 nm from $\Delta\varepsilon \simeq 0.7$ L-(phosphate)$^{-1}$ -cm^{-1} to $\Delta\varepsilon \simeq -0.5$ at r = 0.3 and (2) production of turbidity at higher r values (>0.3 for salt-soluble preparations, >0.2 for water-soluble preparations) accompanied by changes in the protein CD band region of 208-222 nm.

Ionen X when complexed with chromatin afforded extrinsic bands at 300-360 nm (Fig. 4). The $\Delta\varepsilon_{300\text{-}360}$ (L-(phosphate)$^{-1}$ -cm^{-1}) increased with r (Fig. 5) reaching a limiting value by r = 0.3. Up to r = 0.3, $\Delta\varepsilon_{345}$ (L-(phosphate)$^{-1}$ -cm^{-1}) for the salt-soluble chromatin was 60-70% that of DNA and ~50% for the water extracted chromatin (not shown). The profile of

Table I

Extrinsic CD Band Intensities in Ionen-Polydeoxynucleotide Complexes

Ionen	Polydeoxy-nucleotide	r[a]	λ	$\Delta\varepsilon_\lambda$[b]
II(n=8)	DNA	0.145	232.5nm	127
	poly (dAdT)	0.136		136
	poly dA-poly dT	0.14		182
	poly (dGdC)	-[c]		0
	poly dG-poly dC	-[c]		0
X($\bar{n}$=5)	DNA	ALL	345nm	30
	poly (dAdT)	0.13		33
		0.30		33
	poly dA-poly dT	0.14		38
		0.32		37
	poly (dGdC)	0.14		15
		0.38		14
	poly dG-poly dC	0.21		8.3
		0.43		7.1

a) r = ratio of ionen repeating residues to DNA phosphates
b) $\Delta\varepsilon_\lambda$ units = L - (ionen residue)$^{-1}$ -cm^{-1}.
c) $\Delta\varepsilon_\lambda$ was 0 at all values of r examined (0-0.5).

the envelope of peaks constituting the extrinsic CD signal is different for DNA than chromatin, but about the same for both types of chromatin preparations. Intensities of extrinsic bands with homo- and alternating polymers of deoxy AT and deoxy GC are shown in Table I. On the one hand, ionen II octamer gave a large positive $\Delta\varepsilon_{232.5}$ with poly dT · poly dA and poly d(AT) and no detectable extrinsic band with poly dC · poly dG. On the other hand, ionen X ($\bar{n}$=5) afforded large positive $\Delta\varepsilon_{>300}$ with poly-

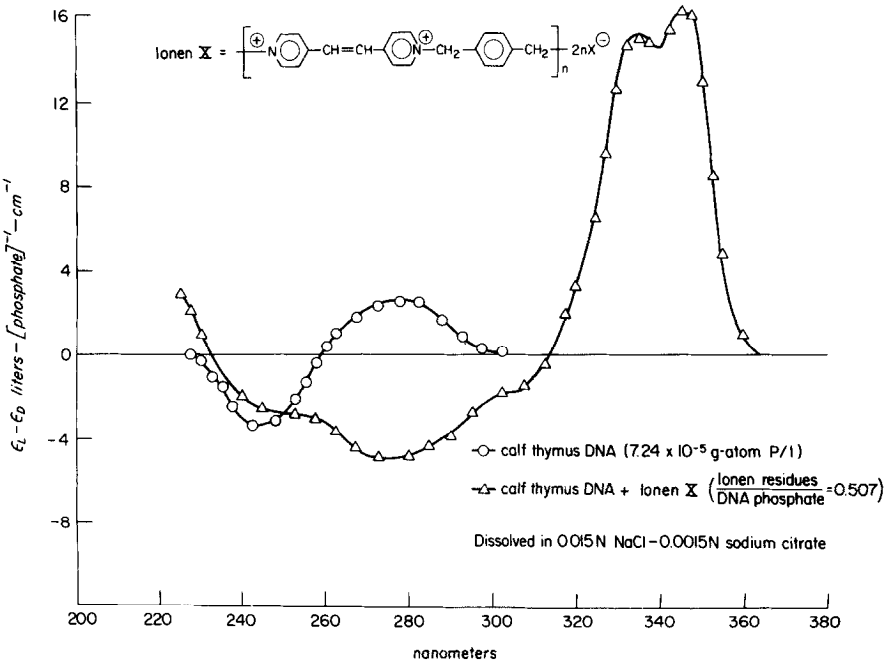

Figure 2. CD Spectra of DNA and of Ionen Polymer X - DNA Complex. See inset for structure and conditions.

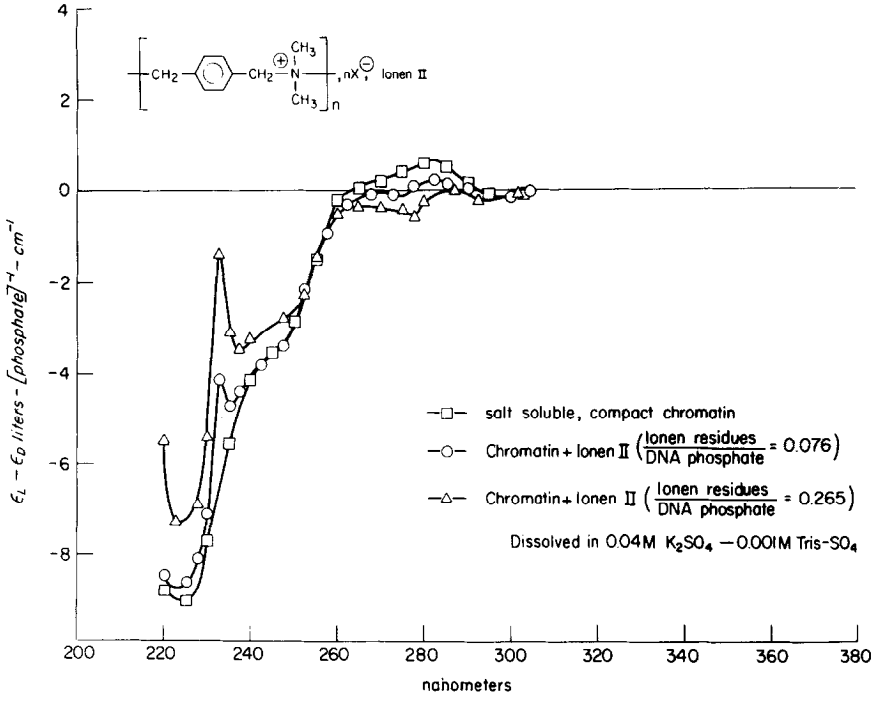

Figure 3. CD Spectra of Chromatin and Chromatin plus Ionen II. The compact chromatin was 3.75 · 10^{-5} M in DNA phosphate and the r values were as shown in the inset.

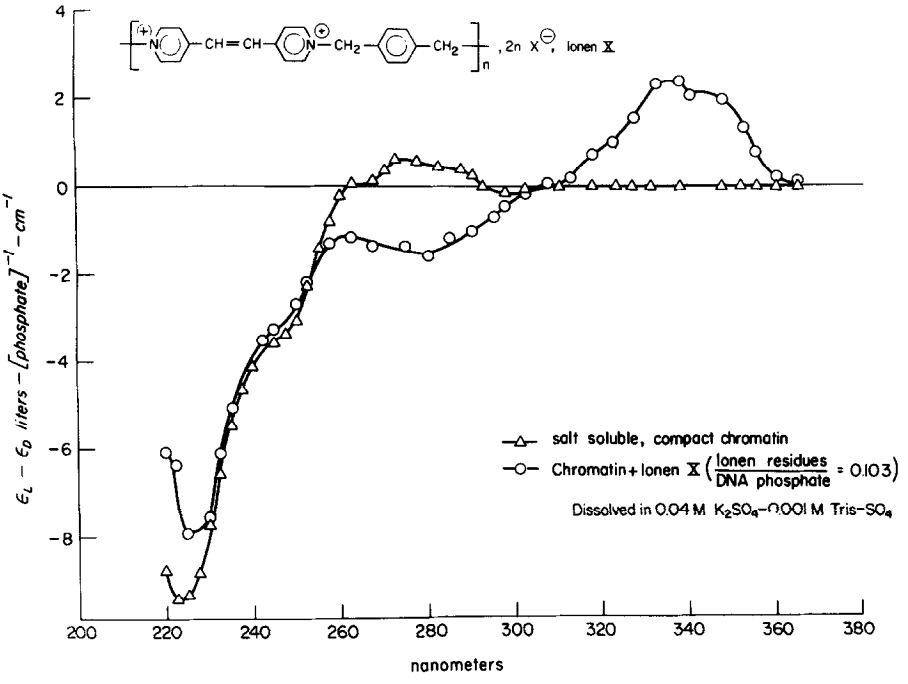

Figure 4. CD Spectra of compact Chromatin and compact Chromatin plus Ionen X.

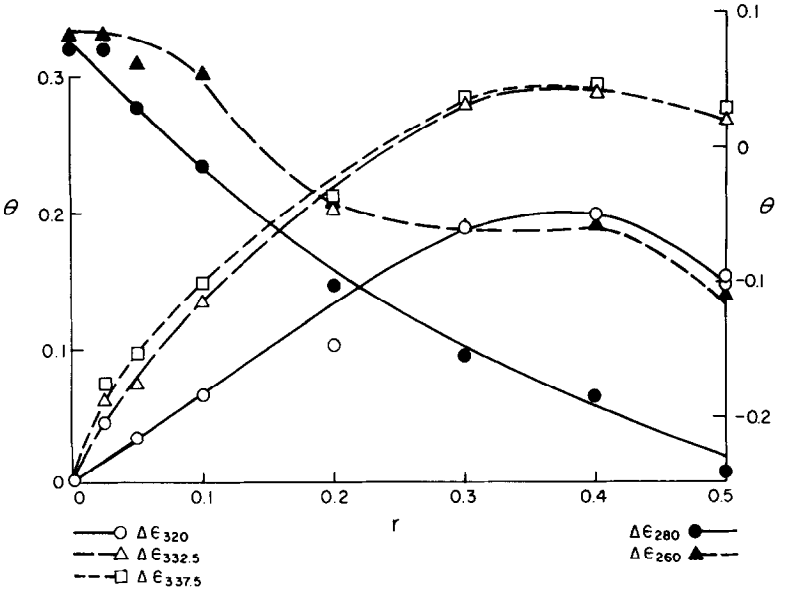

Figure 5. Variation of compact Chromatin Intrinsic and complex Extrinsic Band Intensity with r for Ionen X. The extrinsic ellipticity values are indicated with the open symbols, left hand ordinate scale, and the intrinsic with the filled symbols, right hand ordinate scale. θ corresponds to observed values.

deoxynucleotides derived from either AT or GC; however, ionen X showed smaller bands with the G and C containing polymers.

Discussion

The extrinsic CD bands appear to arise by an exciton splitting mechanism[8] which depends on spatial arrangement of the chromophores.[9] Imposing the exciton chirality rule[9] leads to the conclusion that the chromophores are in a right handed helical array. Our model building studies supported a stacking arrangement of the chromophores of II in the major groove with salt bridge to phosphates. Here the p-xylylene chromophores are proposed to be stacked against the relatively hydrophobic face of the major groove with the cationic groups paired with the phosphates.

The binding of II to chromatin is indicated by the change in intrinsic band intensities, even though the extrinsic signal is very small or undetectable. Such changes in the intrinsic bands are characteristic of salt effects[10] and the association of polycationic compounds with DNA[3,11] and chromatin[11]. The data presented here are consistent with critical regions of the major groove of the DNA in the chromatin being inaccessible to ionen II in a non-random array. Thus, the model of chromatin with proteins -- principally histones -- associated with the major groove is supported by the above data.

Exciton splitting is critically dependent on relative orientation and spacing of chromophores. The differences in the pattern of the partially resolved peaks in the extrinsic band region of DNA-X complexes (particularly at 300-320 nm) is suggestive of a different spatial arrangement of the chromophores of X in the complex reflecting an altered DNA secondary in chromatin structure.

We see a titration with X (Fig. 5). The $\Delta\varepsilon_{345}$ L-(phosphate)$^{-1}$-cm^{-1} has reached a maximum at r = 0.5 with DNA followed by increasing turbidity and decline at higher r values; at r = 0.5 ionen X would just neutralize the negative charges. With both kinds of chromatin preparations the $\Delta\varepsilon_{345}$

has reached limiting values at r = 0.3; further the $\Delta\varepsilon_{345}$ is less at any given r value than for the DNA complex. Thus we may infer that approximately 40% of the binding sites are not available. Furthermore, since the absolute values of $\Delta\varepsilon_{345}$ (or $\Delta\varepsilon$ at any wavelength within the extrinsic band) is less at any given r value, that again, the DNA in the complex with chromatin is significantly different from the B form.

Some of the work leading to the conclusion that histones are predominantly associated with the major groove [1-4] was done with the high viscosity, water-soluble chromatin; the two probes, II and X, provide a link between the data derived from the two types of chromatin preparations. Both show nearly complete exclusion of II and both show 60-70% access to X. A reasonable assumption is the shielding or exclusion from the ionen II binding sites may be due to bound histones. The shielding (>85%) is high when compared with the shielding (<15%) seen by the methylation technique[2] and may be ascribed to the obvious differences in the physical and chemical properties of the probes as well as the conditions of measurement (i.e. equilibrium vs. non-equilibrium).

In conclusion, we have described a pair of CD structural probes for aiding in the characterization of DNA and chromatin. The probes can provide both quantitative and qualitative data with respect to secondary structure and may reflect the amount of available binding sites in the major and minor groove regions of the DNA.

Acknowledgements

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