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Interaction of benz[*a*]pyrene diol epoxide with chromatin studied by flow linear dichroism

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Chromatin isolated from Ehrlich ascites cells was incubated with the tumourigenic compound (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenz[*a*]pyrene [(+)-*anti*-BPDE] at low ionic strength and the modified chromatin was analysed using flow linear dichroism (LD). The results confirm that (+)-*anti*-BPDE preferentially binds to the DNA in the linker regions, and furthermore show that the long axis of the bound pyrenyl chromophore is oriented parallel or close to parallel to the average orientation of the chromatin fiber axis. The data indicate that the binding geometry of (+)-*anti*-BPDE in chromatin is similar to that in pure DNA and deoxyguanosine-containing double-helical oligonucleotides.

Benz[*a*]pyrene diol epoxide; Chromatin, active; Flow linear dichroism

1. INTRODUCTION

The tumourigenic process initiated by benz[*a*]pyrene (BP), a ubiquitous environmental contaminant, is believed to involve sequential activation of BP to (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenz[*a*]pyrene [(+)-*anti*-BPDE] and subsequent covalent binding of this intermediate through the benzoylic carbon 10 to DNA (reviews [1,2]). Results from both in vitro and in vivo experiments have demonstrated that the major binding target in DNA is the exocyclic nitrogen of deoxyguanosine (dG) ([3] and references therein). Structural features of the adducts resulting from incubating (+)-*anti*-BPDE or the less tumourigenic isomers (e.g. (±)-*syn*- and (−)-*anti*-BPDE) with purified DNA or different oligonucleotides have been extensively studied in vitro and in vivo by various spectroscopic techniques [4,5]. From such studies it can be concluded that DNA binding of (+)-*anti*-BPDE results in different types of complexes where the chromophores of the dominating fraction are oriented with their long axes parallel or close to parallel to the helix axis of DNA (defined as type II complexes, see [4,5]). In contrast, a major fraction of the bound chromophores of the less carcinogenic BPDE isomers is oriented more perpendicular to the helix axis of DNA, which is more compatible with the intercalation type of complexes (defined as type I complexes, see [4,5]). Systematic studies on the tumour-initiating potency of all possible bay-region diol epoxides of several polycyclic aromatic hydrocarbons have shown that the (+) enantiomers of the *anti* diastereomers in most cases are by far the most active forms [2]. Interestingly, available data from in vitro experiments show that DNA binding of these intermediates in general results in formation of type II complexes [4,6]. To our knowledge, there is, however, no information demonstrating the relevance of these findings in in vivo-like systems. In a first attempt to obtain structural information, we have incubated isolated chromatin with (+)-*anti*-BPDE and analysed the

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modified preparation by flow linear dichroism (LD).

2. MATERIALS AND METHODS

Micrococcal nuclease solubilized chromatin was isolated from nuclei of mouse Ehrlich ascites mouse tumour cells as in [16], and dialyzed vs 0.25 mM EDTA. The chromatin had the normal content of histones and an average size of 50 nucleosomes.

35–75 nmol (+)-*anti*-BPDE was dissolved in 50 μl ethanol immediately prior to addition to 2.5 ml of chromatin, or DNA, in 0.25 mM EDTA, pH 7.8 (3.0–3.6 *A* units/ml). The binding ratio, *r*, is defined as the number of bound chromophores per phosphate and was calculated using molar absorption coefficients of 29000 $M^{-1} \cdot cm^{-1}$ at 346 nm for BPDE and 6600 $M^{-1} \cdot cm^{-1}$ at 258 nm for chromatin and DNA.

The experimental conditions for LD analysis have been described in earlier chromatin flow LD studies [13,17]. The LD spectrometer was operated according to the principles given in [18].

3. RESULTS

When adding (+)-*anti*-BPDE to chromatin an LD signal arises immediately (within 2 min) in the absorption bands of the pyrenyl chromophore (fig.1) showing that the diol epoxide is bound and oriented by the chromatin fiber. Both positive and negative absorption bands are observed in the 300–370 nm region (corresponding to the L_a transition moment in the pyrene residue), indicating the presence of different populations of (+)-*anti*-BPDE–DNA complexes with more parallel and more perpendicular orientations, respectively, relative to the average fiber axis. Both the absorption and LD features differ significantly from the spectra obtained with (+)-*anti*-BPDE incubated with pure DNA (obtained from the same chromatin batch). The predominant negative LD feature observed with DNA can be due to remaining (+)-*anti*-BPDE being physically intercalated or, which is more likely, tetraols (BPTs) resulting from diol epoxide hydrolysis. The absorption spectrum is also in support of a significant portion of BPTs being intercalated in DNA but not in the corresponding chromatin sample. After dialysis (vs 200 vols of 0.25 mM EDTA overnight) to remove the remaining, physically bound diol epoxide and tetraols, the LD spectrum became entirely positive (except for a weak negative contribution at 356 nm in the DNA sample due to the remaining intercalated BPT, which vanished after further dialysis).

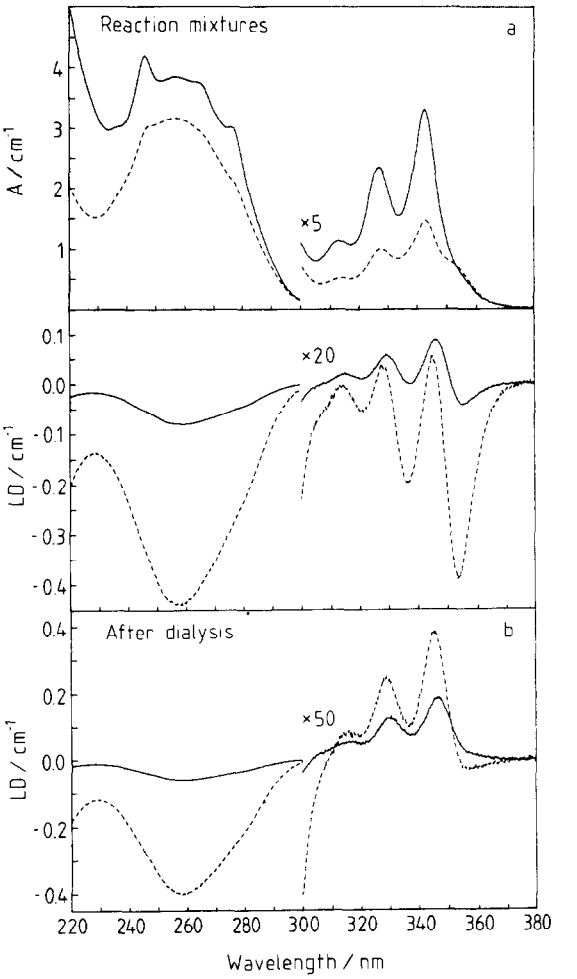

Fig.1. Absorption (*A*) and LD spectra of reaction mixtures (a) and dialysed solutions (b) of (+)-*anti*-BPDE and chromatin [r = 0.0069 (——)], or DNA [r = 0.0044 (– – –)]. Reaction medium, 0.25 mM EDTA, pH 7.8, 8°C; flow gradient, 1800 s^{-1}. Neither of the LD spectra changed significantly with time (h).

The positive LD of (+)-*anti*-BPDE covalently bound to chromatin is consistent with the binding geometry observed for the DNA adduct, with the long axis of the pyrenyl chromophore oriented more or less parallel to the DNA helix axis [7,8]. Table 1 summarizes the LD values for the pyrene residue of (+)-*anti*-BPDE (346 nm) and for the DNA bases (258 nm) observed under different salt conditions in chromatin and in DNA. Table 1 also

Table 1

LD^r and LD features of (+)-*anti*-BPDE covalently bound to chromatin or DNA[a]

	[NaCl]	(+)-*anti*-BPDE/ chromatin (r = 0.0048)	(+)-*anti*-BPDE/ DNA (r = 0.0044)
LD^r_{258}	0 mM	−0.024	−0.137
	2.5 M	−0.050	−0.053
LD^r_{346}	0 mM	0.036	0.136
	2.5 M	0.046	0.056
LD_{346}/LD_{258}	0 mM	−0.031	−0.019
	1 mM	−0.035	
	2 mM	−0.042	
	5 mM	−0.071	
	2.5 M	−0.013	−0.021

[a] $LD^r = LD/A$

All measurements were performed at 8°C in medium comprising 0.25 mM EDTA (pH 7.8) plus the indicated additions of NaCl. Flow gradient, 1800 s^{-1}. At 2.5 M NaCl the chromatin dissociates

lists data for the (+)-*anti*-BPDE–DNA sample remaining after salt-induced dissociation of the histones.

4. DISCUSSION

The positive LD spectrum in the 300–350 nm region of (+)-*anti*-BPDE-modified chromatin is in qualitative agreement with preferential binding of the diol epoxide to linker DNA and with binding geometry similar to that in pure DNA. This conclusion is supported by the fact that the ratio LD_{346}(BPDE)/LD_{258}(DNA) decreases by as much as a factor of 2.3 for chromatin when increasing the salt from 0 to 2.5 M (dissociation) compared to the constant ratio for the modified pure DNA. If the diol epoxide had been homogeneously distributed over nucleosomal and linker DNA, identical ratios would have been obtained (presuming the same local binding geometries). The enhancement in the LD of (+)-*anti*-BPDE is thus explained by a different orientation of the linker DNA vs the average DNA orientation. Previous experiments using BP or racemic *anti*-BPDE and chromatin have clearly demonstrated a pronounced preference for binding to DNA in the linker regions [9–12].

Another interesting observation concerns the behaviour of the LD(BPDE)/LD(DNA) ratio when increasing the ionic strength moderately by addtion of NaCl. At very low ionic strength, chromatin is known to adopt an extended conformation, characterized by a strongly negative LD(DNA); the magnitude of this LD signal decreases drastically with increasing salt concentration and the LD of unmodified chromatin has been observed to change sign in the region of 2–5 mM NaCl [13]. With (+)-*anti*-BPDE-modified chromatin, the negative LD(DNA) amplitude similarly decreases with added salt and becomes positive, however, at a somewhat higher salt concentration (10 mM NaCl) than for unmodified chromatin. The LD(BPDE)/LD(DNA) ratio increases considerably when the salt concentration is increased (by a factor of 2 at 5 mM NaCl). Such behaviour would be expected if the nucleosomal and linker DNA regions contributed to differing extents to the LD for the various degrees of folding of the chromatin structure. Indeed, these results indicate that BPDE covalently bound to chromatin may be used as a sensitive probe of the conformation of the internucleosomal linker DNA of the chromatin fiber. Results concerning this aspect will appear elsewhere.

It is worth noting that the kinetics of diol epoxide binding to chromatin observed here are markedly more rapid than those reported by Dock and McLeod [14]. At the low ionic strength used here maximal amplitude of the LD(BPDE) is observed within 2 min after addition of the diol epoxide to the chromatin preparation, thus implying that the reaction has proceeded to completion. Similar results were obtained in preliminary experiments where chromatin was incubated with tritiated (+)-*anti*-BPDE at low salt concentration and the extent of covalent binding to DNA as well as to histones was determined as a function of time (15 s–30 min). Full details of these experiments will be given elsewhere. The results of Dock and McLeod [14] could be explained by the fact that they used spermine and spermidine in the chromatin preparation, which may lead to more condensed, and hence less penetrable structures [15], as demonstrated by the LD data for such preparations (not shown). These authors also used slightly higher salt concentrations (10 mM) and the polyamines may also react with the diol epoxide. Finally, the less negative LD for freshly reacted

(+)-*anti*-BPDE with chromatin, compared to DNA, indicates lower amounts of intercalated non-covalently bound BPTs in chromatin. This is in agreement with the findings of Dock and McLeod [14].

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