Binding of Indium, Used in the Perturbed $\gamma-\gamma$ Angular Correlation Studies, on DNA and DNA Moieties

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Received June 11, 1985

Abstract

The two successive gamma rays emitted from bound indium (^{111}In) are utilized in the Perturbed $\gamma-\gamma$ Angular Correlation (PAC) method for the study of the molecular dynamics of cellular macromolecules such as DNA. In this paper, evidence is presented indicating that indium binds on both phosphate and the base moieties of DNA and of the nucleotides and that, the binding of indium, under the conditions used in the PAC method, does not alter the hydrodynamic properties of the DNA macromolecule or its UV absorbance spectrum.

Introduction

The radioactive indium nuclide (^{111}In) is currently being used for the study of the molecular dynamics of cellular macromolecules and especially of DNA [1–4]. These studies are of importance as a means of testing models for DNA. They also offer a new way of studying the nature of lesions in this important biomolecule induced by irradiation or other clastrogenic factors.

Although the binding on DNA of other metals close to the indium in the periodic table, such as cadmium, nickel or cobalt has been explored very early [5] no relevant information is available for indium. Thus, following our current work on DNA with the perturbed $\gamma-\gamma$ angular correlations [2, 3], we studied the binding of ^{113}In, the non-radiactive nuclide, on the DNA and the DNA moieties. In this work, results are presented on the electrophoretic properties of DNA moieties (free bases, nucleosides and nucleotides) preincubated with $InCl_3$ and on the hydrodynamic, thermal transition and UV absorbance properties of the mammalian DNA.

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Experimental

Materials and Methods

Sedimentation studies

The sedimentation properties of mammalian DNA in the presence of $InCl_3$ were studied by ultracentrifugation of ^{3}H-labelled DNA. The ^{3}H-labelled DNA was isolated, with the usual methodology, from V-79 cells cultured in the presence of 1 μCi/ml tritiated thymidine. Following ultracentrifugation of 5–20% of the alkaline sucrose ingredients with a SW 65 Ti head in an L-75 Beckman Ultracentrifuge, aliquots were collected on 3MM paper strips, non-soluble in 5% trichloroacetic acid, and the radioactivity was measured in a Tricarb 3385 Scintillation Counter. Details of DNA labelling, isolation, ultracentrifugation and counting procedures are reported elsewhere [6].

Thermal transition spectroscopy

The temperature at which the DNA unfolds, the T_M (DNA melting point), was estimated in 1.5×10^{-5} M DNA-P solutions buffered by $0.01 \times$ SSC (1.55×10^{-3} M sodium chloride, 1.5×10^{-4} M sodium citrate), in the presence or absence of indium chloride, with a Perkin-Elmer S551 Spectrophotometer. The spectrophotometer was coupled with a thermoelectric cell holder and a digital temperature controller. Heating or cooling of the cell holder was carried out by solid-state Peltier thermoelectric modules in conjunction with a platinum resistance sensor monitoring of the cell temperature. The same instrument was used for recording the UV absorbance of the DNA solutions. Details on thermal transition spectrophotometry methodology are given elsewhere [7].

Electrochromatography

Free bases, nucleosides and nucleotides (Sigma Chemical Co.) of adenine, cytocine, guanine and thymine at 5×10^{-2} M solutions were incubated at room temperature in the presence of equimolar

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concentrations of $InCl_3$ for 24, 48 and 72 h. After incubation the samples were transferred on 3MM paper and subjected to electrophoresis in citrate buffer at 20 V/cm [8].

Results and Discussion

The sedimentation profiles of 5×10^{-5} M DNA-P incubated for 24 h at room temperature in the presence of 10^{-4} M, 10^{-6} M and 10^{-8} M $InCl_3$, or 2:1, 0.02:1 and 0.0002:1 $InCl_3$:DNA-P molar ratios (Fig. 1) indicate an increase in the sedimentation velocity of the DNA molecules exposed to the higher $InCl_3$ concentration. The relative molecular weights (M_r) (MW_T of treated DNA: MW_C of non-treated DNA) were estimated according to previously published methodology [9].

A linear function relates the M_r to the $InCl_3$ concentration, in the incubated solutions so that, with a concentration of 10^{-8} M, a change of the order of less than 1% is estimated and observed. In Fig. 2 the estimated linear function and the experimentally observed M_rs are presented. The chi-square criterion, calculated equal to 0.00436 for 3 degrees of freedom, indicates that there is a close agreement between expected and observed values. These results indicate that binding of indium on DNA at low molar ratios does not considerably affect the hydrodynamic properties of this molecule.

The % *Rf* of the electrochromatograms, on the basis of the material not treated with $InCl_3$, for the deoxyriboso-nucleotides, -nucleosides and free bases incubated in the presence of equimolar concentrations of $InCl_3$ are shown in Table I. Changes were not observed in the *Rf* values of the free bases and the nucleosides incubated even for 72 h in the presence of $InCl_3$. On the contrary, when nucleotides were incubated under the same conditions, changes in the *Rf* values occurred after incubation of 24 h. These changes were more prominent in the case of adenine where a considerable decrease in the *Rf* value was observed after 48 or 72 h of incubation.

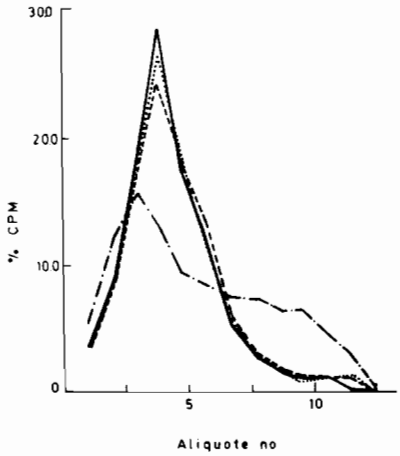

Fig. 1. Ultracentrifugation profiles of mammalian DNA in 5–20% alkaline sucrose gradients in the presence of 10^{-4} M (– – –), 10^{-6} M (- - -), 10^{-8} M (· · ·) $InCl_3$. Control DNA profile (——).

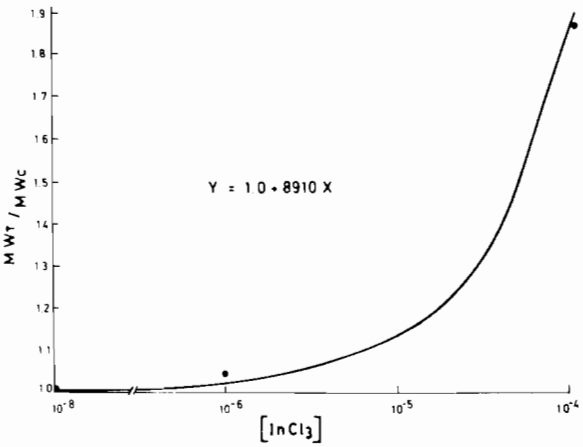

Fig. 2. M_r of DNA (MW_T = molecular weight of treated DNA over MW_C = molecular weight of non-treated DNA) in the presence of different concentrations of $InCl_3$. Dots = experimental points, solid line = theoretical fit for the form $Y = \alpha + \beta X$.

TABLE I. *Rf* Values for Nucleotides, Nucleosides and Free Bases Incubated for 24, 48 and 72 h with $InCl_3$ in Aqueous Equimolar Solutions of 10^{-2} M from Electrochromatograms in Citrate Buffer (% *Rf* on the Basis of Material Incubated in the Absence of $InCl_3$). Means of three replications

Material	Incubation time	% *Rf*			
		Adenine	Cytocine	Guanine	Thymine
Free bases	72 h	99.3	101.2	98.1	100.0
Nucleosides	72 h	100.0	99.1	98.0	102.1
Nucleotides	24 h	90.0	91.7	94.6	93.0
Nucleotides	48 h	72.2	89.6	93.9	95.0
Nucleotides	72 h	73.4	90.0	95.0	89.8

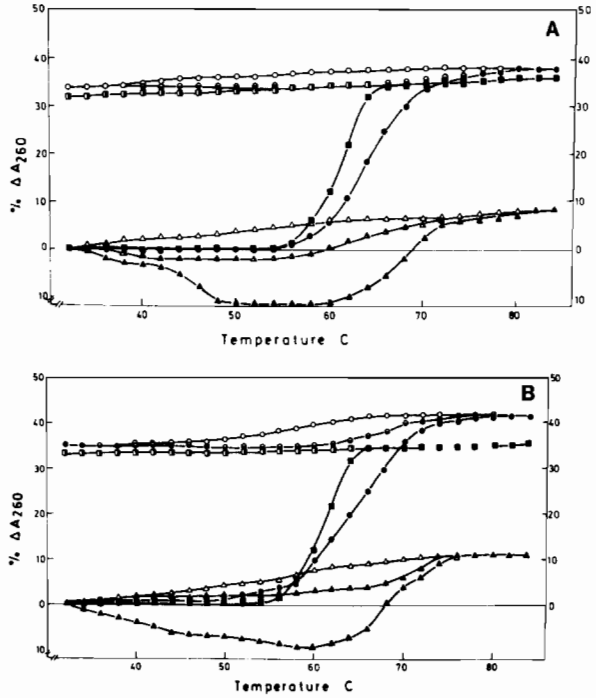

Fig. 3. Heating (●▲■), cooling (○△□) and reheating (◒◭◨) thermal transition profiles of mammalian DNA in the presence of 10^{-3} M (triangles), 10^{-4} M (circles) and in the absence of $InCl_3$ (squares). A: 0 h and B: 24 h incubation.

These results indicate that binding of indium on the DNA moieties is strongly dependent on the phosphate moiety. The differential effect on the adenosine triphosphate also indicates differential recognition of the base moiety during the binding process.

The heating thermal transition profiles (Fig. 3) show a higher temperature shift in the presence of 10^{-4} M or 10^{-3} M $InCl_3$ whether the samples were not incubated for 24 h in room temperature or were incubated. The shift was prominent in the 10^{-4} M and the 10^{-3} M $InCl_3$ solutions although the profiles in the second case were somewhat unusual. The observed decrease in the A_{260} between 30 and 50 °C is under investigation, in these unusual profiles.

The cooling and reheating thermal transition profiles observed during gradual cooling and reheating of the DNA–$InCl_3$ solutions gave no superimposable curves while, in the absence of $InCl_3$, no difference was observed between the A_{260} values measured during cooling and reheating. Changes in the λ_{max} were not recorded during heating, cooling and reheating of the samples (Fig. 4). The higher temperature shift which was observed during the initial heating, coupled with the lack of a superimposable pattern in the cooling-reheating process, indicates a binding of the indium on the phosphate as well as on the bases of the DNA molecule and it is comparable to the behaviour of the DNA in the presence of other transition metals [5].

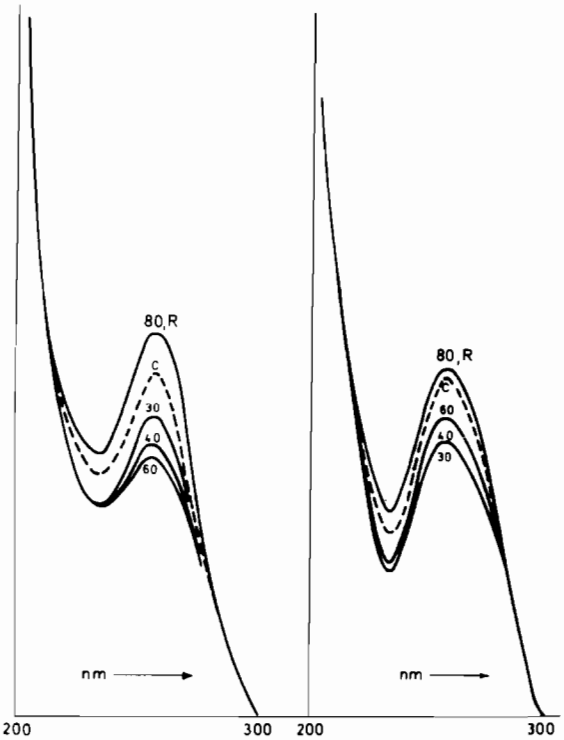

Fig. 4. λ_{max} of DNA–$InCl_3$ solutions during the heating at 30, 40, 60 and 80 °C, cooling (C), and reheating (R) at 80 °C procedures with samples incubated for 24 h in the presence of 10^{-3} M (left) and 10^{-4} M (right) $InCl_3$. Similar results were found from samples of 0 h incubation time.

The conclusions from the electrophoresis work and from thermal transition behaviour, leading to existence of binding sites on the phosphate as well as on the base moieties, are also in agreement with the work on cadmium which indicates a binding of this metal on the N7, O and P atoms of the inosine monophosphate in polymeric stretches [10].

The lack of any significant alteration in the hydrodynamic sedimentation properties of the DNA after exposure to $InCl_3$, at a molar ratio as high as 0.02:1 $InCl_3$:DNA-P and coupled with the lack of λ_{max} changes, encourages the further use of the perturbed γ–γ angular correlations method for the study of the DNA dynamics where the ^{111}In is used at molar ratios of the order of 10^{-8} [2].

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