J. Indian Chem. Soc.,
Vol. 76, January 1999, pp. 13-16

Effects of chelating agents EDTA, phytin and deferoxamine on the heavy metal complexes of DNA

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Manuscript received 13 August 1997, revised 18 May 1998, accepted 19 May 1998

The characteristic CD spectrum of B-conformation of DNA is highly perturbed by Ag^+ and Hg^{2+} but not by Cu^{2+}, Zn^{2+}, Mn^{2+} etc. This perturbation is due to the formation of coordination complexes with the DNA bases within the helix by the former two metals. While the chelating agents deferoxamine and EDTA partially reverse the perturbed CD spectrum of DNA-Ag^+, phytin practically has no effect. But phytin as well as deferoxamine and EDTA effectively but partially reverse the CD spectrum of DNA-Hg^{2+} to that of free DNA. The DNA-Hg^{2+} complex seems to be weaker than DNA-Ag^+. Thus the iron chelating drug deferoxamine has an additional beneficial effect of partially nullifying mercury and silver poisoning; phytin is weakly effective in case of mercury poisoning. EDTA is more effective in silver as well as mercury poisoning compared to the other two chelating agents.

The interaction of Ag^+ with DNA is not restricted just to a counterion binding, as in the electrostatic binding of Na^+, Zn^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+} etc. to the phosphate group of DNA, but involves the formation of distinct coordination complexes with DNA bases[1]. The formation of such complexes is responsible for the heavy metal toxicity and carcinogenic activity[2]. Such specific binding of Ag^+ to DNA results in a significant change in its structure, as revealed in change in viscosity and inferred from various spectroscopies[3]. Hg^{2+} behaves qualitatively similar to Ag^+ in its interaction with DNA[4]. While the effect of Ag^+ ion on the UV absorption spectrum of DNA is rather minor, Hg^{2+} perturbs the DNA absorption spectrum relatively more significantly[5]. However, both Ag^+ and Hg^{2+} affects the characteristic CD spectrum of DNA drastically. In this report we probed the effects of the chelating agents phytin (myo-inositol hexaphosphate) and deferoxamine on the heavy metal-DNA complexes using circular dichroism as a monitoring technique. Phytin is produced during the germination of various seeds[6]. Different pharmaceutical properties including antidiabetic, hypolipidemic, hypocholesterolemic, antioxidant in food preservatives, antineoplastic activity in control of colon cancer etc. have been reported[7]. Deferoxamine is a well-used iron chelating agent and used in chelating out excess iron in thalassemia patients. The metal chelating activities of phytin are well established and this property renders phytin also harmful, as for example, phytin inteferes with the roles of metalloenzymes like alkaline phosphates[8] and carboxypeptidase-A[9]. The affinity of phytin for Ca^{2+} has rendered it an efficient and inexpensive blood anticoagulant *in vitro*[10] as well as *in vivo*[11]. We have made an attempt to probe whether these two drugs phytin and deferoxamine have some additional beneficial role in reducing heavy metal toxicity by chelating out Ag^+ and Hg^{2+} from their DNA-complexes. For comparison we have also included the common chelating agent EDTA in our study. Our results show that while EDTA and deferoxamine can remove the bound Ag^+ and Hg^{2+} from DNA, phytin has only a marginal effect in case of Hg^{2+}.

Results and Discussion

Fig. 1 summarises the UV absorption spectra of 60.0 μM DNA in the presence of $AgNO_3$ at Ag^+/DNA base ratios of 0.0, 0.2 and 0.5. All the spectroscopic data in this and subsequent figures have been collected at pH 7.4 in 5.2 m*M* phosphate buffer and 0.20 m*M* $MgSO_4$. It is obvious that the change in the absorption spectrum of DNA caused by the presence of Ag^+ is rather minor, the lower concentration of Ag^+ reduces the OD of DNA slightly, and the higher concentration enhances it, the λ_{max} being slightly red-shifted. The enhanced absorbance in the shorter wavelength region is just due to absorption by Ag^+ itself; we have checked that aqueous $AgNO_3$ has a λ_{max} around 200 nm.

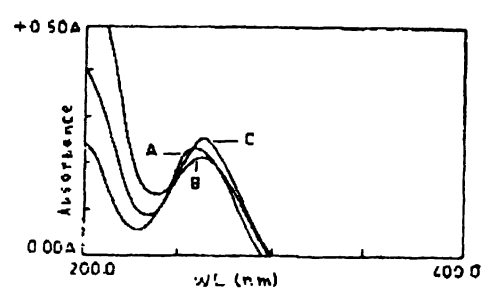

Fig. 1. UV absorption spectra of 60 0 μ*M* DNA in aq buffer at pH 7 4 (A), in presence of Ag^+ at Ag^+/DNA mole ratios of 0 2 (B) and 0 5 (C)

The UV CD spectra (Fig. 2) show that Ag^+ ion perturbs the dichroism of DNA to a large extent. DNA in the native B-conformation depicts the characteristic bisignate CD spectrum in the absorption region of the bases as depicted by the spectrum (A). The sign and ellipticity values depend

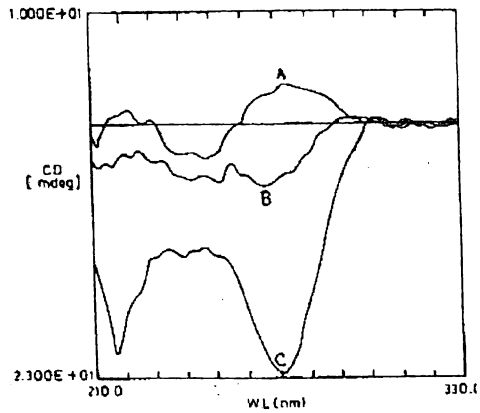

Fig. 2. UV CD spectra of 60.0 μ*M* DNA (A), in presence of Ag^+ at Ag^+/DNA ratios of 0.2 (B) and 0.5 (C).

on the tilting of the bases with respect to the DNA helix. This tilting changes with the conformation of DNA, and DNA in its A conformation, for example, has the CD spectrum characteristically different from the spectrum (A). If the heavy metal ions form coordination complexes with the bases within the DNA helix perturbing the tilting of the bases, this should be reflected in the CD spectra of the DNA-metal complexes. That this really happens is shown by the CD spectra (B) and (C) of Fig. 2. The characteristic positive peak of DNA (A) is converted to a trough in (B) and (C) in the presence of Ag^+ at Ag^+/DNA base ratios (r_b) of 0.2 and 0.5 respectively. In fact the CD spectra (B) and (C) are quite different from (A) all over the wavelength region. The type II Ag complex of DNA[2] shows the remarkable change in the CD spectrum and we restricted our subsequent studies at r_b of 0.5. Norden and Matsuoka[1] from their LD and CD studies of DNA-Ag^+ complexes concluded that Ag^+ causes substantial deviation of the plains of base pair from approximately normal to the helix axis in B-DNA to a tilted orientation, resulting in conformational change of DNA, as reflected in the changed CD spectrum.

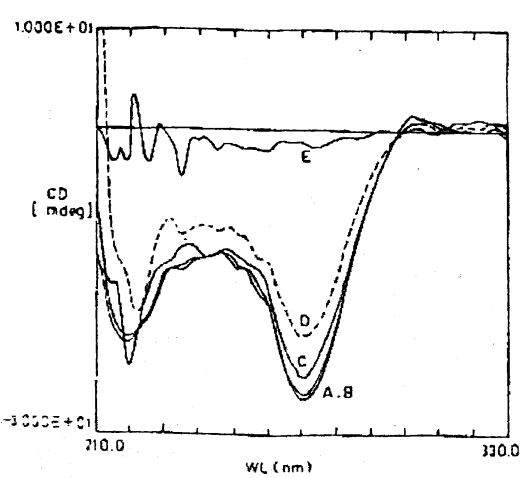

Fig. 3. UV CD spectra of the mixture of 60.0 μ*M* DNA and 30.0 μ*M* $AgNO_3$ in water (A); in 3.0 μ*M* phytin (B); in 60.0 μ*M* (C) and 120.0 μ*M* (D) deferoxamine; in 0.30 μ*M* EDTA (E).

We probed the effects of chelating agents phytin, deferoxamine and EDTA on the CD spectrum of DNA-Ag^+ type II complex over a wide range of concentration of the complexing agents. The CD spectra (Fig. 3) summarise our observations. It is apparent that phytin at a molar concentration hundred times that of Ag^+ does not purturb the CD spectrum (A) of DNA-Ag^+, and the spectra (A) and (B) are almost superimposed. The CD spectra (C) and (D), however, show that the iron chelating drug partly complexes out Ag^+ from DNA even at low concentration – double and four times that of Ag^+. Thus this chelating drug used in the treatment of thalassemia might have some additional benefit of nullifying heavy metal toxicity. This observation deserves further study. A parallel study of the well-known complexing agent EDTA shows that it also complexes out bound Ag^+ from DNA, and a ten-fold molar concentration of EDTA relative to Ag^+ reverts the CD spectrum (E) more than half way to that of DNA alone. It is to be pointed out that since Ag^+ only converts the characteristic CD peak of DNA at 270 nm to a deep trough without any shift of λ_{max}, chelating out Ag^+ from DNA only reduces the ellipticity at the trough without any shift of its position.

We thought that phytin with a high anionic charge density (twelve per inositol molecule) may not approach anionic DNA due to electrostatic repulsion, and hence the observed insensitivity of DNA-Ag^+ CD spectrum to phytin. With the idea that increased ionic strength might reduce the electrostatic repulsion between DNA and phytin, we also recorded the CD spectrum of DNA-Ag^+ in the presence of 3.0 m*M* phytin in 0.15 *M* NaCl (Fig. 4). A comparison of the spectra (A) and (B) momentarily gives us the idea that

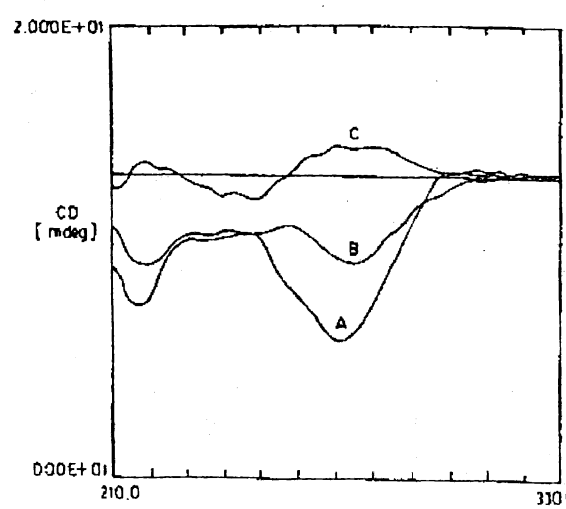

Fig. 4. UV CD spectra of the mixture of 60.0 μ*M* DNA and 30.0 μ*M* $AgNO_3$ in 3.0 μ*M* phytin (A); solution (A) in 0.15 μ*M* NaCl (B); solution (B) without phytin (C).

phytin in 0.15 *M* NaCl is partly effective in reverting the spectrum (A) to that of DNA; but the spectrum (C) shows that NaCl alone completely reverts the UV-CD of DNA-Ag^+ to that of DNA (as also reported earlier). It is obvious

that the observed reversal of spectrum (B) is caused by NaCl; but what is not obvious is why phytin reduces the efficiency of NaCl in reversing the CD spectrum of DNA-Ag^+ [compare spectra (B) and (C)]. This effect of phytin is reproducible, though the reason is not apparent. On increasing the ionic strength by 0.15 *M* $NaNO_3$, The CD spectrum of DNA-Ag^+ in the presence or absence of phytin remains unchanged.

Since the structure of DNA is perturbed by Ag^+ as reflected on the changed CD spectra, we were curious to know whether the induced dichroism in achiral dyes[12] by DNA is also affected by Ag^+ at a concentration much below the concentration required by the common electrolytes for the disruption of metachromasia. We studied the effect of Ag^+ on the absorption and ICD spectra of the respective complexes of acridine orange (AO) and pinacyanol (PCYN) with DNA. The absorption spectra as shown in Figs. 5(A) and 5(C) of AO-DNA and PCYN-DNA in the absence and presence of Ag^+ show that Ag^+ does have minor effect on the absorption spectra. But the corresponding ICD spectra (B) and (D) of the same figure show that the effect of Ag^+ on the respective ICD spectra is more pronounced. This is in harmony with our earlier view[13] that dichroism is more sensitive to the precise geometry of aggregation of dyes induced by polyanions than the corresponding metachromatic shift is; the perturbation of the structure of the host polymer DNA changes the geometry of aggregation of the bound dyes, which is reflected in the changes ICD spectra caused by Ag^+ ion.

The effect of Hg^{2+} on the UV CD of DNA, and the perturbation of DNA-Hg^{2+} complex by the three chelating agents phytin, deferoxamine and EDTA are summarised in

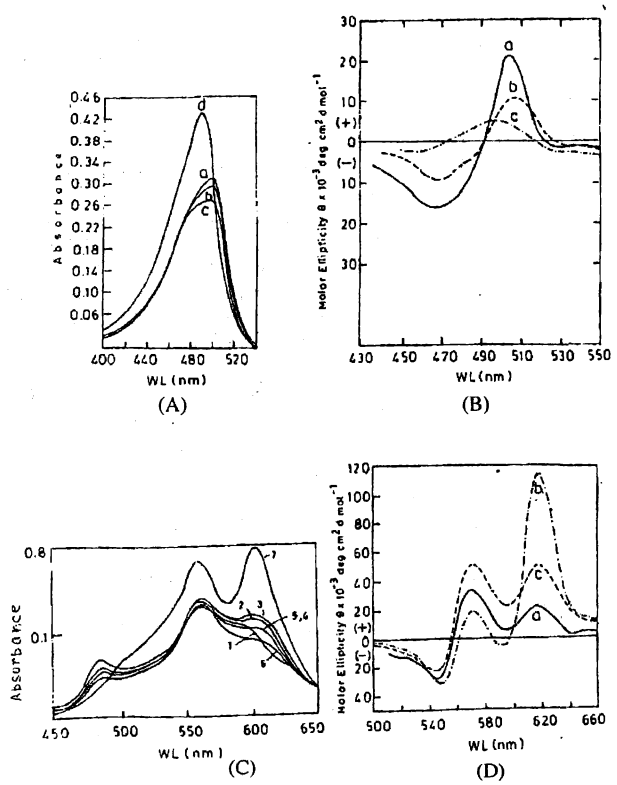

Fig. 5. (A) Effect of Ag^+ on the visible absorption spectra of DNA-AO system : (a) 12.0 μ*M* AO in presence of DNA at a P/D of 5.0; (b), (c) same as (a) in presence of Ag^+/DNA ratios of 0.2 and 0.5 respectively; (d) 12.0 μ*M* aqueous AO.

(B) Visible CD spectrum : (a) 12.0 μ*M* AO in presence of DNA at a P/D of 5.0; (b) and (c) in presence of $AgNO_3$ with Ag^+/DNA ratios of 0.2 and 0.3 respectively.

(C)Effect of Ag^+ on the visible spectra of DNA-PCYN system : (1) 12.0 μ*M* PCYN in presence of DNA at a P/D of 5.0; (2-6) of solution (1) in presence of Ag^+ at Ag^+/DNA ratios 0.10, 0.15, 0.20, 0.30 and 0.50 respectively; (7) 12.0 μ*M* aqueous PCYN.

(D) Same as Fig. 3(B) with PCYN in place of AO.

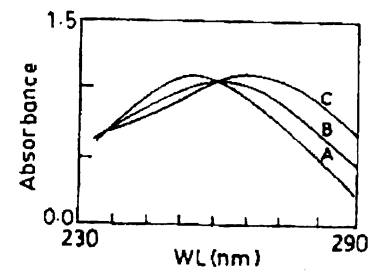

Fig. 6. UV absorption spectra of 60.0 μ*M* DNA in aq. buffer at pH 7.4 (A); in presence of Hg^{2+}/DNA mole ratios of 0.2 (B) and 0.5 (C).

Figs. 6 and 7. Mercury also forms two types of complexes with DNA at Hg^{2+}/DNA base ratios[5] at below and above

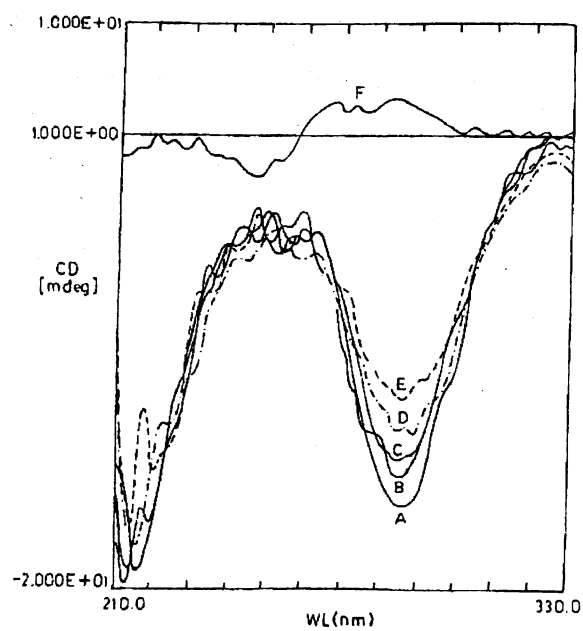

Fig. 7. UV CD spectra of 60.0 μ*M* DNA in the presence of Hg^{2+} at Hg^{2+}/DNA ratio 0.5 (A); 0.30 and 3.0 μ*M* phytin added to solution (A) exhibiting spectra (B) and (C) respectively, 60.0 and 120.0 μ*M* deferoxamine added to solution (A) exhibiting spectra (D) and (E) respectively; (F) CD spectrum of Hg^{2+}-DNA complex at Hg^{2+}/DNA ratio of 0.5 in the presence of 300 μ*M* EDTA.

0.5. We restricted our study to the type of complex at r_b of 0.5. Hg^{2+} also drastically changes the characteristic UV CD spectrum by converting the peak at 270 nm to a deep trough. It is to be noted that unlike Ag^+ the position of the trough is red-shifted to 290 nm (spectrum A). This is in parity with the observation that Hg^{2+} (unlike Ag^+) also red-shifts the absorption maximum of DNA from 260 nm as represented in Fig. 6 and also reported earlier[5]. A comparison of the spectrum (C) of Fig. 7 with the spectrum (B) of Fig. 3 shows that phytin at a high concentration of 3.0 m*M* reverts at least partially the UV CD of DNA-Hg^{2+}. The spectra (D) and (E) show that deferoxamine is a more efficient chelating agent in chelating out bound Hg^{2+} from its DNA complex. EDTA at a concentration ten-fold of the Hg^{2+} concentration totally chelates out the bound Hg^{2+}, as inferred from the total reversion of the CD spectrum (A) of DNA-Hg^{2+} to (E) characteristic of the B-conformation of free DNA.

In conclusion we can say that the iron chelating agent deferoxamine can also have a role in nullifying heavy metal poisoning; phytin is marginally successful in case of Hg^{2+} poisoning, but does not seem to be effective in removing Ag^+ from the DNA complex. EDTA is more efficient in chelating out Hg^{2+} than Ag^+ from their respective DNA complexes.

Experimental

Calf thymus DNA and deferoxamine mesitylate (Sigma), phytin and EDTA (Sisco) were used as received. $AgNO_3$, $HgCl_2$ and all other chemicals were of A.R./G.R. grades. All the solutions were buffered by 5.2 m*M* phosphate buffer at pH 7.4; 0.20 m*M* $MgSO_4$ was also added in all solutions containing DNA to maintain the native conformation of DNA. Molarity of DNA has been expressed in terms of equivalent weight (330, defined as the average weight containing one anionic site). Acridine orange (AO, as $ZnCl_2$ double salt, E. Marck) was made zinc-free[14]. The cationic dye pinacyanol chloride (PCYN; Sigma) was pure sample as checked from the absorbance and absorption maximum of 12.0 μ*M* aqueous solution.

A Shimadzu 160A spectrophotometer was used, using quartz cuvettes of 1.00 cm pathlength. CD spectra were recorded on a Jasco J-710 spectropolarimeter using circular cuvettes of pathlength 1.00 cm.

All the experiments were done at 25° and readings were taken at least 30 min after making up of the solutions.

Acknowledgement

One of the authors (S.S.) thankfully acknowledges the receipt of a research fellowship for GATE qualified scholars from U.G.C., New Delhi.

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