Thermal Stability of DNA Interacting with Furazolidone and Cu(II) Ions

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Furazolidone, on complexing with DNA, led to its thermal stabilization. The increase in transition temperature of DNA (ΔT_m) increased linearly with % A–T content. Increasing concentration of Cu(II) ions progressively lowered the transition temperature of DNA, but Cu(II) ions were not equally effective in lowering the transition temperature of furazolidone-DNA complex. When equimolar amounts of Cu(II) ions and furazolidone were used, the stabilisation effects of furazolidone prevailed over the destabilisation effect of Cu(II) ions.

Introduction

Furazolidone or N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone, one of the members of the group of synthetic nitrofurans, displays a wide spectrum of antibacterial activity [1–4] and has found useful application in human therapy [5]. The mode of action at the molecular level of this drug has been under active study in our laboratory. The drug at the concentration level of 0.5 μg/ml inhibited DNA synthesis while stimulating RNA synthesis at the same time and causing filamentation of the cells [6, 7]. DNA of cholera phage Φ 149 was at least 10 times sensitive to this inhibitory action of furazolidone [8, 9]. In an attempt to explain the basis of the inhibition of DNA biosynthesis it was found that the drug underwent metabolic activation or transformation within the cells and then produced interstrand cross-links in DNA [10, 11]. The drug has recently been shown to exhibit radiomimetic property [12].

It was interesting to note that furazolidone exhibited a different type of interaction with DNA *in vitro*, an intercalative type of binding leading to thermal stabilisation of DNA and inhibition of its digestion by DNase [13]. Such intercalative *in vitro* binding of the drug to DNA led to the detection of its photobiological activity, a property till then unknown in the literature. The drug was found to cause photodynamic inactivation of choleraphages and also to render protection to the same phages against UV inactivation [14–16]. Since this *in vitro* interaction of furazolidone with DNA might have important practical applications, *e.g.*, in the treatment of surface infection by the so called photochemotherapy, further characterization of such *in vitro* interaction seems important and useful. The present communication reports in this context, the effect of Cu(II) ions on the thermal stabilisation of furazolidone-DNA complex and indicates that the drug has a preference for A–T base pairs in DNA.

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Materials and Methods

Chemically pure furazolidone was obtained as a gift sample from Smith, Kline and French (India) Ltd., Bombay. The drug concentrations were determined spectrophotometrically using the molar extinction coefficient of 16 650 $M^{-1}\,cm^{-1}$ at 367 nm [13].

Calf thymus DNA (type I) and *Micrococcus lysodeikticus* DNA were obtained from Sigma Chemical Co., USA and were always tested for their nativeness and purity [11, 13, 17]. *Vibrio cholerae* DNA and *Pseudomonus pyociania* DNA used in this study were isolated, purified and characterised in our laboratory as described elsewhere [13, 17, 18]. DNA concentrations in terms of nucleotide phosphate were determined spectrophotometrically by using molar extinction coefficient (ε) at 260 nm for calf thymus DNA (6600 $M^{-1}\,cm^{-1}$), for *V. cholerae* DNA (6400 $M^{-1}\,cm^{-1}$), for *P. pyociania* DNA (6700 $M^{-1}\,cm^{-1}$) and for *M. lysodeikticus* DNA (6900 $M^{-1}\,cm^{-1}$).

All experiments were conducted either in 5 mM NaCl, pH 6.8 or in 1 mM tris-HCl buffer, pH 7.4. Glass distilled deionised water and analytical grade reagents were used throughout. Cu(II) ions were used in the form of $CuSO_4$, 5 H_2O. The drug obeyed Beer's law in the concentration range used in this study. Addition of suitable amount of Cu(II) ions to DNA or to drug-DNA complex was done following the method described previously [19, 20].

The thermal strand separation profile of DNA in the presence or absence of furazolidone or Cu(II) ions or both was determined as described previously [11, 17, 21]. The temperature of the solution within the reference cell of the spectrophotometer was measured by a calibrated thermocouple system. The absorbance readings were corrected for volume expansion [22]. The transition temperature, T_m was defined as the temperature corresponding to which the hyperchromicity, h (A_T/A_{25}) is given by $h = \frac{1}{2}(h_{max} + 1)$, where h_{max} is the maximum hyperchromicity.

Results

The transition temperature of *V. cholerae* DNA in 5 mM NaCl, pH 6.8 was 72 °C (Fig. 1). When 15 μM furazolidone was allowed to complex with DNA (furazolidone/DNA molar ratio of 0.25) in the same solvent, the transition temperature of DNA was enhanced to 79.5 °C (Fig. 1). The transition was steeper in the presence of furazolidone and was indicative of its more cooperative nature. Further increase of the drug/DNA molar ratio did not increase the transition temperature by any significant degree.

Transition temperature of *V. cholerae* DNA (60 μM) was lowered to 65.5 °C in the presence of 15 μM Cu(II) ions in 5 mM NaCl, pH 6.8. Keeping the DNA concentration fixed, its transition temperature was lowered to 46 °C and 33 °C in the presence of 30 μM and 100 μM Cu(II) ions respectively (Fig. 1; Table I). Cu(II) ions of different concentrations were not equally effective in lowering the transition temperature of furazolidone-DNA complex. When Cu(II) ions of molarity same as that of furazolidone was added to furazolidone-DNA complex, the transition temperature was lowered from 79.5 °C to 76 °C (Table I). This was significantly higher than the transition temperature of DNA complexed with same amount of Cu(II) ions (65 °C) alone. Keeping the concentrations of DNA and furazolidone fixed,

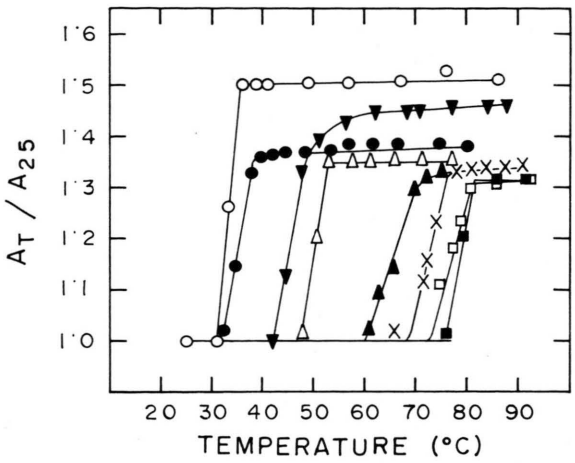

Fig. 1. Thermal transition profiles of native *V. cholerae* DNA (60 μM) and furazolidone-DNA complex (molar ratio of 0.25) in presence of Cu(II) ions in 5 mM NaCl, pH 6.8. Symbols: (○–○), 100 μM Cu(II) ions in presence of DNA; (●–●), 100 μM Cu(II) ions in presence of furazolidone-DNA complex; (▼–▼), 30 μM Cu(II) ions in presence of DNA; (△–△), 30 μM Cu(II) ions in presence of furazolidone-DNA complex; (▲–▲), 15 μM Cu(II) ions in presence of DNA; (×–×), native DNA; (□–□), 15 μM Cu(II) ions in presence of furazolidone-DNA complex; (■–■), furazolidone-DNA complex. The ordinate A_T/A_{25} indicates the ratio of absorbance at any temperature (T) and that at 25 °C.

increasing concentration of Cu(II) ions caused increased lowering of the transition temperature of the furazolidone-DNA complex (Table I), but in all such cases the lowering was not as much as was done by the corresponding amount of Cu(II) ions alone (without furazolidone).

For the same value of ligand to DNA ratio and using DNAs of different base composition, the transition temperatures of the furazolidone-DNA complexes were different. The transition temperature increase linearly with the % A−T content of DNAs (Fig. 2).

Table I. Thermal melting temperatures of native DNA and furazolidone-DNA complex in presence of Cu(II) ions in 5 mM NaCl, pH 6.8. In all cases the concentrations of DNA and furazolidone were 60 μM and 15 μM respectively.

Concentration of Cu(II) ions [μM]	T_m value [°C]	
	Native DNA	Furazolidone-DNA complex
0	72.0	79.5
15	65.5	76.0
30	46.0	51.0
100	33.0	35.0

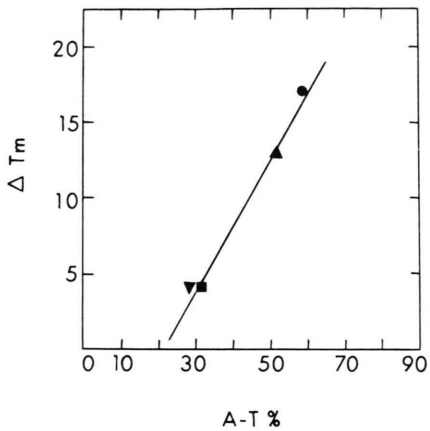

Fig. 2. Plot of enhancement of transition temperature (ΔT_m) of different DNAs resulting from *in vitro* furazolidone binding against their % A−T content in 1 mM tris-HCl buffer, pH 7.4. Concentrations of DNA and furazolidone were 75 μM and 30 μM respectively. Symbols: (●), calf thymus DNA; (▲), *V. cholerae* DNA; (■), *P. pyociania* DNA; (▼), *M. lysodeikticus* DNA.

Discussion

The results presented here have confirmed and extended our previous findings [13] on the *in vitro* interaction of furazolidone with DNA. Furazolidone binding leads to thermal stabilisation of DNA and this has been found true for DNAs of different % A−T content. This study has further confirmed the previous reports on the Cu(II) ions induced lowering of the transition temperature of DNA [19, 20, 23–26]. The present observations on the effects of Cu(II) ions on the thermal stabilisation of furazolidone-DNA complex appears to be interesting. Cu(II) ions were not equally effective in lowering the transition temperature of furazolidone-DNA complex. When Cu(II) ions were added subsequent to the formation of furazolidone-DNA complex, the transition temperatures of furazolidone-DNA-Cu(II) complexes were always significantly higher than the corresponding values of DNA-Cu(II) complexes and this was significant even when the molar concentration of Cu(II) ions was more than 6 fold higher than that of furazolidone. These data indicated that furazolidone and Cu(II) ions did not complete for the same sites in DNA. The nature of the interaction of Cu(II) ions with DNA has been investigated by many workers [23–26] and different interpretations were presented. It is generally believed that at the low ionic strength of the solvent, two different types of interaction between Cu(II) ions and DNA take place simultaneously. Type I interaction has the overall effect of stabilising [24] or at least non-denaturing [25] the DNA and is supposed to be predominant at lower values of the Cu(II) ions/DNA ratio. Type II interaction has an overall destabilisation effect on DNA and is predominant at higher values (>0.3) of Cu(II) ions/DNA ratio [25]. This type II interaction should thus be of great concern in our case where the Cu(II) ions/DNA ratio is greater than 0.25. Although there are differences of opinion in respect of the exact nature of the interaction involved in the type II reaction, it is generally agreed that the destabilisation of DNA structure is initiated by the breakage of bonds between G−C base pairs [24, 25]. It appears that furazolidone binding has very little to do with the G−C base pairs. This idea was supported by the thermal transition profiles of furazolidone-DNA complexes for DNAs of different G−C or A−T content, which clearly indicated that furazolidone has a preference for A−T base pairs.

In conclusion, this study has thus shown that furazolidone on complexing with DNA led to its thermal stabilisation irrespective of its base composition. When equimolar amounts of furazolidone and Cu(II) ions were used, the stabilisation effects of furazolidone prevailed over the destabilisation effect of Cu(II) ions. The furazolidone-DNA complexing had a preference for A−T base pairs.

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