# THE EFFECT OF TEMPERATURE AND THIONINE CONCENTRATION ON DNA STABILITY

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**Interest in studying the interaction of small molecules with DNA is caused by the need to develop new, highly effective, and low-toxic drugs for cancer treatment [1]. The strong and highly specific binding of thionine with DNA makes it a promising candidate for use in medicine and pharmacology [2]. In this study, DNA-thionine complexes in aqueous solutions were investigated using UV-Vis absorption spectroscopy. The thermal stability of native DNA was studied in a broad range of thionine concentrations.**

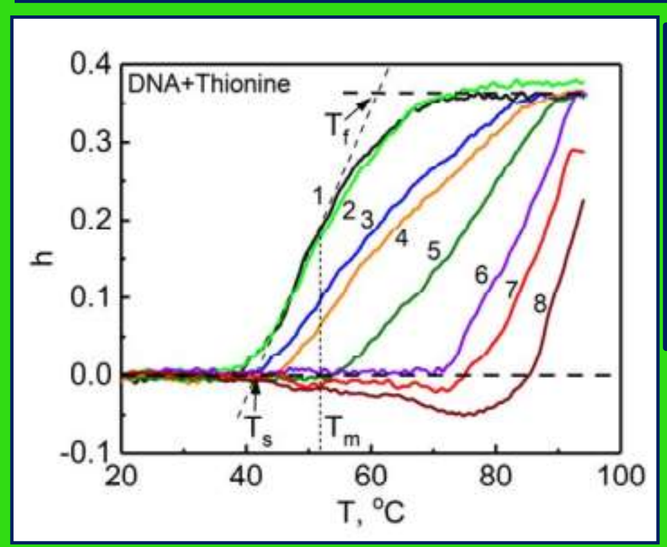


Fig. 2 Temperature dependence of hyperchromic coefficient (h) of DNA without (curve 1) and with (curves 2 - 8) thionine: "1" – $[c_{th}]$ = 0 mg/L; "2" – $[c_{th}]$ = 0.1 mg/L; "3" – $[c_{th}]$ = 1 mg/L; "4" – $[c_{th}]$ = 1.5 mg/L; "5" –$[c_{th}]$ = 2.5 mg/L, "6" – $[c_{th}]$ = 5 mg/L; "7" – $[c_{th}]$ = 7.5 mg/L; "8" – $[c_{th}]$ = 10 mg/L.

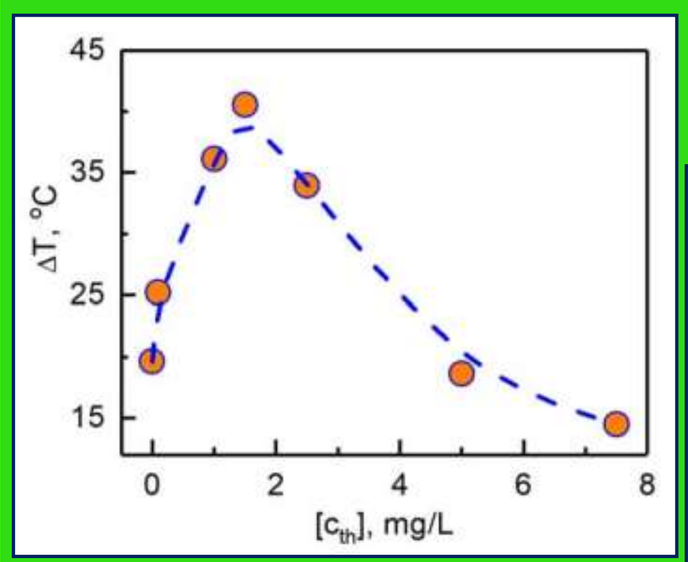


Fig. 3 Melting interval (ΔT) of DNA in the DNA-thionine suspension. The melting interval was determined as follows: $\Delta T = T_f - T_s$, where $T_s$ and $T_f$ are the temperatures at which DNA melting starts and finishes, respectively (Fig. 2).

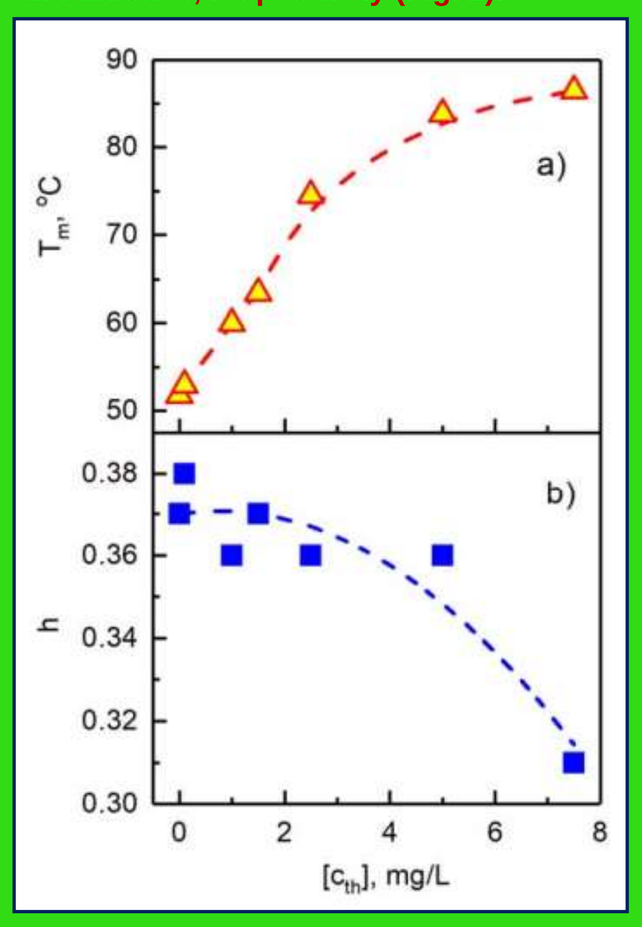


Fig. 4 a) Effect of thionine concentration on the melting temperature $T_m([c_{th}])$ and b) hypochromic coefficient $h([c_{th}])$ of DNA.

**Materials: The calf thymus DNA was added to a buffer solution of $10^{-3}$ M sodium cacodylate from Serva (Germany), at pH7. The effect of thionine on the biopolymer structural conformation was studied by adding the required amount of thionine solution to the DNA buffer solution. The DNA phosphorus concentration [P] was (6.9 ± 0.6)·$10^{-5}$ M, which was determined by the molar extinction coefficient ($\varepsilon_m$ = 6600 $M^{-1}\cdot cm^{-1}$) at the DNA absorption maximum ($\nu_m$=38,500 $cm^{-1}$).**

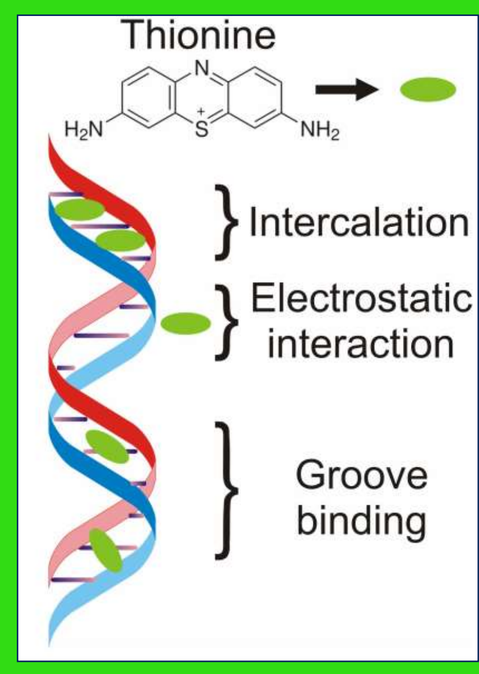


Fig. 1 Chemical structure of thionine in cationic form. The different modes of thionine binding to DNA.

**The melting curve of the pure DNA has a typical S-shape (Fig. 2). The addition of thionine (0.1 mg/L) to the DNA solution does not disrupt the double-stranded structure and conformation of DNA. However, the injection of $[c_{th}]$ = 1 mg/L induces a change in the shape of the melting curve: DNA melting becomes less cooperative, and it occurs in a wider temperature interval (Fig. 2). An increase in thionine concentration leads to a broadening of the melting interval, which peaks near 1.5 mg/L (Fig. 3). A possible reason for the increase in the melting range is the intercalation of thionine molecules into the biopolymer (Fig. 1). At the same time, at $[c_{th}]$≥1.5 mg/L, the DNA melting range narrows (Fig. 3). A possible reason for this effect is the involvement of other types of thionine binding to DNA (groove binding and external electrostatic interaction of thionine with the phosphate backbone of DNA) (Fig.1). Also, it can be noted that in the temperature range of 45 – 75 °C at $[c_{th}]$ ≥ 7.5 mg/L, hypochromism is observed, which is replaced by hyperchromism, upon further heating. A possible reason for the revealed hypochromism may be the formation of the conglomerates consisting of several DNA-thionine complexes [3].**

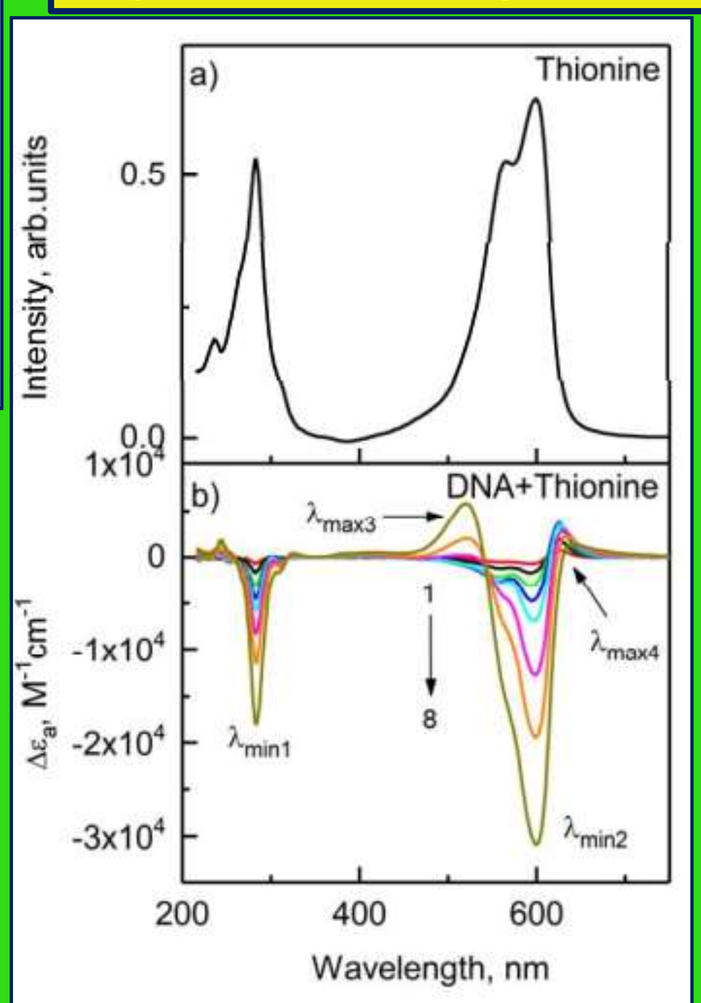


Fig. 5 a) Absorption spectrum of an aqueous solution of thionine ($[c_{th}]$ = 0.12 g/L) and b) DUV spectra of DNA thionine complexes with $[c_{th}]$ = 1, 2.5, 5, 7.5, 10, 15, 20, and 30 mg/L (curves 1-8). All experimental data were obtained at room temperature.

**The analysis of room-temperature DUV absorption spectra of the thionine-DNA complex (Fig. 5b) suggests that thionine binds to DNA primarily via intercalation up to a threshold concentration. As thionine concentration increases, the main absorption minima (at about $\lambda_{min1}$ = 283 nm and $\lambda_{min2}$ = 593 nm revealed at $[c_{th}]$ = 1 mg/L) show hypochromism and a bathochromic shift, which are characteristic of dye binding and similar to other phenothiazine derivatives. This hypochromism is a key indicator of intercalation, which is thought to be complete at about 7.5 mg/L. Above this concentration, the binding mode shifts from intercalation to the minor groove binding or external electrostatic binding to the DNA phosphate backbone, although both modes may coexist. At very high concentrations ($[c_{th}]$ ≥ 20 mg/L), the appearance of new bands suggests the formation of thionine aggregates (possibly H-type dimers) on the DNA surface [4]. The observed threshold concentration for the binding mode change is slightly higher than that found in temperature-dependent studies, where increased DNA flexibility at higher temperatures facilitates intercalation even at lower concentrations.**

**Fig. 4a clearly shows that injecting thionine into the DNA solution raises the DNA melting temperature by 34.7 °C at concentration up to 7.5 mg/L. Thus all types of interaction are accompanied by a stabilizing effect. The injection of thionine up to $[c_{th}]$ = 1.5 mg/L has negligible effect on h parameter, with fluctuations within ±3% (Fig. 4b). With a subsequent increase in the dye concentration, a gradual decrease in h is observed, and at a thionine concentration of $[c_{th}]$ = 7.5 mg/L, h decreases by 16%.**

## Conclusion

**This study employed UV-visible spectroscopy and DNA thermal denaturation to investigate the effects of temperature and thionine concentration on DNA stability in aqueous solutions at pH7. Thionine interaction with DNA was found to increase the melting temperature of DNA across all tested thionine concentrations of 0.1 - 7.5 mg/L. Specific concentration ranges were identified in which a particular mode of thionine binding to DNA predominates. There is a concentration at which the interaction type shifts from intercalation to the groove binding and electrostatic interaction. The results obtained in the present work can help shed light on the molecular mechanisms of small molecule binding to double-stranded DNA. That will stimulate the development of new highly effective, and most importantly, less toxic drugs for the treatment of viral and cancer diseases.**

References:
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