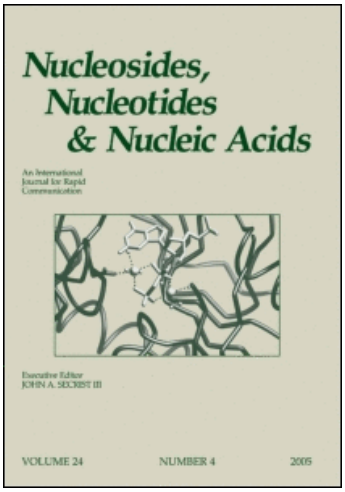

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INTERACTION OF A MINOR GROOVE BINDER WITH A FLUORESCENT DNA OLIGOMER CONTAINING THE Eco RI RECOGNITION SEQUENCE

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ABSTRACT: The minor groove binding drug netropsin quenches the 2-aminopurine (2AP) fluorescence in the duplex $d(CTGA(2AP)TTCAG)_2$. Drug binding constants, $K \sim 10^5$ M-1 were established between 5-25°C. A preliminary evaluation of the thermodynamic data indicated a predominantly entropy driven interaction.

2-Aminopurine (2AP) is an analogue of adenine which has a fluorescence lifetime three orders of magnitude higher than that of adenine. 2AP-substituted oligonucleotides therefore constitute useful model systems for fluorescence studies of the kinetics and dynamics of drug-DNA or protein-DNA interactions. Extensive structural dynamic studies, employing 2-dimensional ^1H n.m.r. spectroscopy, and fluorescence spectroscopy in addition to modelling and molecular dynamics have shown that the oligonucleotide forms a stable duplex adopting the classic B-conformation.[1] The 2AP still participates in the Watson-Crick H-bonding with thymine but places the 2AP, C2-NH_2 in the minor groove. We have studied the interaction of netropsin with this fluorescent oligomer. Netropsin is an antitumour antibiotic which binds specifically in the minor groove of AT sites of 5±1 base pairs.

The oligomer showed a concentration dependent melting at pH 7.5 (0.01 M TRIS-HCl, 0.1 M KCl and 0.001 M EDTA). The melting point was seen as the maximum of the increase in fluorescence with increasing temperature (using λ_{ex} at 315 nm and λ_{em} at 370 nm). Above 0.02×10^{-3} M (in strands), the melting point stabilised at 30°C. In the presence of netropsin the fluorescent oligomer showed an elevated melting indicating its association with the drug.

Titrations of fluorescent oligomer (0.02×10^{-3} M in strands) with netropsin at pH 7.5 (buffer as above) resulted in complete fluorescence quenching of the 2AP probe over the temperature range 5-25°C. Binding isotherms for the netropsin interaction were evaluated, and fitted for binding constant (K) and number of binding sites (n), using a nonlinear least squares regression analysis of the following theoretical expression[2] :

$$\alpha_A = \frac{[A_0] + n[drug] + K^{-1} - \sqrt{([A_0] + n[drug] + K^{-1})^2 - 4n[A_0][drug]}}{2[A_0]}$$

α_A is the fraction of oligomer bound and A_0 is the initial concentration of the oligomer. Best values of $K \sim 10^5$ M^{-1} between 5-25°C, using $n=1$ were derived. A preliminary evaluation of the thermodynamic data using, $K = e^{-\Delta G/RT}$ suggests essentially an entropy driven interaction *i.e.* $\Delta S = +12.2$ cal/mol.°K at 25°C. Comparatively for the decamer $d(CGCAATTCGC)_2$ an enthalpy driven interaction has been reported, with a netropsin binding constant of $K \sim 10^9$ M^{-1} at 25°C.[3] However, a decreased binding affinity with poly $d(GC)_2$ has been observed, resulting in a drop of 10^4-10^5 in the binding constant, and is an entropy driven interaction.[3] Thus netropsin shows a kinetically and thermodynamically similar interaction with d(CTGA(2AP)TTC-AG)$_2$ and an all-GC duplex. This is not unreasonable since in both nucleic acid systems there is an exocyclic C2-NH2 protruding out into the bottom of the minor groove. This probably prevents the tight fitting of the netropsin into the minor groove, which seems essential for a thermodynamically favourable interaction.

Our preliminary time resolved measurements have shown that the netropsin causes complete quenching of the 2AP fluorescence. Thus this drug system is not useful for studying the dynamics of the drug interaction. However the probe in this system can give reliable information concerning the kinetics and thermodynamics of the drug interaction. This study has also shown that netropsin can still bind to the modified oligomer. However, the modified 2AP:T base pair is recognised as a G:C base pair resulting in a decreased binding affinity and the less favoured entropy driven interaction.

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