

A Molecular Dynamics Study of the Bis-Intercalation Complexes of Echinomycin with d(ACGT)₂ and d(TCGA)₂: Rationale for Sequence-Specific Hoogsteen Base Pairing

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The behavior of the complexes of echinomycin with the DNA tetramers d(ACGT)₂ and d(TCGA)₂, in which the terminal AT base pairs are in either a Hoogsteen or a Watson-Crick conformation, has been explored by molecular dynamics taking into account experimental data from NMR studies (Gao and Patel. *Biochemistry* 1988, 27, 1744-1751). The DNA binding specificity of echinomycin appears to be the result of a subtle balance between stabilizing and destabilizing forces. Among the former is a number of hydrogen bonds between the alanine residues of echinomycin and both the N3 and 2-amino groups of the guanine bases which decisively determine the strong affinity of the antibiotic for CpG steps. On the other hand, there appears to be an unfavorable dipolar interaction between the chromophores of the antibiotic and the CpG step. This electrostatic component of the stacking interactions also contributes to explaining the conformational preferences of the flanking sequences: upon Hoogsteen pairing, the dipole moment of an AT base pair is found to increase significantly and alter its relative orientation. In the d(ACGT)₂:echinomycin complex, this arrangement helps to improve the stacking interactions with the quinoxaline-2-carboxamide system, but would lead to unfavorable dipolar interactions in the d(TCGA)₂ complex. The bearing of these findings on the binding of echinomycin to several sequences as well as on the altered binding selectivity of other members of the quinoxaline family of antibiotics is also discussed.

Introduction

The DNA molecule is the primary target of many antitumor agents. Among the binding modes of drugs to DNA, both intercalative and nonintercalative mechanisms have been described, and in both cases a certain degree of sequence selectivity has been shown. Interestingly, minor groove binders show a tendency to bind to AT-rich DNA better than to GC-containing sequences whereas most intercalators get sandwiched at GpC or CpG steps,¹ with the notable exception of TANDEM, which also prefers AT runs.² In addition to the experimental approach, modeling studies have proved of value in order to understand the determinants of specificity involved in the recognition process³ and can help in the design of new compounds with tailor-made binding properties.

Echinomycin is one of several antibiotics produced by *Streptomyces echinatus* that consists of two quinoxaline chromophores attached to a cyclic octadepsipeptide ring with a thioacetal cross-bridge (Figure 1). It displays potent cytotoxic activity that is generally accepted to arise from its capacity to bind to cellular DNA as a bis-intercalator, as shown in a wide range of viscosimetric, X-ray, and NMR studies.⁴ Echinomycin is currently in phase II clinical trials as an antitumor agent.⁵

Footprinting experiments with DNase I⁶ and chemical probes⁷ have demonstrated that echinomycin binds specifically to CpG sequences and shows some preference for AT as the flanking base pairs.

X-ray crystal structures are available for the complexes of echinomycin with the d(CGTCAG)₂ hexamer⁸ and of the closely related analogue triostin A with d(CGTCAG)₂⁹ and d(GCGTACGC)₂.¹⁰ A number of NMR studies have also been undertaken with echinomycin bound to different

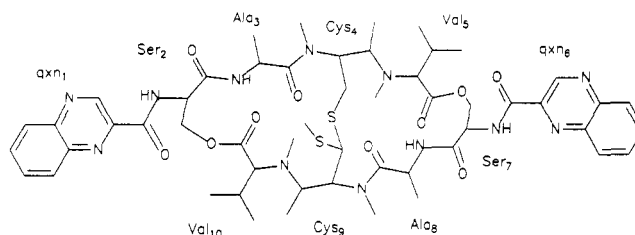


Figure 1. Chemical structure of echinomycin. Echinomycin is one member of the quinoxaline family of antibiotics, which are characterized by a cross-linked octadepsipeptide ring bearing two quinoxaline chromophores (qx). In the echinomycin analogue triostin A, a disulfide bond replaces the thioacetal cross-link.

DNA oligomers: d(ACGT)₂ and d(TCGA)₂,¹¹ d(GCGC)₂, d(CCGG)₂, and d(AAACGTTT)₂,¹² d(ACGTACGT)₂ and d(TCGATCGA)₂,¹³ and d(ACGTATACGT)₂.¹⁴ This experimental work has provided considerable insight into the structural features of these complexes. Most notably, it has shown that in every complex studied the quinoxaline rings of the antibiotic intercalate on both sides of the CpG steps and that three major factors contribute to stabilizing the echinomycin-DNA complexes: (1) van der Waals interactions between the peptide part of the antibiotic and the minor grooves of the oligomers, (2) stacking interactions between the quinoxaline rings and the adjacent base pairs, and (3) several hydrogen bonds between the NH and carbonyl groups of alanines and the N3 and 2-amino groups of guanines, respectively. These hydrogen bonds are widely considered to be crucial for the binding specificity of echinomycin to CpG steps.⁵

The conformation of echinomycin complexed with DNA is similar to that found for the isolated antibiotic in solution.^{15,16} In contrast, the DNA oligomers undergo drastic conformational changes upon binding of the drug. Most important among such changes are the unwinding of the double helices and the induction of a Hoogsteen

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