

DNA Sequence-Specific Reading by Echinomycin: Role of Hydrogen Bonding and Stacking Interactions

José Gallego,[†] F. J. Luque,[‡] Modesto Orozco,[§] Carolina Burgos,^{||} Julio Alvarez-Builla,^{||} M. Melia Rodrigo,[⊥] and Federico Gago^{*†}

Departamentos de Fisiología y Farmacología, Química Orgánica, and Química Física, Universidad de Alcalá de Henares, 28871 Madrid, Spain, and Departament de Farmàcia (Unitat Físico-Química), Facultat de Farmàcia, and Departament de Bioquímica i Fisiologia, Facultat de Química, Universitat de Barcelona, 08028 Barcelona, Spain

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The binding of echinomycin to DNA hexamers of the form GpApXpZpTpC, where the central XpZ step can be CpG, TpA, GpC, or ApT, has been studied by molecular modeling and molecular mechanics techniques. Interaction energies have also been calculated for the complexation of echinomycin with sequences containing the preferred central CpG step and different flanking base pairs. Besides, two more sets of sequences incorporating either 2,6-diaminopurine (DAP) or hypoxanthine in place of adenine or guanine, respectively, have been examined. The aim of this work was to evaluate the relative importance of hydrogen-bonding and stacking interactions in the association of echinomycin with DNA and further rationalize the experimental evidence. The results of these calculations are in consonance with available data from footprinting experiments and appear to support our previous hypothesis that, in addition to the crucial intermolecular hydrogen bonds in the central region, the stacking interactions involving the quinoxaline-2-carboxamide chromophores of the drug and the DNA base pairs play an important role in modulating the binding specificity of this bisintercalating antitumor antibiotic. This is most clearly seen when sequences with similar minor-groove environments are compared (e.g. CpI vs TpA or CpG vs TpDAP). The dipole moment of *N*-methylquinoxaline-2-carboxamide has been measured ($\mu = 4.15 \pm 0.03$ D) and compares very well with the calculated value ($\mu = 4.14$ D). The fact that G:C, I:C, A:T, and DAP:T base pairs are shown to be endowed with distinct van der Waals and electrostatic stacking properties with respect to this heteroaromatic ring system could have important implications for the design of novel DNA mono- and bis-intercalating agents.

Introduction

Echinomycin is a staple-shaped antitumor antibiotic from *Streptomyces echinatus* which binds to DNA as a bifunctional intercalator.¹ Its two quinoxaline rings bisintercalate into double helical DNA whereas the inner part of the bicyclic depsipeptidic linker faces the minor-groove region of the two base pairs comprised between the chromophores where it establishes a number of hydrogen bonds with the DNA bases (Figure 1). The resulting interaction has been compared to that of a vice clamping the inner bases.²

In common with its close relative triostin A, echinomycin shows a marked preference for binding to CpG steps.^{3,4} In this selectivity it is commonly accepted that the hydrogen bonds established between the NH and carbonyl groups of the antibiotic's alanines and the N3 and 2-amino groups of the guanines play a predominant role. Some preference for A:T as the flanking base pairs has also been reported for echinomycin^{3,4} although no definitive rules have been established in this respect.⁵ Interestingly, when a purine nucleoside is on the 5' side of a CpG binding site, its base ring can rotate 180° about the glycosidic bond, giving rise to a Hoogsteen base pairing scheme,⁶ as shown experimentally by X-ray diffraction⁷⁻⁹ and NMR analyses on several oligonucleotides.¹⁰⁻¹² For longer DNA sequences,

however, no evidence exists that Hoogsteen pairing occurs at internal base pairs surrounding an isolated binding site.⁵

In a recent theoretical study,¹³ we have suggested that the origin of the different conformational behavior of the bases flanking the echinomycin binding site in d(ACGT)₂ and d(TCGA)₂¹⁰ could lie in the drastic change in dipole moment taking place in an A:T base pair when going from Watson-Crick to Hoogsteen pairing, which results in better stacking interactions with the drug's chromophores in the former complex but not in the latter. This same study highlighted an unfavorable electrostatic interaction between the quinoxaline-2-carboxamide system of the antibiotic and the sandwiched G:C base pairs, which led us to hypothesize that modulation of the dipole moments of the intercalating chromophores could be an additional element to be taken into account in the recognition process.

Traditional work in structure-affinity relationships (SAR) for the quinoxaline family of antibiotics has dealt with the effects that introduction of new substituents or removal of existing ones have on the binding properties of a given drug, as assessed mainly by DNA footprinting experiments.¹⁴⁻¹⁷ The best known examples are probably those provided by des-*N*-tetramethyltriostrin A (TANDEM) and [*N*-MeCys³,*N*-MeCys⁷]TANDEM (CysMeTANDEM), triostrin A analogues lacking either all or half of the *N*-methyl groups of the cysteines and valines, respectively, which bind better to TpA.^{18,19} More recently, a series of elegant experiments utilizing molecular biology techniques have gone one step further: modification of the DNA itself. This has turned out to be another very important aspect of SAR studies. Thus, it has been shown that removal of the exocyclic amino group of guanine (by

[†] Departamento de Fisiología y Farmacología, Universidad de Alcalá de Henares.

[‡] Departament de Farmàcia, Universitat de Barcelona.

[§] Departament de Bioquímica i Fisiologia, Universitat de Barcelona.

^{||} Departamento de Química Orgánica, Universidad de Alcalá de Henares.

[⊥] Departamento de Química Física, Universidad de Alcalá de Henares.

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