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Conformation of the Deoxyribonucleic Acid-Binding Peptide Antibiotic Echinomycin Based on Energy Calculations

GIOVANNI UGHETTO AND MICHAEL J. WARING

Department of Pharmacology, University of Cambridge Medical School, Cambridge CB2 2QD, England

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SUMMARY

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Potential energy calculations and geometrical requirements for ring closure limit the number of allowed conformations for the peptide ring of echinomycin to a very few. Refinement by a method of steepest descent leads to the selection of a minimum-energy conformation having substantial elements of twofold rotational symmetry, with the quinoxaline chromophores spaced approximately 10.2 A apart in a configuration which is compatible with simultaneous (bifunctional) intercalation into the DNA helix.

INTRODUCTION

Echinomycin is a cyclic peptide antibiotic whose antitumor and other biological effects are attributable to inhibition of DNA template activity (1–3). The binding of echinomycin to DNA was recently shown to occur by a novel bifunctional mechanism in which both quinoxaline chromophores of the antibiotic can intercalate simultaneously between the base pairs (4, 5). In order to gain deeper insight into the molecular nature of the interaction, we have undertaken a conformational analysis of the echinomycin molecule (Fig. 1), using the method of energy calculations successfully applied to predict the conformation of actinomycin D in previous studies (6, 7).

The approach is semiempirical, based on calculation of potential energy in terms of internal conformational parameters, namely, the angles of rotation about chemical bonds (8). The most stable conformations are selected by optimizing the potential energy with regard to constraints imposed by experimental evidence as to dihedral angles in agreement with coupling constants from NMR data, formation of hydrogen bonds, geometrical conditions for ring closure, and symmetry arguments (6, 7, 9). This approach provides a general method for studying the conformation of molecules for which the number of constraints is large enough with respect to the number of conformational variables. Most importantly for cyclic molecules, the condition of ring closure drastically limits the number of allowed conformations. Although the approximations necessarily introduced preclude evaluation of small differences in energy minima, there is the advantage that all sterically allowed conformations may be studied and compared within a range of increasing potential energy. This can be helpful as a means of examining possible interconversions when the molecule is in different environments, e.g., the free and DNA-bound states.

Since a complete, multidimensional

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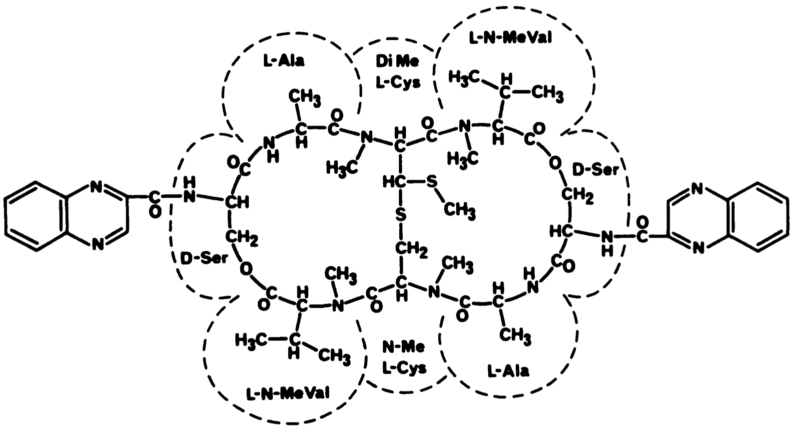

FIG. 1. *Structural formula of echinomycin*

analysis of the potential energy of echinomycin is impossible, we began by making a few simplifying assumptions, in particular that the molecular conformation can be derived as an assemblage of sterically allowed conformations of fragments of the peptide chain (6, 7, 9). Fixed geometry was adopted, taking standard values for bond angles and lengths (10–12), and electrostatic interactions were neglected (there are no charged atoms in echinomycin). Thus the total potential energy was approximated to the expression $E = E_{\mathrm{vdW}} + E_{\mathrm{T}} + E_{\mathrm{HB}}$, where E_{vdW} is the van der Waals energy calculated as the sum of pairwise additive interatomic interactions between nonbonded atoms (9, 13, 14), E_{T} is the intrinsic torsional potential for rotation about bonds (9, 10), and E_{HB} is the hydrogen bond energy calculated using a Stockmayer function (9).

In the first step we calculated the energy of dipeptide units in terms of rotation angles ϕ, ψ, and χ about $C_\alpha-N$, $C_\alpha-C'$, and $C_\alpha-C_\beta$ bonds. The angle ω about $N-C'$ peptide bonds was kept *trans* except for *N*-methylated amino acid residues, both the *cis* and *trans* isomers of which were considered. Energy maps corresponding to the four amino acid residues of echinomycin are shown in Fig. 2. In the second step we analyzed tripeptide fragments, using angles within the allowed regions of energy maps and in the ranges compatible with NMR coupling constants (15–20), restricting further the number of possible conformations by rejecting those unsuitable for ring closure. In the third step we considered the molecule as composed of two identical halves except for the *S*-methyl group, which was not included at this stage. Conformations which permitted ring formation by the half-molecule were selected by calculating the distance between the sulfur atom of the cross-bridge, considered belonging to one cysteine residue, and the same atom considered belonging to the other cysteine residue. In this way the angle of rotation about the $C_\beta-O$ bond of serine, not included in the energy calculations for dipeptide fragments, is implicitly defined. Conformations so selected were taken as the starting point for a process of refinement by a steepest-descent method, in which the total van der Waals and torsional energy and the end-to-end distance were minimized simultaneously by perturbation of rotational angles (6, 7, 9). Lacking experimental evidence, no conditions were imposed for the formation of hydrogen bonds, but where it appeared during refinement that such bonds might be formed, their energies were included in the calculations. Finally, the conformations obtained for the half-molecule were combined together, the *S*-methyl group was included in four possible configurations (each time replacing one of the C_β hydrogens of the cysteine residues), and the complete molecule was refined by the same procedure. By analogy with actinomycin (6, 7, 19), the bonds connecting to

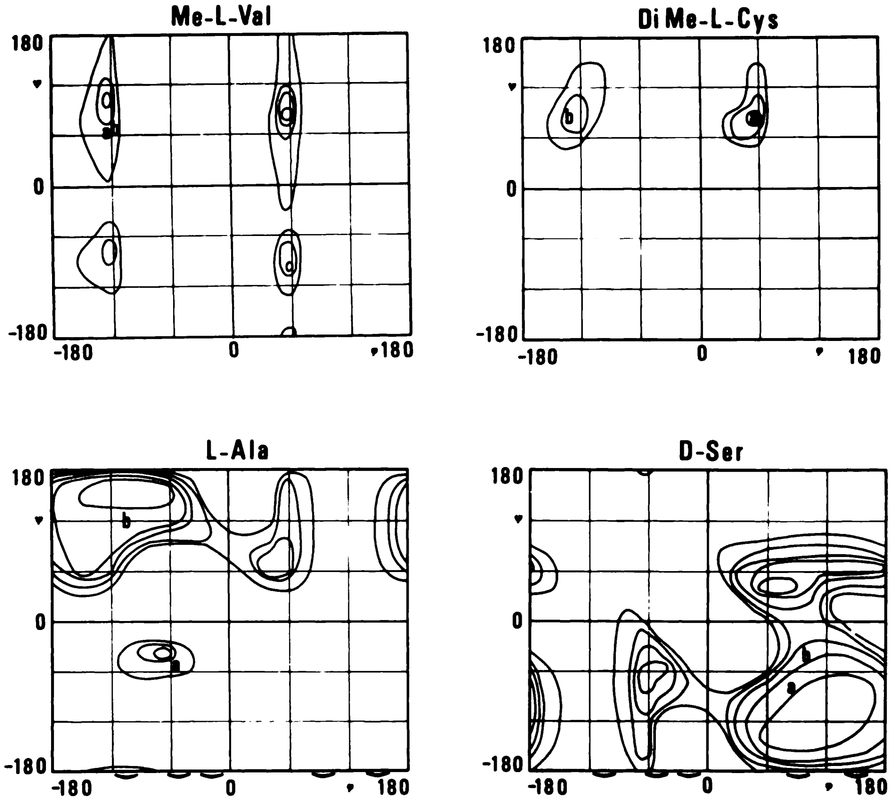

FIG. 2. *Van der Waals and torsional potential energy diagrams*

Me-L-Val, *N*-methyl-L-valine peptide lactone unit (C–CO–[L-MeVal]–O–C); DiMe-L-Cys, *N*, *S*-dimethyl-L-cysteine dipeptide unit (C–CO–[L-DiMeCys]–NCH_3–C); L-Ala, L-alanine dipeptide unit (C–CO–[L-Ala]–NCH_3–C); D-Ser, D-serine dipeptide unit (C–CO–[D-Ser]–NH–C). The external contours correspond to $E = 8$ kcal/mole, and the contours inside are at intervals of 4, 2, 1, and 0.5 kcal/mole, going downward. For the rotational angle ϕ about N–C_α, zero corresponds to C_α–C *cis* to N–C; for rotation about C_α–C′, $\psi = 0$ corresponds to C_α–N *cis* to C′–N (8). In each case the peptide bond C–N is *trans*. The diagrams are sections of three-dimensional maps corresponding to energy minima along the *z* axis, which represents the rotation angle χ about the C_α–C_β bond. The methylvaline diagram shows the unique energy minimum for χ corresponding to H–C_β *trans* to H–C_α, in agreement with NMR data [$J = 10$ Hz for both residues (15)]. The dimethylcysteine diagram shows the unique energy minimum for χ corresponding to H–C_β *trans* to H–C_α ($J = 9$ Hz). For *N*-methyl-L-cysteine there are two energy minima for χ, where one H–C_β is *trans* to H–C_α and the other is *trans* to C_α–N or to C_α–C, respectively; in both cases the dihedral angle with H–C_α is 60°, in agreement with NMR data ($J = 9$ Hz and 4 Hz). For L-alanine the energy map is two-dimensional because the methyl group of the side chain was treated as a single atom. For D-serine there are three energy minima for χ, corresponding to the three staggered conformations. The diagram shows that corresponding to H–C_β *trans* to H–C_α. Brackets on the abscissae indicate the allowed ranges from NMR coupling constants $J_{NH-C_\alpha H}$.

the quinoxaline chromophores were assumed to be nearly free. Calculations revealed two large energy minima corresponding to C═O at right angles to the aromatic plane; these angles are not affected by the conformation of the peptide chain.

For the half-molecule ring only two conformations are energetically favored, designated *a* and *b* (Fig. 3), with all peptide bonds *trans* and rotational parameters defined by the letters in the energy diagrams of Fig. 2. In conformation *a* two atoms are sufficiently close to form a hydrogen bond

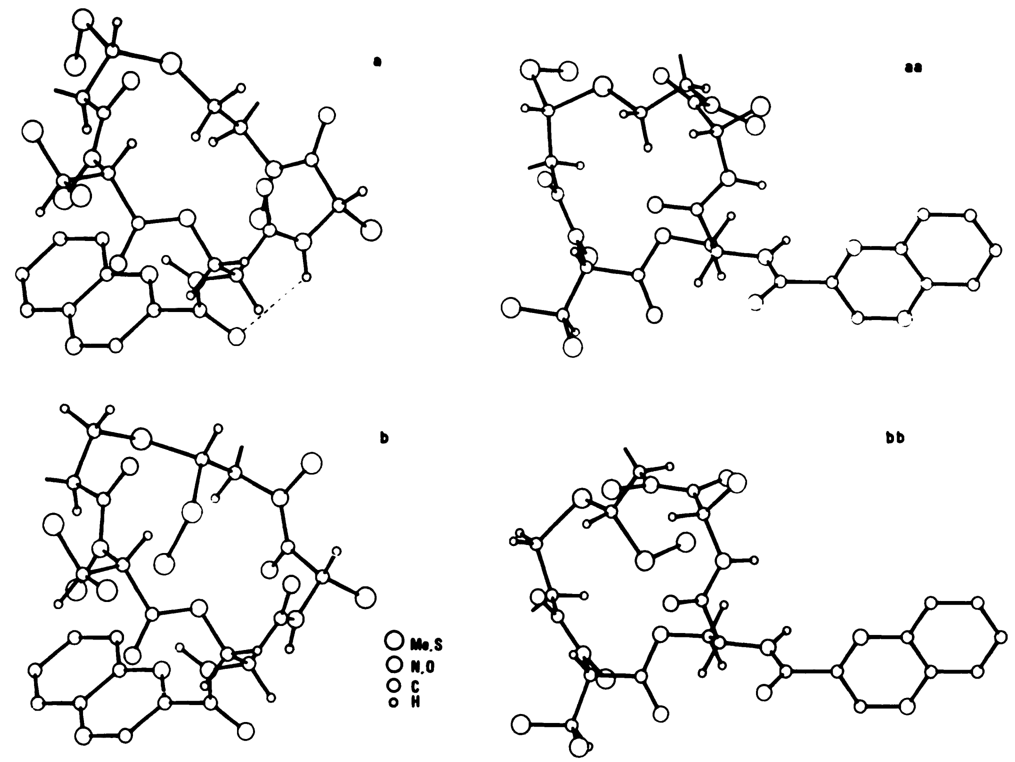

FIG. 3. *Conformations of the half-molecule ring of echinomycin*

a = 5.8 kcal/mole; b = 6.0 kcal/mole; aa = 8.8 kcal/mole; bb = 9.5 kcal/mole. Broken bonds show the junction with the other half-molecule. The dashed line in conformation a represents a possible hydrogen bond: $E_{\mathrm{HB}} = -2.4$ kcal/mole; H···O = 2.14 A. Hydrogens on aromatic rings are omitted.

(broken line). Two other conformations, aa and bb (Fig. 3), are equivalent to conformations a and b, respectively, except for the rotational angles about the $C_\alpha - C_\beta$ and $C_\alpha - C'$ bonds of serine. They are less preferred because of their substantially higher conformational energy. The best conformation for the entire molecule, lying in the lowest potential energy minimum and compatible with all experimental data, was obtained by refinement of a structure having one ring in conformation a and the other in conformation b (Figs. 4 and 5). The nonequivalence of the two halves in this structure agrees with NMR evidence (15, 20), although substantial elements of symmetry are retained. There is

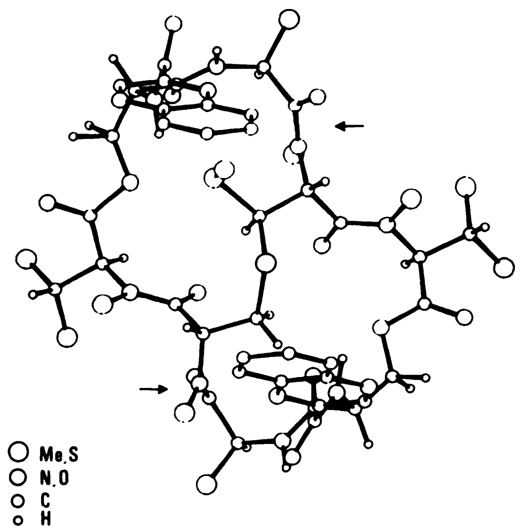

FIG. 4. *Proposed molecular structure for echinomycin*

The molecule is viewed along the quasi-dyad axis, with the chromophores projecting forward toward the viewer. Arrows indicate the L-alanyl-N-methyl-L-cysteine peptide bonds.

FIG. 5. *Corey-Pauling-Koltun model of proposed conformation for echinomycin*

The model is viewed along the quasi-dyad axis (outer panels) or at right angles to it (center). The view at left corresponds to the projection shown in Fig. 4.

a quasi-dyad axis passing through the central sulfur atom of the cross-bridge. The major asymmetrical feature is the reversal through approximately 180° of the peptide bond linking L-alanine to *N*-methyl-L-cysteine (arrowed in Fig. 4). A structure having both rings in the *b* conformation appears sterically hindered. With both rings in the *a* conformation the *S*-methyl group cannot be incorporated without considerable distortions from symmetry, resulting in the chromophores lying practically perpendicular to each other.

While we cannot claim a unique solution of the conformation based purely on theory and necessarily approximate energy calculations, an attractive feature of the proposed structure is its compatibility with the evidence for bifunctional intercalative binding to DNA (4, 5). The quinoxaline chromophores are almost ideally placed to engage in such interaction with DNA; they project well clear of the peptide ring mass, with their planes not far from parallel (the dihedral angle of inclination is 35° and can be reduced to zero by small deviations from ideality of bond lengths and angles). Their separation, assessed as the distance between the C-2 atoms by which they are linked to the peptide moiety, is 10.2 A – the exact value required to accommodate two stacked DNA base pairs between them. In addition, the whole molecule presents the same chirality about its quasi-dyad axis as does DNA.

The validity of the proposed structure can be examined if and when a crystal structure becomes available. Thus far all attempts to crystallize echinomycin have failed, although diffraction data have been collected for the related antibiotic (1, 21) triostin A. Further NMR experiments, using triostin A as well as echinomycin, should also shed light on the conformation in solution.

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