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Structure of TANDEM and its implication for bifunctional intercalation into DNA

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Quinoxaline antibiotics (Fig. 1*a*, *b*) form a useful group of compounds for the study of drug–nucleic acid interactions[1,2]. They consist of a cross-bridged cyclic octadepsipeptide, variously modified, bearing two quinoxaline chromophores. These antibiotics intercalate bifunctionally into DNA[2,3] probably via the narrow groove, forming a complex in which, most probably, two base pairs are sandwiched between the chromophores[4,5]. Depending on the nature of their sulphur-containing cross-bridge and modifications to their amino acid side chains, they display characteristic patterns of nucleotide sequence selectivity when binding to DNAs of different base composition and to synthetic polydeoxynucleotides[4,6,7]. This specificity has been tentatively ascribed to specific hydrogen-bonding interactions between functional groups in the DNA and complementary moieties on the peptide ring[2,4,5]. Variations in selectivity have been attributed both to changes in the conformation of the peptide backbone[6] and to modifications of the cross-bridge[7]. These suggestions were made, however, in the absence of firm knowledge about the three-dimensional structure and conformation of the antibiotic molecules. We now report the X-ray structure analysis of the synthetic analogue of the antibiotic triostin A, TANDEM (des-*N*-tetramethyl triostin A) (Fig. 1*c*), which binds preferentially to alternating adenine–thymine sequences[7]. The X-ray structure provides a starting point for exploring the origin of this specificity and suggests possible models for the binding of other members of the quinoxaline series.

Hydrated crystals of TANDEM, $C_{46}H_{54}N_{12}O_{12}S_2.12H_2O$, were obtained from a mixture of dimethyl formamide/ethanol/water. Crystal data: orthorhombic, $P2_12_12_1$, $Z=4$, $a=18.400$, $b=22.126$, $c=16.435$ Å. Diffractometer measurements for 5,422 independent reflections were obtained with MoKα radiation to a resolution of 0.85 Å at 7 ± 1 °C. The structure was solved with some difficulty by a combination of direct methods and a novel iterative Fourier procedure[8]. Refinement, with all non-hydrogen atoms anisotropic, converged at $R=14.7\%$ for 4,111 data with $F>2\sigma(F)$. Full details will be published elsewhere.

Figure 2 shows the molecular structure of TANDEM. The octadepsipeptide ring is held in a relatively rigid conformation through intramolecular hydrogen bonds NH(L-Val) ... O(L-Ala). The quinoxaline chromophores and the disulphide cross-bridge project on opposite sides of the peptide ring. The carbonyl groups of L-Cys and L-Val also project from the side opposite to the chromophores. The molecule displays a helical twist and has an approximate 2-fold axis of symmetry. Bond lengths and angles fall within the normal ranges.

The mean planes of the two chromophores are inclined at an angle of 14°. The distance between the Ser α-carbon atoms is 12.2 Å. Assuming that the intercalated complex is of the Fuller–Waring type[9], with minimal distortion from a classical B-DNA helix, we would expect the chromophore planes to lie parallel, approximately orthogonal to a line joining the Ser α-carbon atoms and separated by a perpendicular distance of 10–11 Å, to allow for bifunctional intercalation[10]. These geometrical constraints are readily imposed on the molecule derived from the X-ray analysis by rotations of approximately 20° about the C_α–N bonds of D-Ser. Such rotations do not result in steric hindrance with neighbouring groups[5,11]. The resultant structure (Fig. 3) can be fitted into the narrow groove of a B-DNA helix,

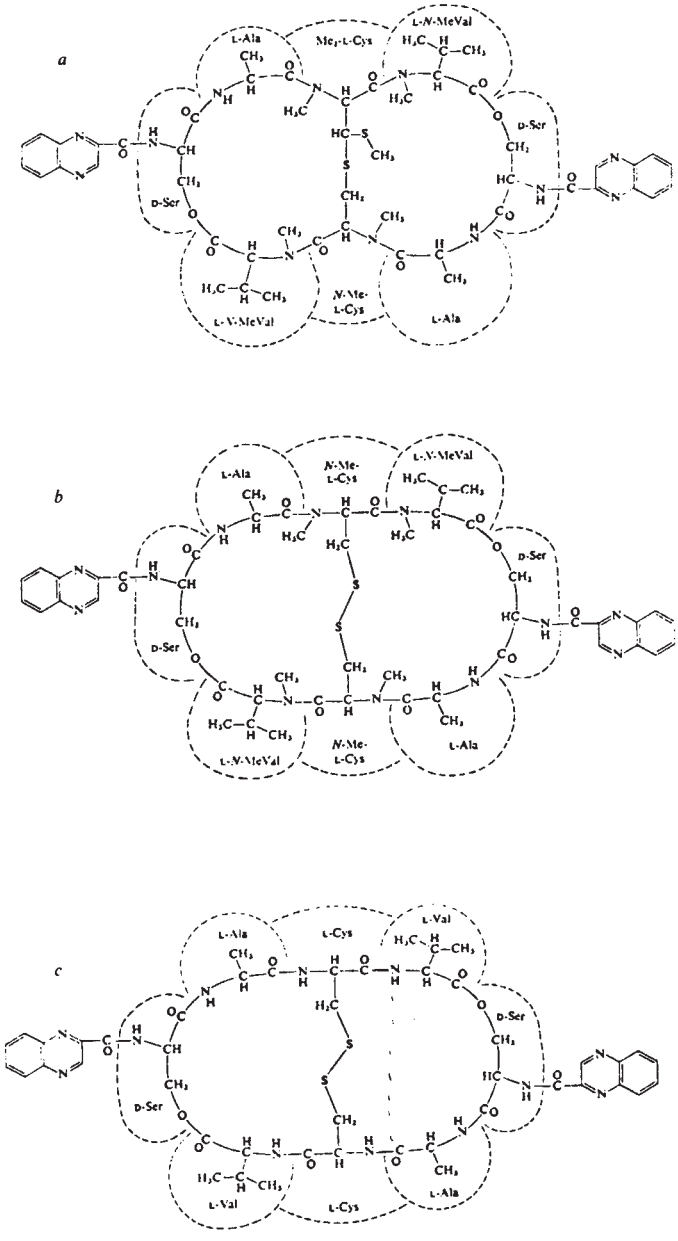

Fig. 1 Structural formulae of echinomycin (quinomycin A) (*a*), triostin A (*b*), TANDEM (des-*N*-tetramethyl triostin A) (*c*).

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which is known from solution experiments to be unwound by 45.5° on intercalation of TANDEM[2,7] (based on an unwinding angle of 26° for ethidium[12]). In this bis-intercalated model the disulphide cross-bridge projects outwards away from the helix, two base pairs are sandwiched between the chromophores and various functional groups of the cyclic peptide are placed in the vicinity of potential hydrogen-bonding sites on the DNA bases. The pseudo-2-fold axis of TANDEM is aligned with the 2-fold axis between the base pairs (Fig. 4).

Binding experiments with DNAs of varying base composition and with synthetic polydeoxynucleotides have revealed that TANDEM binds at least 7,500 times more tightly to poly(dA-dT) than to poly(dG-dC) and there are marked differences in the binding constants for DNAs of different nucleotide composition[7]. This specificity might be explainable in terms of hydrogen

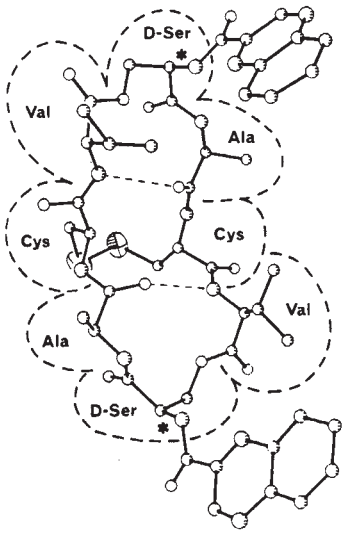

Fig. 2 Molecular structure of TANDEM determined by X-ray analysis at room temperature to a resolution of 0.85 Å. Dotted lines indicate intramolecular hydrogen bonds between the NH groups of L-Val and the O atoms of L-Ala. These bonds hold the molecule in a relatively stable conformation. Asterisks indicate the N–C_α (D-Ser) bonds through which the chromophores are attached to the octapeptide ring.

bonding between the peptide backbone of the drug and the DNA bases, using the model derived from the X-ray structure.

There are two potential hydrogen-bond donors and one potential acceptor in each half of the molecule (Fig. 3) which could be directed towards the interior of the helix on intercalation. The donors are the NH groups of L-Ala and D-Ser and the acceptors are the carbonyl O atoms of L-Ala. The latter are already involved in an intramolecular H bond with the NH group of L-Val (Fig. 2) and in an unfavourable position to accept a second hydrogen bond from the available donors of the DNA base pairs. Of the potential donor groups of TANDEM the NH groups of L-Ala seem to be well placed, as planes parallel to the chromophores oriented as in Fig. 3 and passing through these N atoms are separated by a perpendicular distance of 3.5–4 Å. In the intercalated complex, therefore, the NH groups of L-Ala lie co-planar with the sandwiched base pairs. Experiments with CPK models indicate that a reasonable fit can be obtained with hydrogen bonds of ~3 Å between the NH groups of L-Ala and the O-2 atoms of thymine in the narrow groove (Fig. 4). In this intercalated model an ApT sequence is sandwiched between the chromophores. The unwinding angle, spread over four base pairs, is 40°–50°, consistent with the experimentally determined value for DNA[2,7]. Attempts to build alternative intercalated models involving the N-3 atoms of adenine or the NH groups of D-Ser in hydrogen bonding were unsuccessful, nor could we achieve satisfactory intercalation via the major groove. It is known that binding of the related antibiotic, echinomycin, to phage T2DNA is not prevented by the presence of bulky sugar substituents which occlude the wide groove of the helix[4].

The X-ray structure of TANDEM prompts some suggestions about the character of the intercalation process. Interestingly, the isolated chromophore, quinoxaline-2-carboxamide, unlike simple monofunctional intercalators, shows no detectable interaction with DNA and does not affect the supercoiling of circular DNA[4,10]. Thus, the driving force for intercalation cannot be ascribed simply to a tendency to form stacking interactions between the chromophores and base pairs. In TANDEM, as shown by the crystal structure, the chromophores are held at the approximate position required for bifunctional intercalation by the relatively rigid depsipeptide backbone, and thus a very important geometrical constraint is already 'locked into' the structure of the ligand molecule. Moreover, all but two of the carbonyl O atoms are directed away from the chromophore side of the drug, where they are best placed to retain their hydrogen-bonding interactions with the surrounding solvent after binding to DNA. Such interactions are apparent in the present crystal structure where all but two of the carbonyl oxygens make at least one hydrogen bond with the surrounding water molecules and there is no direct hydrogen bonding between neighbouring TANDEM molecules. The hydration spheres are even more perfect in a structure of TANDEM determined at −135 °C (D. van der Helm, personal communication).

The naturally occurring antibiotics echinomycin and triostin A (Fig. 1*a*, *b*) display patterns of selectivity in binding to DNA and synthetic polydeoxynucleotides which, while characteristic for each antibiotic, are quite different from the marked preference of TANDEM for A + T-rich sequences[2,6,7]. For instance, echinomycin binds well to poly(dG-dC), approximately twice as strongly as it binds to poly(dA-dT), whereas the converse is true of triostin A. These differences from TANDEM are probably

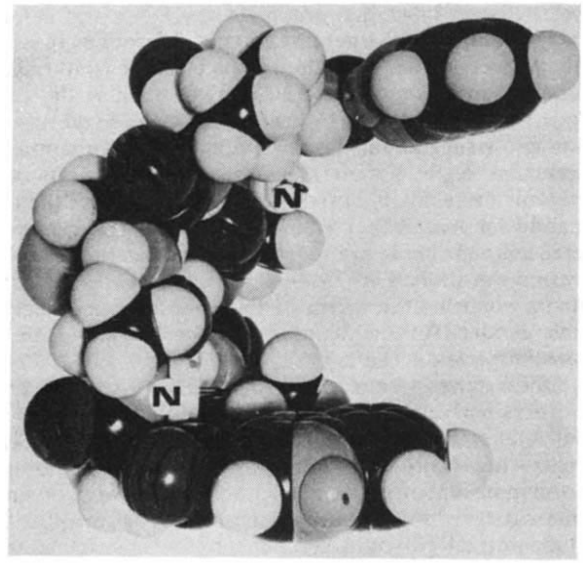

Fig. 3 CPK model of the TANDEM molecule with the quinoxaline chromophores rotated about the N–C_α (D-Ser) bonds from the crystal position to allow for bifunctional intercalation. The rotation is about 20° for each chromophore. The nitrogen atoms of L-Ala donating hydrogen bonds to the O-2 atoms of the thymine bases are indicated.

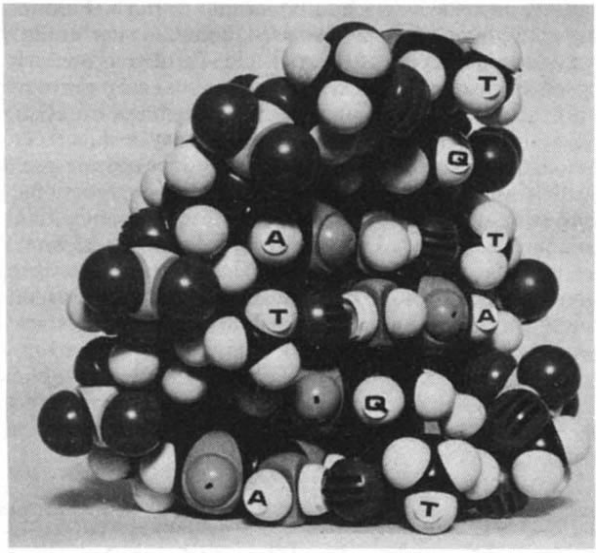

Fig. 4 CPK model of the intercalated complex between TANDEM and a partially unwound B-DNA helix showing the major groove side. The quinoxaline groups (Q) and the bases (adenine, A, and thymine, T) are labelled.

related to the effects of *N*-methylation on the precise conformation of the octapeptide ring and thus the availability of its functional groups for hydrogen bonding[2,4,5,13]. *N*-methylation of the L-Cys and L-Val residues in the natural antibiotics precludes formation of the intramolecular H bonds observed in TANDEM. Moreover, methylation of the L-Val residues necessarily results in conformational differences which will cause the carbonyl oxygens of L-Ala to project in the same direction as the chromophores. From this position they become available as effective hydrogen-bond acceptors for interaction with donors in the minor groove, that is, the 2-amino groups of guanine nucleotides, which may explain the ability of the antibiotics to bind to sequences containing G.C as well as A.T base pairs.

Extrapolation from the crystal structure of TANDEM to detailed molecular models for quinoxaline antibiotic–DNA complexes must be approached very cautiously. Although various lines of evidence (NMR[5,13], conformational calculations[5,11]) suggest that the peptide backbone in all these compounds adopts a relatively rigid and well defined conformation, there is no experimental evidence that this conformation does not alter on intercalation. Indeed, the significant differences found between the present room-temperature X-ray study and the similar one at −135 °C indicate that the orientation of some bonds, particularly those involving carbonyl groups, can be significantly affected by hydrogen bonding to solvent molecules[14]. There is also a degree of uncertainty about local DNA conformation[15,16] and about the orientation of the base pairs with respect to the helix axis in solution[17–19]. The recent X-ray study of the daunomycin–hexadeoxynucleotide complex[20] has shown that widespread changes can occur in the conformation of the backbone and the positioning of the bases on intercalation. Thus, although the present study gives well defined information about TANDEM itself, only a generalized model can be put forward for the DNA-drug complex. We are continuing our attempts to co-crystallize TANDEM with synthetic A-T deoxyoligonucleotides in the hope of establishing the structure of an intercalated complex directly by X-ray analysis.

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1. Katagiri, K., Yoshida, T. & Sato, K. in *Antibiotics* Vol. 3 (eds Corcoran, J. S. & Hahn, F. E.) 234–251 (Springer, Heidelberg, 1975).
2. Waring, M. J. in *Antibiotics* Vol. 5, Pt 2 (ed. Hahn, F. E.) 173–194 (Springer, Heidelberg, 1979).
3. Waring, M. J. & Wakelin, L. P. G. *Nature* **252,** 653–657 (1974).
4. Wakelin, L. P. G. & Waring, M. J. *Biochem. J.* **157,** 721–740 (1976).
5. Cheung, H. T. *et al. J. Am. chem. Soc.* **100,** 46–54 (1978).
6. Lee, J. S. & Waring, M. J. *Biochem. J.* **173,** 115–128 (1978).
7. Lee, J. S. & Waring, M. J. *Biochem. J.* **173,** 129–144 (1978).
8. Sheldrick, G. M. (in preparation).
9. Fuller, W. & Waring, M. J. *Ber. Bunsenges. phys. Chem.* **68,** 805–808 (1964).
10. Waring, M. J. in *Drug Action at the Molecular Level* (ed. Roberts, G. C. K.) 167–189 (Macmillan, London, 1977).
11. Ughetto, G. & Waring, M. J. *Molec. Pharmac.* **13,** 579–584 (1977).
12. Wang, J. C. *J. molec. Biol.* **89,** 783–801 (1974).
13. Kalman, J. R., Blake, T. J., Williams, D. H., Feeney, J. & Roberts, G. C. K. *JCS Perkin* **I,** 1313–1321 (1979).
14. van der Helm, D. *et al.* (in preparation).
15. Wang, A. H. J. *et al. Nature* **282,** 680–686 (1979).
16. Drew, H., Takano, T., Tanaka, S., Itakura, K. & Dickerson, R. E. *Nature* **286,** 567–573 (1980).
17. Levitt, M. *Proc. natn. Acad. Sci. U.S.A.* **75,** 640–644 (1978).
18. Hogan, M., Dattagupta, N. & Crothers, D. M. *Proc. natn. Acad. Sci. U.S.A.* **75,** 195–199 (1978).
19. Hogan, M., Dattagupta, N. & Crothers, D. M. *Biochemistry* **18,** 280–288 (1979).
20. Quigley, G. J. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).

Transglutaminase modifies the carboxy-terminal intracellular region of HLA-A and -B antigens

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The HLA-A and -B antigens, encoded in the major histocompatibility complex, are transmembrane proteins whose carboxy termini project inwards from the cell membrane. The intracellular region of the HLA-B7 antigen is known to include two glutamine residues[1]. We have used guinea pig transglutaminase to show that *in vitro* the enzyme couples amines specifically to glutamine residues in the carboxy-terminal region of the HLA-B7 and -A2 antigens. The specificity of this reaction has an immediate practical significance, but may also have a wider biological interest.

HLA-A and -B antigens contain an invariant extracellular polypeptide subunit (β_2-microglobulin or p12) of molecular weight (MW) 12,000 noncovalently attached to a polymorphic 44,000-MW transmembrane, glycosylated, heavy chain (p44)[2]. The heavy chain has three major regions: a 34,000-MW glycosylated, extracellular amino-terminal segment (p34) that is released from the membrane by papain in a complex with the light chain (p34, 12), a transmembrane hydrophobic segment of about 25 amino acid residues, and an intracellular hydrophilic carboxy-terminal region of about 30 amino acid residues[3]. Interactions of the HLA-A and -B antigens with intracellular

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