Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 41 (2006) 27–39
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Original article

Structure of daunomycin complexed to d-TGATCA by two-dimensional nuclear magnetic resonance spectroscopy

Ritu Barthwal [a,*], Uma Sharma [a], Nandana Srivastava [a], Monica Jain [a], Pamita Awasthi [a], Manpreet Kaur [a], Sudhir Kumar Barthwal [b], Girjesh Govil [c]

[a] *Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee 247 667, India*
[b] *Department of Physics, Indian Institute of Technology Roorkee, Roorkee 247 667, India*
[c] *Department of Chemical Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Navy Nagar, Colaba, Mumbai 400 005, India*

Received 15 December 2004; received in revised form 22 August 2005; accepted 8 September 2005
Available online 15 November 2005

Abstract

The anthracycline antibiotic daunomycin, having four fused rings and an amino sugar, is being used in the treatment of acute leukemia. Binding to DNA is generally believed to be essential for its activity. We have studied the interaction of daunomycin with DNA hexamer sequence d-$(TGATCA)_2$ by titrating up to two drug molecules per duplex using nuclear magnetic resonance spectroscopy. The solution structure of 2:1 drug to DNA complex based on two dimensional nuclear Overhauser enhancement (NOE) spectroscopy and molecular dynamics calculations has been studied. The change in conformation of drug molecule on binding to DNA, deoxyribose conformation and glycosidic bond rotation has been obtained. The absence of sequential NOE connectivities at d-T1pG2 and d-C5pA6 sites shows that the drug chromophore intercalates between these two base pairs. This is substantiated by intermolecular NOEs observed between nucleotide base protons and aromatic ring protons of drug molecule. A set of 17 intermolecular NOE interactions allowed the structure to be derived by restrained molecular dynamics simulations, which have been compared with that obtained by X-ray analysis. Several specific interactions between the drug and DNA protons are found to stabilize the formation of drug–DNA complex.

Keywords: Daunomycin-d$(TGATCA)_2$ complex; Solution structure; Nuclear magnetic resonance spectroscopy; Interproton distance constraints; Restrained molecular dynamics; Drug–DNA interactions; Intercalative binding

1. Introduction

The anthracycline antibiotic daunomycin (Fig. 1a) and closely related adriamycin, are used in the treatment of acute leukemia and solid tumors, respectively [1,2]. Both the compounds have an aglycon chromophore containing four fused rings and amino sugar. They have been the subjects of intense chemical and biological research. A variety of biochemical evidence suggests that the anthracyclines function primarily at the DNA level by blocking the process of replication and transcription [3]. Although their interaction with other cellular targets may play a role in the selective cytotoxicity of these drugs, it is the binding to DNA that is generally believed to be essential for the activity. Due to their importance in cancer chemotherapy, many attempts have been made to understand the key features responsible for the biological activity of this family of antibiotics [4], particularly their interaction with chromosomal DNA.

Several physicochemical investigations [5–10] like absorbance, fluorescence, circular dichroism and foot printing titrations have been used to analyze the structure–activity relationship quantitatively. The thermal stabilization and fluorescence quenching techniques [11,12] have shown that daunomycin binds to DNA with some preference for alternating pyrimidine–purine tracts. Binding properties of daunomycin and its analogue were examined by using 16 bp long oligonucleotides [13]. The site and sequence specificity of the daunomycin-DNA interactions were examined by equilibrium binding methods; foot printing and restriction endonuclease cleavage studies

* Corresponding author. Tel.: +91 1332 28 5807; fax: +91 1332 27 3560.
E-mail address: ritubarthwal@yahoo.co.in (R. Barthwal).

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2005.09.005

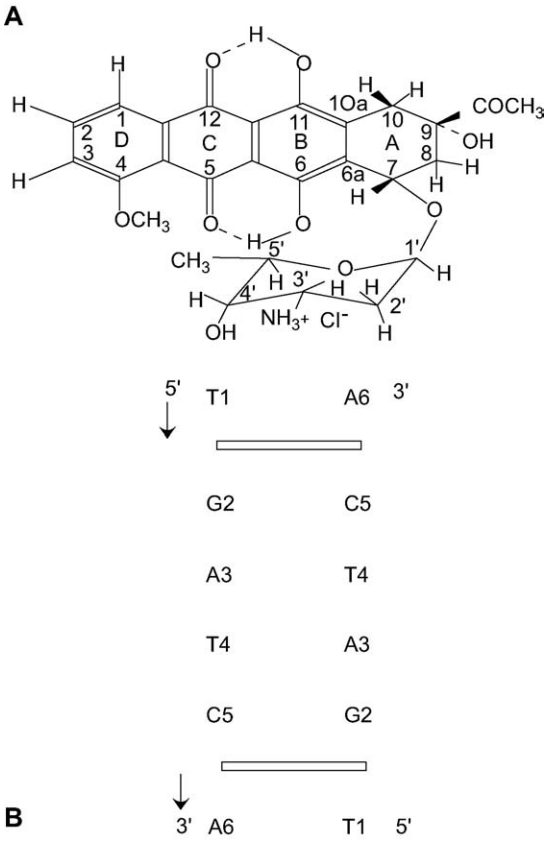

Fig. 1. (a) Molecular structure of daunomycin and (b) schematic representation of the 2:1 daunomycin–d-$(TGATCA)_2$ complex showing two drug molecules D1 and D2 intercalated in one hexanucleotide duplex molecule.

[14,15]. The changes in physical properties of both DNA and drug upon binding were consistent with the intercalation of the chromophore, preferentially at CpG steps [16,17]. The results of equilibrium binding and kinetic studies have shown that the binding of daunomycin to DNA involves three distinct steps [18]. First step is a rapid outside binding, followed by intercalation and then conformational changes of drug, DNA or both. The conformation and self association of daunomycin, its derivative adriamycin and N-acetyl daunomycin has been studied by NMR [19–21]. The complete solution structure of daunomycin and adriamycin was first reported by Barthwal et al. [22,23]. Preliminary nuclear magnetic resonance (NMR) studies have been carried out on the binding of daunomycin to poly (dA-dT), $d(pTpA)_3$ and d-GCGC and d-CGCG [24–27]. Subsequently interaction of daunomycin/analogues with di- [28], tetra- [29] and hexamer DNA sequences [30–34], containing d-CpG binding sites has been studied by two-dimensional proton NMR techniques.

Several anthracycline antibiotics have been crystallized and their structures are determined by X-ray diffraction analysis [35–38]. In these structures, the semi saturated ring A is in a half chair conformation with C-8 farthest out of plane of the ring. The first X-ray diffraction studies of oriented fibers of a daunomycin-DNA complex showed that the drug chromophore is inserted between base pairs [39,40]. The same was also suggested by theoretical potential energy calculations [41,42] (Fig. 1b). The detailed interaction between daunomycin and hexamer sequences, d-CGATCG and d-CGTACG, were later revealed by a single crystal X-ray structure of daunomycin-DNA complex [43–45]. The local mobility of daunomycin and d-CGTACG, formaldehyde cross-links of daunomycin to DNA and studies of X-ray crystallographic structures of daunomycin/other analogues have also been attempted [46–50]. Subsequently daunomycin and 4′-epiadriamycin have been co-crystallized with sequences d-TGATCA and d-TGTACA [51–53]. The X-ray structure analysis shows significant differences in interaction of amino sugar, O5 and $4OCH_3$ moieties in the minor groove of DNA containing d-TpG binding sites. There is no study of solution structure of these drugs with d-TpG containing DNA sequences. In this paper, we present the solution structure of d-$(TGATCA)_2$ complexed with daunomycin by NMR spectroscopy and analyze the intermolecular interactions.

2. Materials and methods

The deoxyribonucleotide sequence d-$(TGATCA)_2$ was purchased from DNA Chemical International, USA. Deuterium oxide (D_2O) with isotopic purity 99.96% and daunomycin were purchased from Sigma Chemical Co., USA. The nucleotide and drug samples were used without further purification. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was purchased from Merck Sharp and Dohme Canada Ltd., Canada and used as an internal NMR reference. All other chemicals like Na_2HPO_4 and NaH_2PO_4 and ethylene diamine tetra acetic acid (EDTA) are of analytical grade and purchased from E. Merck, India Ltd. A solution of 3.68 mM d-$(TGATCA)_2$ (duplex concentration) was prepared by dissolving a known quantity of sample in deuterated phosphate buffer (16.25 mM) of pH 7.0 containing 15 mM Na salt. The sample was lyophilized and redissolved in D_2O and the process was repeated twice. Finally d-$(TGATCA)_2$ was dissolved in 0.4 ml of D_2O and its concentration was determined by absorbance measurements at 260 nm. EDTA, 0.1 mM, was added to suppress any paramagnetic impurity, which may cause line broadening during NMR measurements. 1 μl of 0.1 M solution of DSS was added as internal reference. A complex of d-$(TGATCA)_2$ and daunomycin was prepared by titrating it with daunomycin. A stock solution of daunomycin having concentration of 32.66 mM was prepared by checking absorbance at 480 nm using extinction coefficient (ε) of 11500 M^{-1} cm^{-1}. Ninety microliters of 32.66 mM daunomycin was added in steps of 10 μl to 0.4 ml of 3.68 mM d-$(TGATCA)_2$ in order to make a final 2:1 complex of daunomycin: d-$(TGATCA)_2$. All proton NMR experiments were carried out at National High Field NMR Facility located at Tata Institute of Fundamental Research (TIFR), Mumbai and were recorded on 500 MHz high resolution Bruker AM 500 FT-NMR spectrometer equipped with aspect computer. Typical parameters for one-dimensional NMR experiments are: pulse width = 10–12.5 μs (60° pulse); number of data points = 8–16 K; spectral width = 4000 Hz; number of scans = 64–128 and digital resolution = 0.25–0.5 Hz per point.