Journal of Molecular Graphics and Modelling 74 (2017) 251–264

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Internal abstraction of dynemicin A: An MD approach

Angus Beane [a], Bill R. Miller III [b], Carol A. Parish [a,*]

[a] Department of Chemistry, Gottwald Center for the Sciences, University of Richmond, 28 Westhampton Way, Richmond, VA 23173, United States
[b] Department of Chemistry, Truman State University, 100 E. Normal Ave, Kirksville, MO 63501, United States

ARTICLE INFO

Article history:
Received 8 October 2016
Received in revised form 15 March 2017
Accepted 16 March 2017
Available online 11 April 2017

Keywords:
Dynemicin A
Molecular dynamics
Intercalation
DNA
Binding
Mechanism

ABSTRACT

Dynemicin A has the ability to undergo the Bergman cyclization, forming a *para*-benzyne moiety with the ability to induce DNA strand scission. This property of dynemicin A makes it a promising anti-tumor agent. Past research has shown conclusively that dynemicin A binds to and abstracts a hydrogen atom (H5′) from the DNA backbone, but the molecular mechanism of the binding event is not fully understood. We have used AMBER Molecular Dynamics simulations to investigate the dynamics associated with the reaction mechanisms. Previously, two binding mechanisms have been proposed, of which the second is more supported: (1) dynemicin A intercalates between two base pairs and directly abstracts a hydrogen atom from DNA and (2) dynemicin A inserts into the minor groove and directly abstracts a hydrogen atom from DNA. We propose a third mechanism, where dynemicin A intercalates, then undergoes a proximate, intramolecular hydrogen atom abstraction (internal abstraction). While not studied here, the resulting radical would then subsequently abstract a hydrogen atom from DNA.

1. Introduction

Dynemicin A is a member of the large family of enediynes that includes esperamicins, neocarzinostatin, and calicheamicins, shown in Fig. 1. These enediynes are of great interest because of their ability to induce cell apoptosis. After undergoing Bergman cyclization (Scheme 1, **B** → **C** or **E** → **F** reactions), the enediyne moiety is converted to a diradical *para*-benzyne. This diradical is responsible for abstracting a hydrogen atom from the DNA backbone, resulting in single-strand scission. Strand scission leads to the unwinding of DNA, which induces cell apoptosis. This characteristic of dynemicin A makes it particularly useful as an anti-tumor drug, and dynemicin A has been shown to have high potency against P388 leukemia and B16 melanoma cells [1,2].

Dynemicin A is isolated from a fermentation broth of *Micromonospora chersina* [3]. The structure of dynemicin A (Fig. 1), first reported in 1990 by Konishi et al. [2], provides advantages over other enediynes. Because dynemicin A is a 10-membered cyclic enediyne (as opposed to a 9-membered cyclic enediyne), it does not contain an apoprotein. The absence of an apoprotein makes its structure more stable [4]. Dynemicin A targets the DNA sequences 5′-CTACTACTGG-3′, 5′-AG-3′, 5′-AT-3′, and 5′-GC-3′, presumably because of binding geometries at these sequences [5]. While most enediynes contain an oligosaccharide tail, dynemicin A has an anthraquinone moiety, making computational studies of the drug more accessible [6].

One major problem with the use of enediynes as drug candidates is that they are indiscriminant in their interaction with cancerous and non-cancerous cells, and therefore induce cell apoptosis in both healthy and afflicted cells. One of the goals of current research is to develop a method for targeting enediyne drugs specifically for malignant cells. It is known that dynemicin A can easily bind to and abstract a hydrogen atom from the DNA backbone. Targeting cancerous cells is still an open problem. However, recent research indicates that certain enediynes may use the pH difference between cancerous and non-cancerous cells such that they only cyclize in cancerous cells, thus only inducing cell apoptosis in cancerous cells [7]. Understanding the dynamics associated with the radical abstraction process may aid in these studies.

Once dynemicin A binds to DNA, it must be triggered to induce the Bergman cyclization. The epoxide ring stores a large amount of strain energy in the molecule (see Fig. S1). When this epoxide ring is opened, the strain is released, providing sufficient energy for the

Abbreviations: AMBER, Assisted Model Building with Energy Refinement; CHELPG, charges from electrostatic potentials using a grid-based method; COM, center of mass; MD, molecular dynamics; ONIOM, our own n-layered integrated molecular orbital and molecular mechanics; MMPBSA/MMGBSA, molecular mechanics energies combined with the Poisson-Boltzmann or generalized Born and surface area continuum solvation; TIP3P, three-site transferrable intermolecular potential.

* Corresponding author.
E-mail address: cparish@richmond.edu (C.A. Parish).

http://dx.doi.org/10.1016/j.jmgm.2017.03.012
1093-3263/

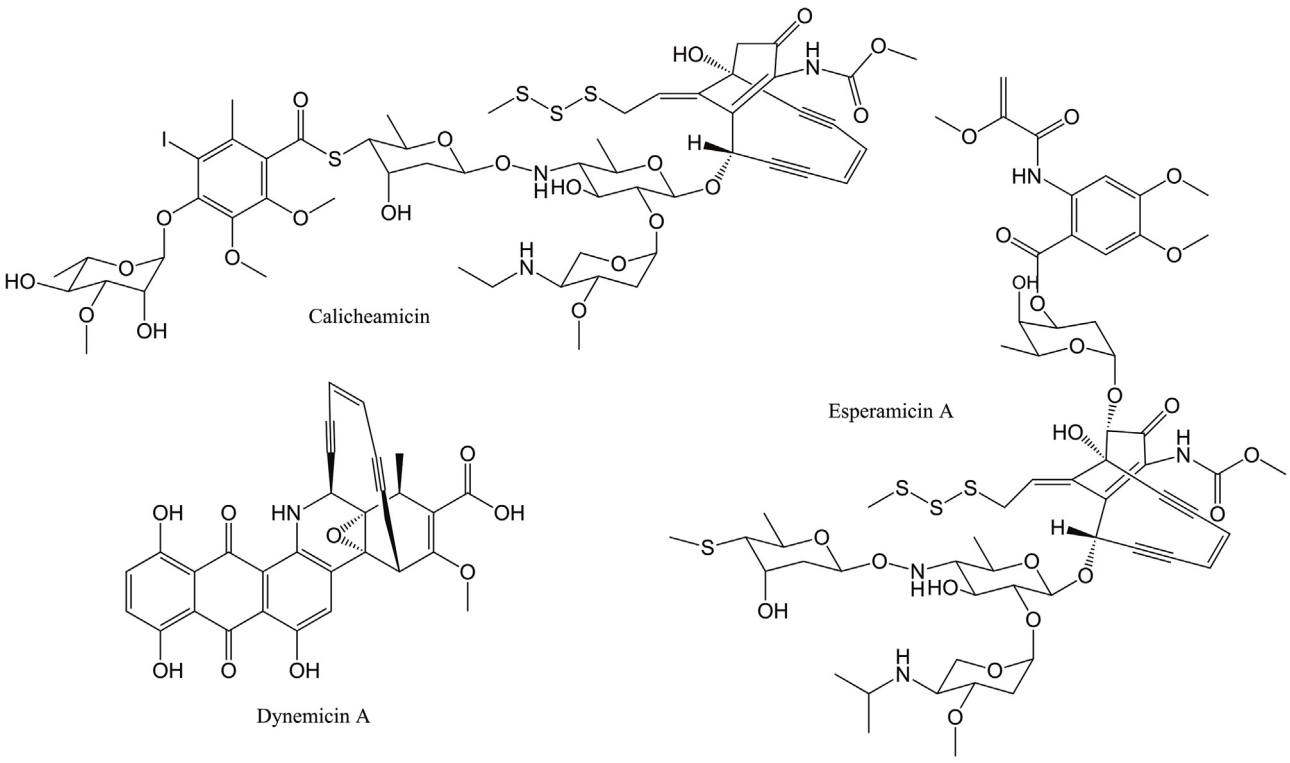

Fig. 1. Structures of well-known micins, including calicheamicin, esperamicin A_1, and dynemicin A.

molecule to cyclize. This ring opening is well-studied [6,8–10] and releases approximately 88 kcal/mol of energy [6].

Many computational studies, including Quantum Mechanics/Molecular Mechanics approaches, have been performed on dynemicin A. In 2001, Feldgus and Shields performed an ONIOM investigation on small enediyne systems [9]. They found that ONIOM accurately matches experimental data for the Bergman cyclization of model enediynes and provided evidence that the release of epoxide ring strain triggers the cyclization. In 2005, Tuttle et al. investigated the docking mechanism of dynemicin A, and suggested that the binding mode that leads to strand scission is an insertion and not an intercalation (see Fig. 2) and that dynemicin A is kinetically stable once cyclized [6]. Tuttle et al. suggested that once intercalated, dynemicin A is unable to abstract hydrogen from DNA. In a subsequent QM/MM study, the same authors showed that inserted dynemicin A undergoes the Bergman cyclization under normal physiological conditions with $\Delta E_a = 19.4$ kcal/mol and $\Delta E_{rxn} = -2.1$ kcal/mol [10]. That study also showed that the protonation of the dynemicin A O8 oxygen atom has little effect on the Bergman cyclization. This is noteworthy as the dynemicin carboxyl group is expected to be deprotonated (anionic) at physiological pH (Scheme 1).

There have been previous Molecular Dynamics (MD) studies on the binding position of dynemicin A. In 1991, Langley et al. performed 115-ps simulations starting from dynemicin A intercalated to examine the stereochemistry of dynemicin A bound to DNA [11]. Their findings supported the intercalation model, suggesting that dynemicin A intercalates after it is triggered. Also in 1991, Wender et al. performed 30-ps simulations starting from dynemicin A intercalated to examine the energetics of dynemicin A [12]. They found that triggered dynemicin A had a much lower energy than the untriggered state. In 1993, Cardozo and Hopfinger performed 10-ps MD simulations on different intercalated structures to determine favorable docking geometries [13]. They were unable to investigate the binding transition discussed in the present work due to the short simulation times possible then with available hardware and software.

In 1995 Myers et al. experimentally showed that when the C30 carboxyl group is modified by the addition of a methyl group, rendering dynemicin A neutral rather than anionic at physiological pH, the binding affinity is increased while the biological activity is reduced [14]. The charge-neutral methyl analog cyclized rapidly in the presence of reducing agent and in the absence of DNA. In 1995 the working assumption was that intercalation was the only plausible binding mode for dynemicin A and these results suggested that DNA binding prevented efficient reductive activation via epoxide ring opening. This led to a mechanistic picture of dynemicin A first binding to DNA, dissociating prior to reductive activation and subsequent cyclization, followed by a second binding event to induce DNA cleavage. In 2005, Tuttle et al. suggested that this mechanism seemed chemically unlikely [6]. The high reactivity of the *p*-benzyne diradical would cause dynemicin A to readily abstract protons from another source before binding a second time to DNA. The literature is replete with evidence that the *p*-benzyne moiety in triggered and cyclized dynemicin A could be quenched by abstraction of a proton from water or reaction with ubiquitous halide anions [6,14–16]. Based on their modeling work, Tuttle et al. proposed that dynemicin A is unable to abstract hydrogen from DNA while in the intercalated state, and instead must be in the inserted state. They argued that in the experimental work of Myers et al., the methyl addition allowed dynemicin A to more easily intercalate, quenching its biological activity. They reasoned that dynemicin A cannot be triggered in the intercalated state, since NADPH (or another similar reductant) cannot access the carbonyl of the anthraquinone tail and therefore cannot begin the cascade to open the epoxide ring [6]. Based on our work described below, we propose a third explanation of Myers et al.'s results based on the premise of internal abstraction.

Internal abstraction is a relatively recently considered process for enediynes whereby the *p*-benzyne radical abstracts a