Designed metalloenediyne warheads damage DNA and outpace DNA polymerase

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Normal cells eventually undergo a highly regulated process of programmed cell death, or apoptosis, while abnormalities in this process may lead to uncontrolled cell proliferation and cancer. Cancer cells differ from normal cells with respect to their resistance to signals that control both cell growth and apoptosis. Furthermore, they divide more rapidly than normal cells, resulting in the development of cancerous tumors and metastasis. This has led to tremendous research efforts that aim to identify the various causes of cancer and aggressively treat this deadly disease. One strategy for combating cancer focuses on the inhibition of DNA polymerases, which function to synthesize DNA and are essential for DNA replication and damage repair. An orthogonal approach centers on the development of chemotherapeutic agents that can damage DNA in a manner that does not allow repair or replication by DNA polymerases. In PNAS, Zaleski and coworkers (1) use this latter approach and describe studies of new enediyne transition metal complexes (metalloenediynes) that function as cytotoxic metal-mediated diradical generators.

Enediynes (Fig. 1) are highly toxic bacterial natural product compounds that contain the 3-ene-1,5-diyne conjugated unit as part of a 9- to 10-member ring system (2). These compounds are susceptible to thermal-activated and photoactivated Bergman cycloaromatization reactions that result in the formation of 1,4-didehydrobenzene diradical derivatives. The diradicals generated from enediynes are highly reactive chemical "warheads" that can abstract hydrogen atoms from the DNA sugar backbone as a mechanistic component of their DNA cleaving reactivity. As such, they are among the most potent naturally occurring cytotoxic compounds that function as antitumor agents (2). Thus, many natural enediynes possess limited therapeutic utility due to their high toxicity. This has led to extensive research efforts focused on the design of new enediyne agents (3), the attachment of the enediyne warhead to drug delivery systems that specifically target DNA (2), and how to trigger a specific event or cascade of events that result in site-selective diradical formation (4). DNA double-strand breaks that are induced by enediyne activity can trigger a DNA damage protein response leading to cell cycle arrest. The activation of checkpoint pathways that regulate the mechanism of DNA repair can then lead to apoptotic cell death if the DNA lesion is not repairable (5).

The cycloaromatization reaction that leads to diradical formation is similar to Woodward–Hoffmann [2+2] cycloadditions (6). Key to the reactivity of enediynes is the proximity (7, 8) of the terminal -yne C_1–C_6 carbon atoms, which interact along the reaction coordinate to form the cyclized diradical intermediate (Fig. 1 *B* and *C*). For example, ab initio computations indicate that the parent (*Z*)-hex-3-ene-1,5-diyne possesses a C_1–C_6 distance of 4.32 Å (9) and is quite stable at physiological temperature with an activation enthalpy ($\Delta H^{\ddagger}$) of 28.2 kcal/mol. Nicolaou et al. (7, 10) have provided an empirical rule indicating that a critical C_1–C_6 distance of ~3.20–3.31 Å is required for spontaneous Bergman cyclization reactivity at 36.5–37.5 °C (i.e., physiological temperature). In addition to the C_1–C_6 distance, other contributions to enediyne reactivity include ring strain effects (11, 12) and the underpinning enediyne electronic structure (e.g., configuration interaction, pseudo-Jahn–Teller effects, etc.) (6, 9). Taken together, these effects contribute to both transition state and diradical stabilization. A novel way to affect the reactivity of enediynes is to use them as components of metal binding ligands (Fig. 1*D*). Since the geometry of the enediyne-containing ligand will change upon binding to a metal ion, chelation may be able to affect the enediyne electronic structure and trigger the activation of Bergman cyclizations. This approach would provide a powerful way to modulate proximity, strain, and electronic structure effects on reactivity.

Zaleski and his team are pioneers in the synthesis, spectroscopy, and electronic structure characterization of metalloenediynes. They have designed redox-mediated Bergman cyclization reactions for the development of new metalloenediyne prodrugs (13) and have photothermally activated metalloenediyne cyclizations using near-infrared excitation into low-energy ligand-to-metal charge transfer bands (14). Additionally, they have shown that both the thermal (15) and

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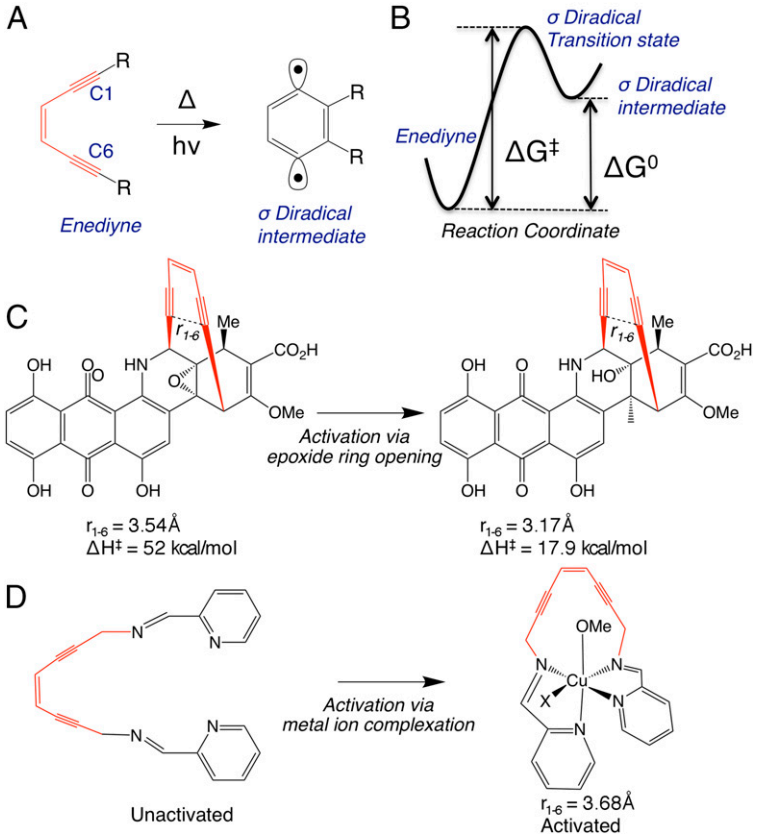

Fig. 1. (*A*) Thermal and photochemical activation of enediynes to the diradical intermediate. (*B*) Simple potential energy diagram indicating the intermediate nature of the diradical. (*C*) Activation of dynemicin A via epoxide ring opening. (*D*) Activation of the PyED ligand by metal ion complexation. In *C* and *D*, the enediyne warhead is depicted in red.

photochemical (16, 17) reactivity of enediynes are affected when the enediyne is a component of a ligand that is bound to a transition metal ion. Their MLX_2 metalloenediyne complexes (X = halogen; L = enediyne-based ligand) display Bergman cyclization temperatures that range from 136° to 225 °C in the solid state, with the reactivity differences deriving from both electronic and steric interactions between the halogen donor and the enediyne π system. The study showed both ancillary ligand and intraligand effects on metalloenediyne thermal reactivity at approximate parity of the terminal alkyne C_1–C_6 separation distance, highlighting electronic influences on reactivity (18, 19).

In PNAS, Zaleski and coworkers (1) synthesize and spectroscopically characterize new Cu(II), Zn(II), and Fe(II) complexes coordinated by an enediyne-containing chelating ligand. Bergman cyclization rates for these M(**PyED**)•2Cl complexes are expected to increase in the order Zn(II) < Fe(II) < Cu(II), which parallels the reduction in enediyne C_1–C_6 distances determined from density functional theory computations (1). Their spectroscopic analysis of the Cu(**PyED**)•Cl_2 complex shows that the Jahn–Teller distortion present in this d^9 system is responsible for the shorter interalkynyl distance and increased reactivity. This underscores how coordination number, geometry, and the d-electron configuration of transition metal ions may conspire to provide a remarkable electronic structure control of metalloenediyne reactivity. Importantly, they have shown that transition metals can effectively tune the diradical activity of Bergman cyclized enediynes to initiate cell cycle arrest at the G_2/M checkpoint. HeLa cells that were treated with Cu(**PyED**)•SO_4 provide evidence that effective DNA damage occurs in vivo, with an IC_{50} cellular toxicity of 10.5 μM. Remarkably, Cu(**PyED**)•SO_4 cleaves the DNA template strand before the polymerase can extend it. The ability of Cu(**PyED**)•SO_4 and related Cu(II) enediyne complexes to damage DNA is important since Cu(II) is required for tumorigenesis and is observed in malignancies at increased levels (20, 21). The implication is that, once the enediyne ligand has entered the cell, it can bind to, and be activated by, Cu(II) for diradical formation (1). Since the **PyED** ligand itself is stable, as evidenced by minimal DNA degradation at physiological temperature (1), the metal ion effectively acts as a trigger to activate the enediyne for diradical formation. Great potential exists to further exploit this strategy in the design of relatively unreactive prodrug molecules that can be activated site specifically in cancer cells with high cytotoxicity.

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