Conversion of Enediynes into Quinones by Antibody Catalysis and in Aqueous Buffers: Implications for an Alternative Enediyne Therapeutic Mechanism

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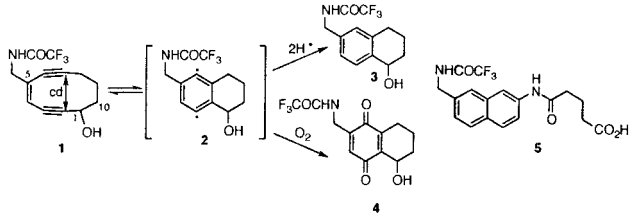
The Bergman cycloaromatization reaction[1] is of tremendous interest to organic chemists and chemical biologists alike because of its key role in the mechanism of action of the naturally occurring enediyne antitumor antibiotics such as calicheamicin γ_1, dynemycin A, and esperamicin A_1.[2] At present there is no known synthetic or naturally occurring catalyst for this reaction. However, selective activation of therapeutically relevant enediynes at their site of action *in vivo* is imperative due to their potent and nonselective cytotoxicity and thus remains an intensive area for research.[3]

Given that antibodies can be programmed to catalyze reactions thought beyond the scope of both biological and chemical catalysis,[4] we were interested in eliciting antibody catalysts for the Bergman cyclization of stable enediyne substrates, thus offering a new approach for the "triggering" of enediynes. Herein we report the generation of an antibody, 5H7, that catalyzes the Bergman cycloaromatization reaction of the 10-membered ring monocyclic enediynol **1** (Figure 1). Remarkably, the diyl intermediate **2** of the antibody-catalyzed reaction is not trapped by 2[H•] to give tetralin **3**, but in an unprecedented manner by molecular oxygen to give quinone **4** as the exclusive product. This finding is even more significant because we have discovered that this formal oxidation of enediynol **1** to quinone **4** occurs spontaneously under biologically relevant conditions and that quinone **4** is more cytotoxic than its enediyne parent **1**.

The starting point for our investigation began with the choice of a suitably stable enediyne substrate. Monocyclic enediynol **1**—the core enediyne structure from calicheamicin γ_1 was chosen because, extrapolating from available data in organic solvents,[5] it was hypothesized that there should be a low but detectable rate of cycloaromatization in aqueous buffer at 37 °C. The propargylic hydroxyl group was included to enhance cycloaromatization[2a] and improve aqueous solubility. The trifluoroacetamide group was included to elicit a key locus for substrate **1** recognition within the antibody combining site.[6]

Scheme 1. Bergman Reaction of Enediynol **1** in the Presence of a Suitable H• Donor (in Toluene) or Molecular Oxygen (in Aqueous Buffer);[7] Antibody 5H7, Elicited to the Transition-State Analogue Hapten **5**, Catalyzes the Formation of Quinone **4** rather than Tetralin **3**



Several reports have delineated the importance of strain, strain release, and distance control in determination of activation barriers for the cycloaromatization reaction.[8] It was anticipated, therefore, that antibodies elicited to naphthalene hapten **5** would possess binding sites capable of compressing the substrate enediynol **1** into a more active ground-state conformation by enforcing a reduced *cd* distance. Concurrently, these antibodies would also stabilize the highly aromatic nature of the Bergman transition state.[8a,9] The structural differences between **5** and the diyl intermediate **2** were hoped to be sufficient that **2** would bind only weakly to antibodies elicited to **5** and would enable **2** to dissociate into solution to abstract 2[H•] from either bulk solvent or a suitable donor to give the stable tetralin **3**. Similarly the low structural homology between **5** and substrate **1** would mean that the antibodies elicited would selectively stabilize the transition state for the Bergman reaction relative to the ground state.

Hapten **5** was synthesized[7] and coupled to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA), via its sulfo *N*-hydroxysuccinimide ester, and the KLH-**5** conjugate was used to immunize 129Gix mice. Monoclonal antibodies were generated using hybridoma technology[10] to give 35 clones with BSA-**5** binding specificity.

In toluene and in the presence of 1,4-cyclohexadiene (1,4-CHD) as an H• donor, enediynol **1** smoothly cycloaromatizes to give tetralin **3** ($t_{1/2}$ = 6 h, 37 °C). However, this is not mirrored in aqueous buffer [pH 6.8, 500 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), 5% DMSO, 37 °C].[11] Preliminarily, this was rationalized as being due to water being a poor H• donor. However, with 1,4-CHD present (in excess) and now under biphasic conditions[12] still no tetralin **3** is formed. In fact, only uncharacterized polymerization products are observed.

Instead of the expected tetralin **3**, a product of unknown constitution was consistently generated from enediynol **1** under the aqueous conditions described above (in the absence of 1,4-CHD). This product was also formed in a range of other aqueous buffers: phosphate-buffered saline (PBS, pH 7.4), morpholinoethane sulfonic acid (MES, pH 6.5), and bicine (pH 8.4). Two antibodies from the hapten-specific panel, IgGs 5H7 and 3H3, significantly enhanced the observed rate of formation of this unknown (10^4- and 10^3-fold respectively) and thus warranted its

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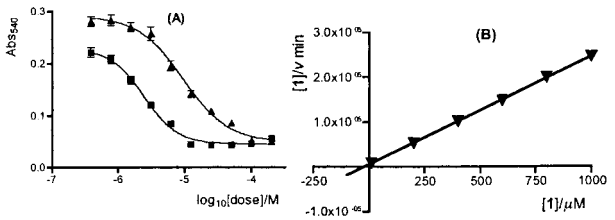

Figure 1.[11] (A) Comparative cytotoxicity of enediynol **1** (▲; $EC_{50} = 9.6 \pm 1.7$ μM) and quinone **4** (■; $EC_{50} = 2.5 \pm 0.3$ μM) against a human pancreatic adenocarcinoma cell line (SW1990). Each point is the mean ± SEM of n=8 determinations. The y-axis is absorbance (measured at 540 nm) of sulforhodamine B (SRB) dye (which is proportional to cell concentration). x-Axis is log[dose] of either **1** or **4**. **(B)** Hanes−Woolf plot of initial rates of formation (v) (▼) of quinone **4** from enediynol **1** (10−1000 μM) in the presence of IgG 5H7 (5 μM): $y = 204.8x + 5631r^2 = 0.998$. V_{max} = 1/gradient ($4.2 \pm 0.1 \times 10^{-3}$ μM/min); $K_m = -(x$-intercept) (23.4 ± 1.2 μM). Each point is the mean ± SEM value of at least duplicate determinations.

structural elucidation.[13] LCMS and ^{1}H NMR characterization both supported the structure as being quinone **4**, and this was further confirmed by independent synthesis.[7]

The rate of formation of **4**, $k_2 = 6.05 \times 10^{-4}$ M^{-1} min^{-1} [pH 6.8, 500 mM MOPS, 5% DMSO, 22 °C],[14] is proportional to dissolved oxygen concentration, with no formation of **4** occurring under degassed conditions. This suggests that molecular oxygen is intercepting the diyl intermediate **2** formed during the Bergman reaction of **1** to give **4**. Previous examples of quinone generation from enediynes involve diyl trapping by the radical spin-probe 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO), followed by heating.[15] To our knowledge, this is the first example of a diyl intermediate produced during the Bergman reaction that is trapped by molecular oxygen, and the mechanism is under further investigation.

Given the known cytotoxicity of both enediynes[3,16] and quinones[17] the relative cytotoxicity of both **1** and **4** were investigated against a human pancreatic adenocarcinoma cell line (Figure 1A). Against this cell line quinone **4** is more toxic ($EC_{50} = 2.5 \pm 0.3$ μM) than its parent enediyne **1** (9.6 ± 1.7 μM). This observation may have ramifications in terms of the overall therapeutic mechanism of the naturally occurring enediyne antibiotics. Perhaps in addition to their accepted therapeutic mechanism, which is known to proceed via H$^\bullet$ abstraction from DNA[18] and proteins,[19] their quinone derivatives may be playing a significant role.

The most efficient antibody at catalyzing the formation of quinone **4**, IgG-5H7, was studied in some detail. The antibody displays saturation kinetics with enediynol **1** [k_{cat}app = $1.0 \pm 0.1 \times 10^{-3}$ min^{-1}; $K_m$app(**1**) = 23.4 ± 1.2 μM] and a rate enhancement under ambient aerobic conditions (k_{cat}app/k_{uncat})[20] of 6.2×10^5 (Figure 1B).[21] IgG 5H7 is stoichiometrically inhibited by hapten **4**, supporting the notion that this catalytic process is occurring within the antibody combining site.

Catalytic antibodies are known to accept reactive intermediates, such as 1,3-dipoles[4f] and carbocations.[22] However, generation of a biradical intermediate, such as **2**, in an antibody combining site has not to date been observed and breaks new ground for the classes of reactive molecules that antibodies can process. IgG 5H7 performs multiple turnovers with neither an appreciable reduction in rate, nor structural modification (confirmed by SDS-gel and ELISA analysis).[11] This is of particular significance because enediynes are known to cause proteolysis and protein agglomeration.[23] We speculate therefore that 5H7 may be binding oxygen so that as the diyl intermediate **2** is generated in the antibody combining site it may be intercepted by the proximate oxygen molecule, thus protecting the immunoglobulin from damage.[24]

In conclusion, we have discovered a unique conversion of enediynes into quinones that occurs in aerated aqueous solution and is of interest not only from a mechanistic standpoint but also may be of relevance to the therapeutic mechanism of naturally occurring enediyne antibiotics. In addition we have evolved an antibody that catalyzes the conversion of the stable cyclic enediyne substrate **1** via the diyl intermediate **2** into the quinone product **4**. This result pushes back the boundary of antibody-catalyzed reactions and the reactive intermediates they are known to handle. In addition, it opens up a new strategy for effective activation of enediyne systems that, with suitable kinetic parameters, may ultimately find use in vivo.

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Supporting Information Available: Complete synthetic information for **1** and **3**−**5**; experimental details of kinetic studies, SRB assay, ELISA and SDS-page analyses (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) In a typical experiment the enediynol **1** (10 μL of a stock in DMSO) is added to a glass vial containing MOPS (100 mM, pH 6.8) either in the presence or absence of antibody (20 μM) and allowed to stand at 37 °C. At times throughout the assay, aliquots (20 μL) are removed and injected directly onto an RP-C18 Vydac HPLC column to allow separation of substrate **1** and products (detection at 254 nm). Enediynol **1** R_T = 11.8 min, tetralin **3** R_T = 14.2 min, **4** R_T = 6.3 min [mobile phase 28:72 acetonitrile:water (0.1% TFA) 1 mL/min).

(14) The observed rate and oxygen-dependence were investigated by pseudo-first-order kinetics in which the oxygen concentration was varied (200−1000 μM) while holding enediynol **1** at a fixed and higher concentration (2 mM). For a graphical representation of the rate data, please see the Supporting Information.

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(24) This hypothesis is strengthened by the observation that during the screening of the hapten **5**-specific antibody panel for catalytic activity a number of clones were degraded in the presence of enediynol **1** (data not shown). Attempts to measure oxygen binding to 5H7 have been hampered by experimental limitations. To a best approximation, the K_m of O_2 for 5H7 is 1.07 mM (Supporting Information). This value is approaching the maximum concentration of dissolved oxygen possible in our buffer system. Therefore, we are unable to explore O_2 binding above the theoretical K_m and cannot prove that oxygen binds to the antibody combining site.