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Activities of recombinant human bleomycin hydrolase on bleomycins and engineered analogues revealing new opportunities to overcome bleomycin-induced pulmonary toxicity

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ABSTRACT

The bleomycins (BLMs) are widely used in combination therapies for the treatment of various cancers. Dose-dependent and cumulative pulmonary toxicity is the major cause of BLM-associated morbidity, limiting the broad uses of BLMs as anticancer drugs. The organ specificity of BLM-induced toxicity has been correlated with the expression of the *hBLMH* gene, encoding the human bleomycin hydrolase (hBLMH), which is poorly expressed in the lung. hBLMH hydrolyzes BLMs into the biologically inactive deamido BLMs, thereby protecting organs from BLM-induced toxicity. Here we report (i) expression of *hBLMH* and production and isolation of recombinant human bleomycin hydrolase (rhBLMH) from *E. coli*, (ii) structural characterization of deamido BLM A2 and B2 isolated from rhBLMH-catalyzed hydrolysis of BLM A2 and B2, and (iii) kinetic characterization of the rhBLMH-catalyzed hydrolysis of BLM A2 and B2, in comparison with five BLM analogues. rhBLMH from *E. coli* catalyzes rapid and efficient hydrolysis of all BLMs tested, exhibiting a superior catalytic efficiency for BLM B2. These findings reveal new opportunities to overcome BLM-induced pulmonary toxicity in chemotherapies, potentially by exploring BLM B2 as the preferred congener, engineering designer BLMs with optimized activity for rhBLMH, or co-administrating rhBLMH directly into the lung as a potential protein therapeutic.

Introduction

The bleomycins (BLMs) are a family of glycopeptide antitumor antibiotics first isolated in 1966 from several *Streptomyces* species.[1] Other members of the BLM family of natural products that have been discovered since include the tallysomycins (TLMs),[2] phleomycins,[3] and zorbamycin (ZBM).[4] The BLMs are thought to exert their biological effects through a sequence-selective, metal-dependent oxidative cleavage of DNA and RNA in the presence of molecular oxygen.[5–8] Structurally, the BLMs have been divided into four functional domains: (i) the *N*-terminally located pyrimidoblamic acid subunit and the adjacent β-hydroxyhistidine for metal binding and molecular oxygen activation, (ii) the C-terminal amines and the adjacent bithiazole moiety for DNA binding and recognition, (iii) a linker region connecting the metal-binding and DNA-binding domains, and (iv) the disaccharide moiety for cell recognition, cellular uptake, and metal ion coordination (Fig. 1).[5–8] There have been continuing efforts to develop new BLM analogues, by both organic synthesis[6,9–11] and combinatorial biosynthesis,[12–17] to define the fundamental functional roles of the individual domains and search for anticancer drugs with better clinical efficacy and low toxicity.

The BLMs are widely used in combination therapies for the treatment of various cancers.[5–8,18] Under the trade name of Blenoxane®, the commercial drug consists of BLM A2 (~70%) and BLM B2 (~30%). The broad uses of the BLMs as anticancer drugs, however, is limited by their dose-dependent and cumulative pulmonary toxicity, which is associated with significant morbidity, affecting up to 46% of the total population, and a mortality rate of 3%.[7,19,20] The organ specificity of BLM-induced toxicity has been correlated with the expression of the *hBLMH* gene, which encodes the human BLM hydrolase (hBLMH), a cysteine aminopeptidase that detoxifies BLMs by catalyzing the hydrolysis of the β-aminoalanine amide moiety of BLMs to afford the biologically inactive deamido BLMs

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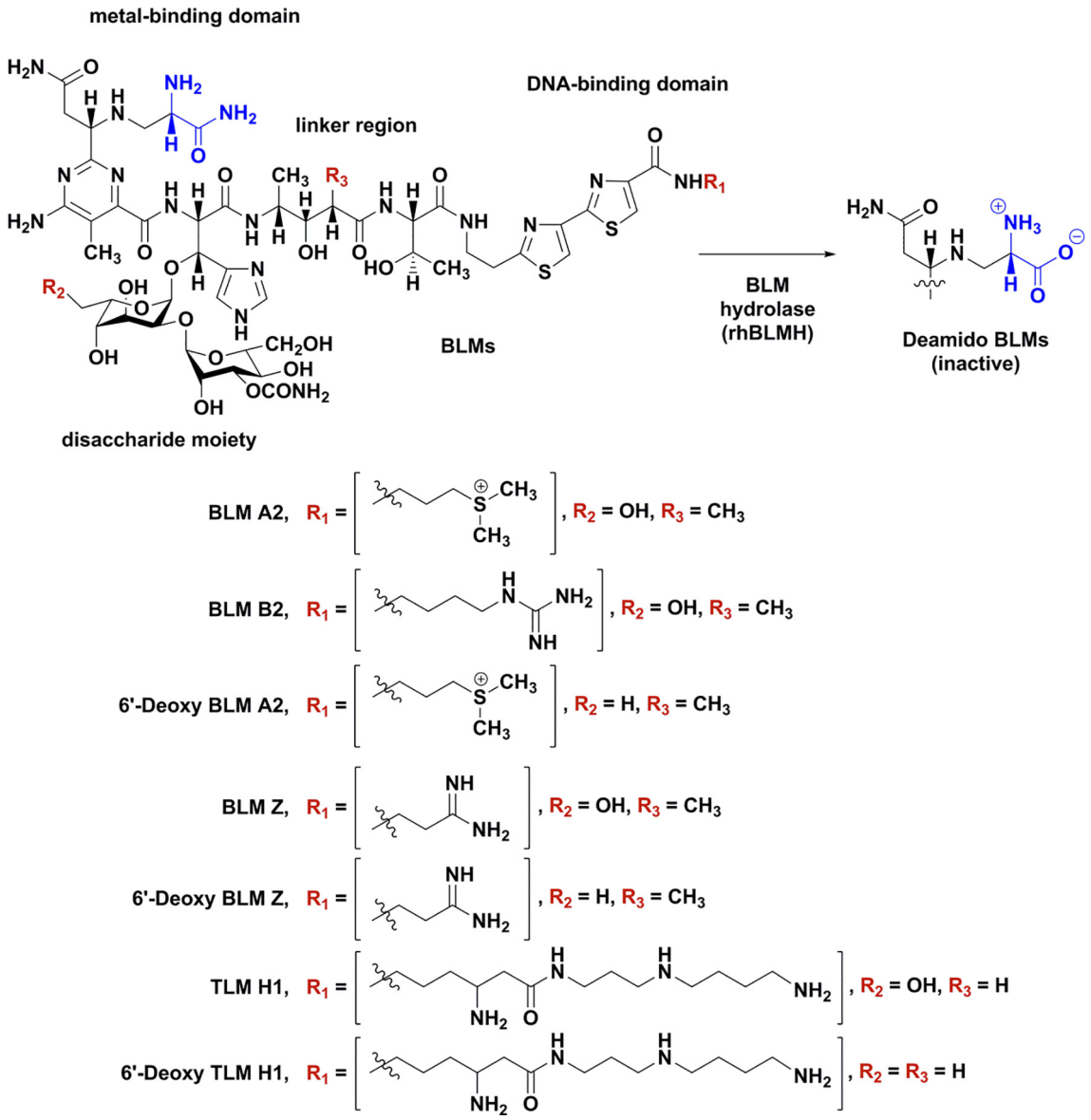

Fig. 1. The structures of BLM A2 and B2, featuring the four functional domains, in comparison with the five engineered BLM analogues with the structural variations highlighted in red. rhBLMH-catalyzed hydrolysis of BLMs to deamino BLMs, with the β-aminoalanine amide moiety of BLMs hydrolyzed by rhBLMH to the corresponding β-aminoalanine shown in the zwitterion form highlighted in blue.

(Fig. 1).[21–24] BLM hydrolases (BLMHs) are widely distributed through nature with high sequence identities.[25] While the exact physiological role of hBLMH remains unknown,[26,27] hBLMH is predominantly found in the liver, spleen, bone marrow, and intestine, but is presented at a low level in skin and lung, a finding that correlates well with the BLM-induced cutaneous effects and pulmonary toxicity during BLM chemotherapies.[20,23,28,29] hBLMH therefore represents an underexplored target related to BLM chemotherapy, exploitation of which may reveal new opportunities to overcome BLM-induced pulmonary toxicity.

In our continued effort to study biosynthesis of the BLM family of anticancer drugs as a model system for hybrid peptide-polyketide natural products and manipulate their biosynthetic machineries for BLM structural diversity,[12] we have previously produced five BLM analogues, featuring structural alterations at the C-terminal DNA binding domain, the linker region, and the disaccharide moiety (Fig. 1). In vitro assays, with BLM A2 and B2 as positive controls, confirmed that they maintain or exhibit superior DNA cleavage activity, highlighting their potential as anticancer drugs.[12–17] Here we report (i) expression of the *hBLMH* gene and production and isolation of recombinant human bleomycin hydrolase (rhBLMH) from *E. coli*, (ii) isolation and structural characterization of deamido BLM A2 and B2 from rhBLMH-catalyzed hydrolysis of BLM A2 and B2, and (iii) kinetic characterization of rhBLMH-catalyzed hydrolysis of BLM A2 and B2, in comparison with five engineered BLM analogues 6′-deoxy BLM A2,[16] BLM Z,[15] 6′-deoxy BLM Z,[15] tallysomycin (TLM) H1,[14] and 6′-deoxy TLM H1.[17] rhBLMH from *E. coli* catalyzes rapid and efficient hydrolysis of all the BLM analogues tested, exhibiting a superior catalytic efficiency for BLM B2, and these findings reveal new opportunities to potentially overcome BLM-induced pulmonary toxicity in chemotherapies by exploiting hBLMH.

We first expressed *hBLMH* in *E. coli*, purified the overproduced rhBLMH to homogeneity, and confirmed its activity in vitro using the commercial drug Blenoxane® as a substrate. Recombinant hBLMH has been produced previously in *Baculovirus* and confirmed