Communication

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Inactivation of the Carbamoyltransferase Gene Refines Post-Polyketide Synthase Modification Steps in the Biosynthesis of the Antitumor Agent Geldanamycin

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Geldanamycin (1) and its closely related analogues, herbimycin B (6) and macbecin, are naturally occurring antitumor antibiotics.[1] Geldanamycin binds to the N-terminal ATP binding site of heat-shock protein (Hsp) 90, inhibiting its chaperone activity.[2] Because Hsp90 client proteins are important in signal transduction and transcription, geldanamycin and its derivatives have the potential of serving as chemotherapeutic agents in a number of diseases.[3] One semisynthetic analogue of these compounds, 17-allylamino-17-demethoxygeldanamycin, demonstrated antitumor activity in vivo and was subjected to Phase I clinical trials.[4]
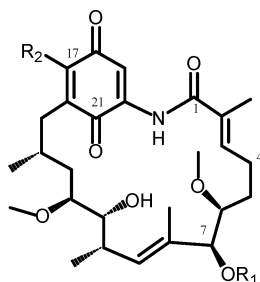
1 R₁=CONH₂, R₂=OCH₃, Δ⁴⁽⁵⁾
2 R₁=CONH₂, R₂=OH, Δ⁴⁽⁵⁾
3 R₁=H, R₂=OCH₃
4 R₁=H, R₂=OH
5 R₁=CONH₂, R₂=OCH₃
6 R₁=CONH₂, R₂=H, Δ⁴⁽⁵⁾

Geldanamycin biosynthesis is of interest as a means of introducing structural diversity by reprogramming biosynthetic assembly lines to create novel antibiotics with optimal properties. Geldanamycin is a 19-membered macrocyclic lactam and is related to ansamycin antibiotics, such as rifamycins and ansamitocins.[5] The biosynthesis of this class of compounds involves the assembly of 3-amino-5-hydroxybenzoic acid (AHBA) as a starter unit, followed by the sequential addition of extender units such as acetate, propionate, and glycolate to form a polyketide backbone, which then undergoes further downstream processing. The genes required for geldanamycin biosynthesis, including a set of type-I polyketide synthase (PKS) gene, have been cloned, sequenced, and analyzed from several streptomycetes independently.[6,7] On the basis of predictions from sequence homology and the results of feeding experiment with ¹⁴C-labeled precursor,[8] it was proposed that the successful production of geldanamycin requires the modification of several steps, which include the *O*-carbamoylation, hydroxylation, *O*-methylation, and oxidation of the initial polyketide synthase product. However, beyond determining sequences and deducing putative functions from sequence homologies, little had been learned about the post-PKS modification genes and the tailoring processes leading from initial polyketide to geldanamycin.

Analysis of the geldanamycin biosynthetic gene cluster revealed a series of putative post-PKS modifying genes.[6,7] Of these, *gel8*

(identical to *gdmN*[6]) encodes a protein highly homologous to carbamoyltransferases, which carry out the *O*-carbamoylation step in the biosynthesis of novobiocin, ansamitocin, and cephamycin. Therefore, the putative function of *gel8* could be to encode carbamoyltransferase.

To verify the assumption that the *gel8* gene is responsible for the carbamoylation step in the geldanamycin biosynthesis, we inactivated the *gel8* gene in *Streptomyces hygroscopicus* subsp. *duamyceticus* JCM4427, a geldanamycin producer. The mutated gene with an insertion of a kanamycin resistance gene was introduced into *S. hygroscopicus* JCM4427 to replace the wild-type gene by a sequential homologous recombination (see Supporting Information). The *gel8* gene-inactivated mutant grew normally in YEME medium containing kanamycin and was comparable to the wild-type strain, but completely lost its ability to produce geldanamycin (1) and 17-*O*-demethylgeldanamycin (2), which are the two major metabolites of the wild-type strain. Instead, two major metabolites, **3** and **4** (*m/z* 519 and 505, respectively), were detected and isolated from the gene-inactivated mutant. Compounds **3** and **4** displayed ESIMS patterns resembling those of compounds **1** and **2**. An analysis of the 1D and 2D NMR spectra of **3** suggested that it is a derivative of **1**. From the ¹H and ¹³C NMR spectra of **3**, the upfield shift of C-7 signals at δ_H 3.86 (1H, d, $J = 6.0$ Hz) and δ_C 78.23 indicated that **3** has a free hydroxy group at C-7 rather than a carbamoyl group, as expected. Furthermore, two olefinic methine signals (C-4 and C-5) of **1** were not detected, suggesting that its cis double bond had been hydrogenated. This was consistent with the molecular formula C₂₈H₄₁O₈N obtained by positive HRFABMS. A combination of COSY, HMQC, and HMBC NMR data were used to assign the ¹H and ¹³C NMR data unambiguously. Therefore, the structure of this new metabolite was elucidated as 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxygeldanamycin (**3**). The ¹H and ¹³C NMR spectra of **4** were almost superimposable with those of **3**, except for the absence of one phenolic methoxy signal in the later compound, and were consistent with the molecular formula C₂₇H₃₉O₈N obtained by positive HRFABMS. Accordingly, the structure of this new metabolite was determined as 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxy-17-*O*-demethylgeldanamycin (**4**).

The accumulation of descarbamoylated compounds in the mutant lacking a functional Gel8 confirmed that the *gel8* encodes a carbamoyltransferase. Identification of **3** also confirmed that the *gdmH* gene-inactivated mutant produced a compound with high-resolution mass spectral data similar to that of compound **3** due to a partial *gdmN* (identical to *gel8*) gene read-through mistake.[6] Interestingly, **3** and **4** do not contain the cis double bond between C-4 and C-5. This result suggests that module 6 of geldanamycin PKS contains a functional enoylreductase (ER) domain that reduces
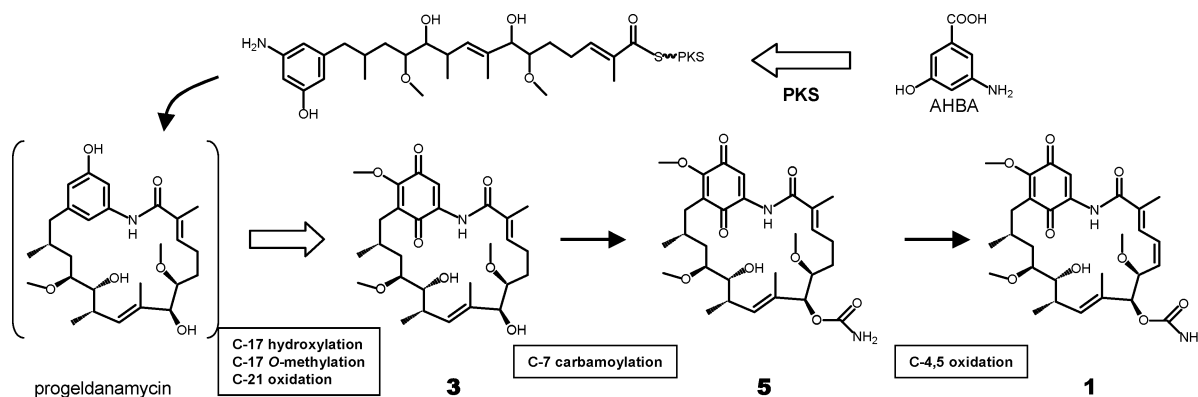
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Scheme 1

the double bond during polyketide assembly, as could be expected by sequence comparison of the ER domain in module 6 with other functional ER domains. Putative NADPH binding sites, GxGxx-AxxxA, of the ER domains in animal fatty acid synthase and erythromycin PKS are well-conserved in the corresponding ER domains of modules 1, 2, and 6 in the geldanamycin PKS (see Supporting Information). Taken together, these results indicate that C-17 hydroxylation, 17-*O*-methylation, and C-21 oxidation occur prior to the carbamoyltransferase reaction, and the hypothetical progeldanamycin does not possess a double bond at the C-4 and C-5 positions (Scheme 1). However, they do not provide information on the nature of the last step between carbamoylation and C-4,5 oxidation.

To determine whether **3** is a direct intermediate in the biosynthesis of **1**, a bioconversion experiment was performed using a geldanamycin PKS gene-inactivated mutant.[7] This mutant cannot produce **1** and **2** because of the inactivation of the loading domain in the PKS gene by the insertion of the kanamycin resistance gene; however, this mutant has a full complement of post-PKS processing genes. The mutant converted **3** to **1** effectively, confirming not only that **3** is an intermediate in the main pathway, but also that C-4,5 oxidation is a post-PKS modification step. In addition, a prospective intermediate, 4,5-dihydrogeldanamycin (**5**), was detected in the bioconversion experiment (see Supporting Information). The ES-IMS/MS profile of **5** was comparable to that of **1** (559 [M − H]⁻ → 516 [M − CONH₂]⁻), which included the UV and the fragmentation pattern of its molecular ion (561 [M − H]⁻ → 518 [M − CONH₂]⁻). The presence of **5**, but no detection of 7-*O*-descarbamoyl-7-hydroxygeldanamycin, indicates that C-4,5 oxidation is likely to be the final post-PKS modification step in the biosynthesis of geldanamycin.

Many steps in the biosynthesis of geldanamycin are still unclear; however, on the basis of the present results, we were able to refine the post-modification steps in the biosynthesis of geldanamycin, such as that C-17 hydroxylation, 17-*O*-methylation, and C-21 oxidation precede *O*-carbamoylation and that the hypothetical progeldanamycin does not carry a double bond at the C-4 and C-5 positions. Moreover, our results revealed that **3** and **5** are newly confirmed biosynthetic intermediates on the main pathway, indicating that *O*-carbamoylation occurs prior to the C-4,5 cis double bond formation in geldanamycin biosynthesis (Scheme 1).

The above results open a way for the selective derivatization of geldanamycin with a mutant, in which the post-polyketide synthase modification step is selectively inactivated.

Supporting Information Available: Experimental methods for the construction of *gel8* mutant and geldanamycin PKS mutant and spectroscopic data for **3** and **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) DeBoer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot.* **1970**, *23*, 442−447. (b) Omura, S.; Iwai, Y.; Takahashi, Y.; Sadakane, N.; Nakagawa, A.; Oiwa, H.; Hasegawa, Y.; Ikai, T. *J. Antibiot.* **1979**, *32*, 255−261. (c) Muroi, M.; Izawa, M.; Kosai, Y.; Asai, M. *J. Antibiot.* **1980**, *33*, 205−212.

(2) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239−250.

(3) Neckers, L.; Schulte, T. W.; Mimnaugh, E. *Invest. New Drugs* **1999**, *17*, 361−373.

(4) Schulte, T. W.; Neckers, L. M. *Cancer Chemother. Pharmacol.* **1998**, *42*, 273−279.

(5) (a) August, P. R.; Tang, L.; Yoon, Y. J.; Ning, S.; Muller, R.; Yu, T. W.; Taylor, M.; Hoffmann, D.; Kim, C. G.; Zhang, X.; Hutchinson, C. R.; Floss, H. G. *Chem. Biol.* **1998**, *5*, 69−79. (b) Yu, T. W.; Bai, L.; Clade, D.; Hoffmann, D.; Toelzer, S.; Trinh, K. Q.; Xu, J.; Moss, S. J.; Leistner, E.; Floss, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7968−7973.

(6) Rascher, A.; Hu, Z.; Viswanathan, N.; Schirmer, A.; Reid, R.; Nierman, W. C.; Lewis, M.; Hutchinson, C. R. *FEMS Microbiol. Lett.* **2003**, *218*, 223−230.

(7) Hong, Y.-S.; Kim, C.-G.; Sohng, J. K.; Liou, K. K.; Kim, W. C.; Seo, W.-M.; Song, K.-I.; Lee, J. J. Book of Abstracts, 13th International Symposium on the Biology of Actinomycetes, 1−5 December 2003, Ipeck Kurtboke, Ed.; Published by ISBA'13, Melbourne, Australia; p 33.

(8) (a) Arthur, H.; Ronald, D. J.; Kenneth, L.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 3541−3544. (b) Johnson, R. D.; Haber, A.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 3316−3317. (c) Kenneth, L.; Rinehart, K. L., Jr.; Potgieter, M.; David, A. W. *J. Am. Chem. Soc.* **1982**, *104*, 2649−2653.

JA047769M