SnoaW/SnoaL2: A Different Two-Component Monooxygenase

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The hydroxylation of C-1 in nogalamycin is an essential step in its biosynthesis. Siitonen and colleagues, in this issue of *Chemistry & Biology*, reveal an unconventional mechanism for the hydroxylation of the 3′,4′-demethoxy-nogalose-nogalamycinone by a two-component monooxygenase SnoaW/SnoaL2.

Aromatic polyketides synthesized by type II polyketide synthases (PKSs) comprise an important class of natural products. According to their polyphenolic ring structures, type II PKS-derived aromatic compounds are classified as anthracyclines, angucyclines, aureolic acids, tetracyclines, tetracenomycins, pradimicin-type polyphenols, and benzoisochromanequinones (Hertweck et al., 2007). The anthracycline family includes several clinically important drugs, such as doxorubicin, daunorubicin, and aclacinomycin A, which are among the most effective anticancer drugs available with antitumor activity against a variety of solid tumors, like carcinomas and sarcomas, and hematological malignancies, such as leukemias and lymphomas (Weiss, 1992). Anthracyclines consist of a planar tetracyclic aglycone moiety with adjacent quinine-hydroquinone groups in rings C-B, a methoxy substituent at C-4 in ring D, and a short side chain at C-9 with a carbonyl at C-13, as well as one or two sugar moieties, most commonly attached at the C-7 position via a glycoside bond (Figure 1).The structural diversity of anthracyclines is generated by different modification patterns of the aromatic polyketide scaffold. The reactions lead to different substitutions on the aglycone, including methylation, hydroxylation, reduction, and glycosylation. Nagalamycin, an anthracyline produced by *Streptomyces nogalater*, was reported to possess high activity against several cancer cell lines, but its clinical trial was stopped due to high toxicity (Wiley et al., 1977). Nogalamycin differs from most other anthracyclines in several unique characteristics, including different stereochemistry at C-9 (C-9S rather than C-9R), a methyl instead of an ethyl group at C-9, and a highly unusual nogalamine (3,6-dideoxy-3-dimethylaminosugar) moiety; C-1′ of nogalamine is attached to the oxygen at C-1 and C-5′ is connected to C-2 by a C-C bond.

The antitumor activity of anthracyclines is mainly attributed to their ability to rapidly diffuse to the nucleus and form the anthracycline-DNA-topoisomerase ternary complex by DNA intercalation. The formation of this complex causes a double-strand DNA break and interferes with biosynthesis of DNA, RNA, and protein, leading ultimately to cell death (Minotti et al., 2004). Both the planar ring and the deoxy sugar moiety of anthracyclines are important for their DNA intercalation. X-ray and NMR structures of nogalamycin-DNA complexes have shown that the tetracyclic ring of nogalamycin intercalates into the DNA, with its nogalose sugar found in the DNA minor groove while its tetracyclic aglycone and bicyclic amino sugar occupy the DNA major groove (Williams and Searle, 1999). Menogaril, a semisynthetic derivative of nogalamycin, differs from the latter compound only in the nogalose sugar. However, this difference changes the cellular activity and toxicity. Nogalamycin is specifically lethal to the S-phase cells, while menogaril is toxic to cells of all phases. Nogalamycin inhibits RNA synthesis more than DNA synthesis while menogaril inhibits DNA synthesis more than RNA synthesis (Li and Krueger, 1991).

Although some anthracyclines are commonly used in the treatment of many malignancies, their clinical effectiveness is greatly limited by the important side effects, such as dilative cardiomyopathy and congestive heart failure (Singal and Iliskovic, 1998), and by the development of multidrug resistance (Kaye and Merry, 1985). Attempts to find anthracyclines with improved activity or reduced toxicity has resulted only in a few valuable compounds. With the advent of molecular techniques and the fast-increasing knowledge about the pathways and enzymes for polyketide biosynthesis, post-PKS modification of the anthracycline aglycones might be possible to generate a series of ''better anthracyclines''.

A remarkable feature in nogalamycin biosynthesis is the hydroxylation at the C-1 position, catalyzed by the two-component monooxygenase system SnoaW/SnoaL2 (Siitonen et al., 2012, in this issue of *Chemistry & Biology*). Hydroxylation at C-1 of 3′,4′-demethoxy-nogalose-nogalamycinone provides a site for the following attachment of the nogalamine sugar. Modifications at C1/C2 of anthracyclines are quite attractive, because these positions have not been touched in generating novel anthracyclines so far (Preobrazhenskaya et al., 2006). Monooxygenases catalyze the incorporation of one oxygen atom from molecular oxygen into the organic substrate. In order to perform this reaction, monooxygenases have to activate O_2, as no reaction will occur without activation due to the ''spin- barrier'' of molecular oxygen (Fetzner and Steiner, 2010). In order to overcome the barrier, most monooxygenases depend on transition metal ions [most often iron (II) or iron (III) or organic cofactors, such as flavin and pterin] for catalysis. However, more and more monooxygenases have been characterized that require neither a cofactor nor a metal ion, which raises the intriguing question of how these enzymes work.

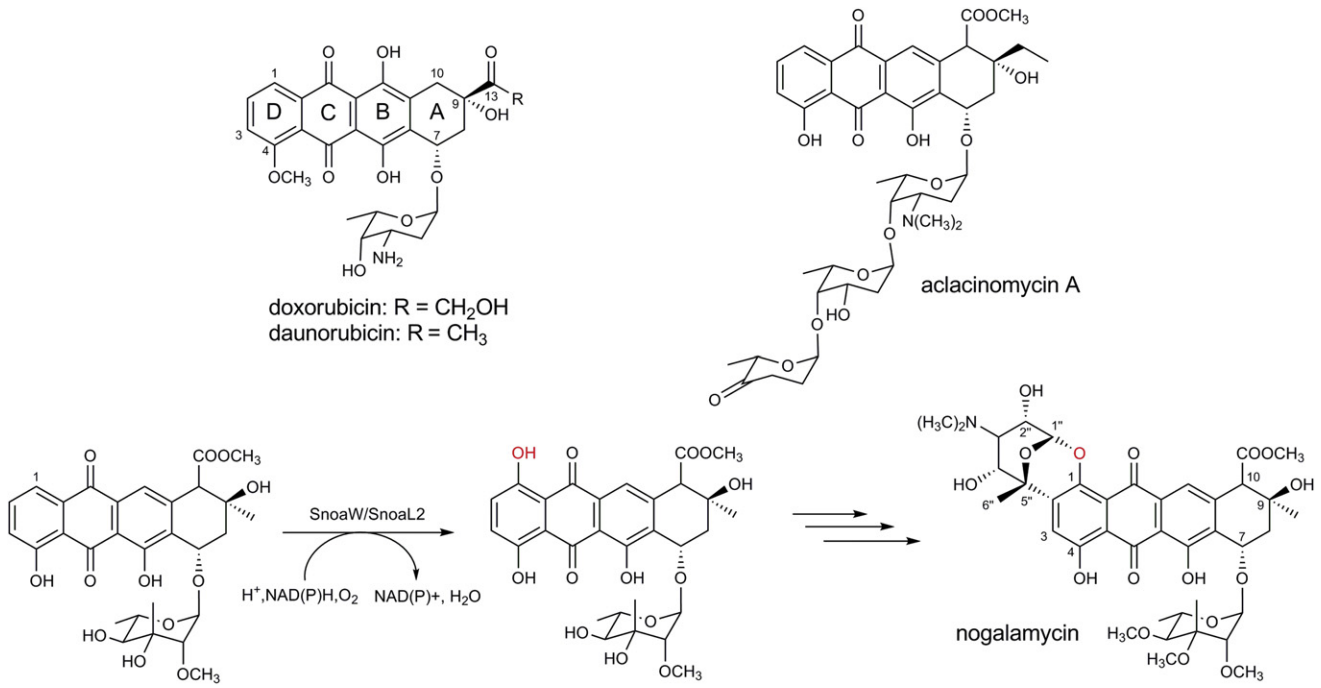

Figure 1. Structures of Selected Anthracyclines and the C-1 Hydroxylation Reaction Catalyzed by SnoaW/SnoaL2
Upon incubation, 3′4′-demethoxy-nogalose-nogalamycinone undergoes a hydroxylation, resulting in the introduction of an oxygen atom at C-1.

In this issue of *Chemistry & Biology*, Siitonen et al. (2012) report a two-component monooxygenase SnoaW/SnoaL2 which is responsible for the C-1 hydroxylation in nogalamycin biosynthesis (Figure 1). The authors reconstitute this reaction in vitro using the substrates, NAD(P)H and the two enzymes SnoaW/SnoaL2. No hydroxylation reaction was observed with only one enzyme. An interesting aspect is the detection of H_2O_2 in the nonproductive SnoaW reaction, which unequivocally demonstrates the presence of a hydroperoxy intermediate during the C-1 hydroxylation reaction. In the absence of SnoaL2, the hydroperoxy intermediate is readily oxidized back to its original substrate. The striking similarity between the formation of FMN hydroperoxide in flavin-dependent monooxygenases (Valton et al., 2006) and the formation of anthracycline peroxy intermediate in nogalamycin C-1 hydroxylation suggests a route for the activation of molecular oxygen by the substrate in its reduced dihydroquinone form, with the help of SnoaW. Moreover, the authors also show that the SnoaW reaction doesn't proceed without molecular oxygen. Together with the detection of H_2O_2 in the SnoaW one-enzyme reaction system, these results imply that the formation of the hydroperoxy intermediate happens in the active site of SnoaW and that this intermediate is the substrate for SnoaL2. In the presence of SnoaL2, the highly active intermediate is protonated to form the final 1-hydroxylated product 3′,4′-memethoxy-nogalose-1-hydroxy-nogalamycinone. From the above observations, the authors deduce that the mechanism for molecular oxygen activation in the SnoaW/SnoaL2 system is different from that in other characterized two-component FMN monooxygenases in which the flavin is first reduced by the reductases, transferred to the monooxygenase component, and then reacts with O_2 to form the C4a-(hydro)peroxyflavin intermediate (Ballou et al., 2005). Therefore, Siitonen et al. (2012) classify SnoaW/SnoaL2 as a novel two-component monooxygenase system.

In the in vitro assay, Siitonen et al. (2012) also show that the SnoaW/SnoaL2 system prefers the mono-glycosylated anthracycline over the nogalamycinone aglycone as substrate. This substrate preference suggests that the C-1 hydroxylation of nogalamycin occurs at a rather late stage. Crystallization analysis of the enzymes-substrate complexes in the future might help to reveal the catalytic mechanism behind this new family of monooxygenases.

The inspiring characterization reported by Siitonen et al. (2012) describes a new type of two-component monooxygenase with a different pattern for molecular oxygen activation from other well studied two-component FMN monooxygenases. This is significant progress in revealing the mechanism of action of cofactor-independent monooxygenases as well as in understanding the biosynthesis of polyketide compounds. These findings will greatly promote the discovery and generation of novel clinically important anthracyclines.

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