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Macrolactam formation catalyzed by the thioesterase domain of vicenistatin polyketide synthase

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Abstract—The excited thioesterase (TE) domain from the vicenistatin polyketide synthase (PKS) efficiently catalyzed the macrolactam formation of the *N*-acetylcysteamine thioester of the *seco*-amino acid of the aglycon vicenilactam. This result indicates that the vicenistatin PKS TE domain cyclizes the extended polyketide chain on the ACP domain in the PKS. Furthermore, the simple ethyl ester of the *seco*-amino acid was also found to be used as a substrate of the TE domain with similar efficiency.

Vicenistatin **1** was isolated from *Streptomyces halstedii* HC34 as an antitumor antibiotic, and consists of a unique 20-membered macrolactam aglycon, vicenilactam **2**, and an aminosugar visenisamine.[1] The characteristic macrolactam **2** was proposed to be biosynthesized by a standard polyketide synthase (PKS), which uses the unique amino acid starter unit, 3-amino-2-methylpropionate, as shown in Figure 1.[2,3] On the aspect of the polyketide macrolactam formation, the post PKS amide bond formation was suggested in the biosynthesis of ansamycin antibiotics including rifamycin and ansatrienin, in which an amide synthase just after the PKS was proposed to be responsible to the macrolactam formation.[4] On the other hand, the vicenistatin PKS obviously contains the thioesterase (TE) domain at the end of polypeptide, so that the TE domain was anticipated to be responsible to the macrolactam formation of the extended polyketide leading to the macrocyclic vicenilactam.[2,3]

In fact, the thioesterase-catalyzed macrolactone formation appears in many PKS system.[5] The isolated PKS TE domains in the epothilone PKS[6] and the pikromycin PKS[7] have recently shown to catalyze the macrolactonization using the suitable *N*-acetylcysteamine (NAC) thioester analogues of the natural polyketide substrate. In addition, some of the isolated nonribosomal peptide synthetase (NRPS) TE domains have been reported to catalyze the macrocyclic peptide formation of linear peptides as well.[8] These reports, therefore, encouraged us to investigate the function of the isolated TE domain of the vicenistatin PKS to confirm the biosynthetic role and to envision the synthetic potential as a macrocycle forming enzyme.

At first, the expected linear amino acyl chain substrate was prepared from vicenilactam **2** (Scheme 1). Activation of vicenilactam **2** as the *tert*-butyl carbamate was necessary to hydrolyze the amide bond giving the desired linear amino acid. Vicenilactam **2** was not hydrolyzed at all or decomposed under the several basic conditions. The protected *seco*-amino acid was then converted to the corresponding NAC thioester and then deprotected under the acidic condition giving the NAC thioester of *seco*-vicenilactam to mimic the S-ACP substrate in the vicenistatin PKS.[9]

The TE domain of the vicenistatin PKS was designed based on the crystal structure of the TE domain of 6-deoxyerythronolide B synthase (DEBS) and the pikromycin PKS.[10] Although the DEBS TE domain has not been shown to have the macrocyclic forming activity, the hydrolytic activity for the specific NAC substrates has been reported.[11] The structural studies clearly showed that the linker region between the TE domain

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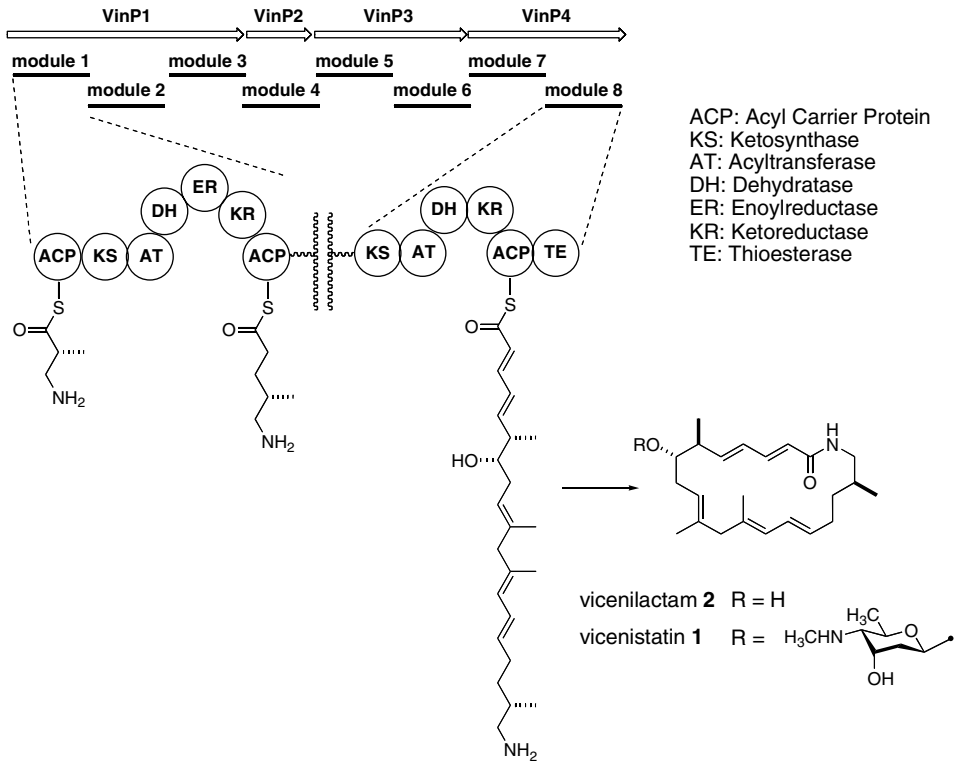
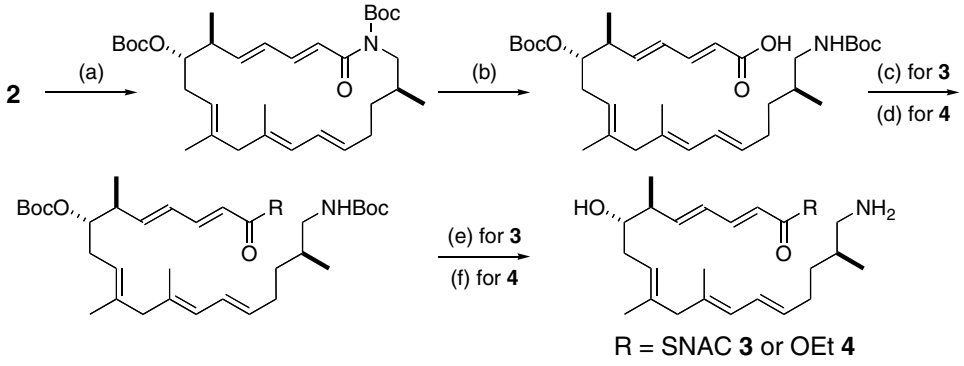


Figure 1. A part of modular organization of the vicenistatin (Vin) PKS in *Streptomyces halstedii*.



Scheme 1. Synthesis of the *seco*-vicenilactam esters. Reagents and conditions: (a) $(Boc)_2O$, pyridine, 98%; (b) LiOH, dioxane, H_2O, 82%; (c) DCC, DMAP, NAC–SH, CH_2Cl_2, 96%; (d) DCC, DMAP, ethanol, 65%; (e) $BF_3{\cdot}OEt_2$, 4 Å—MS, CH_2Cl_2 67%; TsOH, EtOH, reflux; 74%; (f) TsOH, EtOH, reflux; 61%.

and the acyl carrier protein (ACP) domain is important to form the hydrophobic interface of the dimeric TE domain. Thus, the position close to the ACP of module 8 in the vicenistatin PKS was chosen as the N-terminal of the TE domain in the present study.[12] The DNA sequence for the vicenistatin TE domain was amplified by a standard PCR and cloned into the expression vector (pET30, Novagen, USA) after confirmation of the DNA sequence. The TE domain was overexpressed in *Escherichia coli* BL21(DE3) by addition of isopropyl β-D-thiogalactoside and the cells of *E. coli* was homogenized by sonication. The TE domain was purified by the DEAE ion chromatography and the gel filtration chromatography to homogeneity with yield of 20 mg/L of culture.

The TE reaction was carried out with the NAC thioester of *seco*-vicenilactam **3** as shown in Figure 2.[13] The formation of the macrolactam product **2** was confirmed by HPLC equipped with a photodiode array detection system and LC–ESI mass spectrometry. Using 1.25 mM of substrate (apparently saturated) and 0.13 mM of the TE domain, 37% of NAC thioester **3** was converted to vicenilactam **2** for 40 min at 28 °C. It should be noteworthy that the hydrolyzed compound was not detected at all under the present reaction conditions. Thus, it was clearly proved that the TE domain is responsible to the macrolactam formation in the biosynthesis of vicenilactam **2**. This result also clearly suggested that the unique amino acid starter unit, 3-amino-2-methylpropionate, is somehow loaded on