Potent Oligomerization and Macrocyclization Activity of the Thioesterase Domain of Vicenistatin Polyketide Synthase

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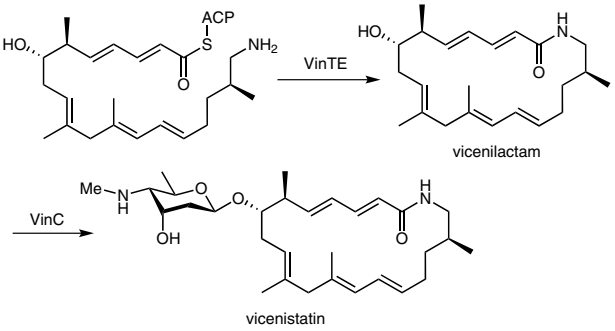
Abstract: The thioesterase domain of the polyketide synthase involved in the biosynthesis of the 20-membered macrolactam antibiotic vicenistatin (VinTE) was found to catalyze oligomerization and macrocyclization of ω-hydroxy fatty acid ethyl esters to afford 17–28-membered macrocyclic lactones. The ring sizes of the macrocycles appear to be limited to the more moderate sizes because of the space limitation of the active site of VinTE. It was also verified that the initially formed linear dimer is first released from the active site of VinTE and then is recognized again by VinTE prior to its transformation to the cyclic dimer.

Key words: macrocycles, cyclization, oligomerization, polyketide synthase, thioesterase domain

Macrocyclic natural products such as macrolide polyketide antibiotics (e.g., erythromycin, avermectin) and nonribosomal peptide antibiotics (e.g., gramicidin, daptomycin) are biosynthesized by polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS), respectively. In general, the C-terminal thioesterase (TE) domain of PKS and NRPS is responsible for the macrocyclizations.[1,2] The TE domain catalyzes the cleavage of the thioester bond of the elongated polyketide-acyl carrier protein (ACP) or polypeptide-peptidyl carrier protein (PCP) via a catalytic active-site serine residue, and the subsequent intramolecular nucleophilic attack of a hydroxy or amino group to form a macrocycle. Walsh et al. have demonstrated the synthetic potential of the truncated NRPS-TE domains by showing that they can catalyze the formation of various unnatural macrocyclic polypeptides.[3–5] In addition, several truncated PKS TE domains including vicenistatin PKS,[6] picromicin PKS,[7,8] and epothilone PKS,[9] are reported to catalyze the macrocyclization of linear polyketides, although a comprehensive analysis of the macrocycle forming activities of PKS TE domains using unnatural types of polyketide chains has never been investigated. Only the hydrolytic activities of the PKS TE domains have been reported to date.[10–12] In the present paper, we describe the oligomerization and macrocyclization activities of the vicenistatin PKS TE domain (VinTE) in the presence of several ω-hydroxy fatty acid ethyl esters (Scheme 1). Although TE have been reported to be active towards thioesters including *N*-acetylcysteamine (NAC) thioester and phenyl thioester (a mimic of ACP-phosphopantetheine thioester),[13] VinTE also recognizes the simple ethyl ester of the *seco*-amino acid of the aglycon vicenilactam in addition to the NAC thioester to form a macrolactam.[6] Only VinTE and TyrC TE[4] are known to recognize ester substrates. Thus we prepared a more stable ethyl ester substrate rather than NAC thioester for the examination of specificity.



Scheme 1 Function of VinTE in vicenistatin biosynthesis. VinTE: thioesterase (TE) domain of VinP4 polyketide synthase (PKS), VinC: vicenisaminyltransferase, ACP: acyl carrier protein.

The VinTE protein was prepared according to a modified method from a previous report.[6] The N-terminal His-tagged VinTE was expressed in *Escherichia coli* and purified with Ni-affinity chromatography. After removal of imidazole and concentration by ultrafiltration, VinTE was found to have the same activity as the native protein. To examine the macrocyclization activity of VinTE, ω-hydroxy fatty acid ethyl esters in DMSO (final 1–3 mM and 10% DMSO) were mixed with VinTE in 100 mM sodium phosphate buffer (pH 7.0), and the mixtures were incubated at 28 °C for 24 hours. Enzymatic reaction products were extracted with excess amounts of chloroform, and the extract was purified by silica gel chromatography to isolate the reaction products (see details in the Supporting Information).

The shortest examined substrate, ethyl 6-hydroxyhexanoate (**1**), was polymerized and macrocyclized to afford cyclic trimer **12** and tetramer **13** in 49% and 10% yield, respectively (Scheme 2). Ethyl 8-hydroxyoctanoate (**2**) and ethyl (*E*)-8-hydroxy-2-octenoate (**3**) were converted into the corresponding cyclic dimers **14** and **16** and trimers **15** and **17**. In the case of the C-8 substrates **2** and **3**, significant amounts of the linear polyesters were also iso-

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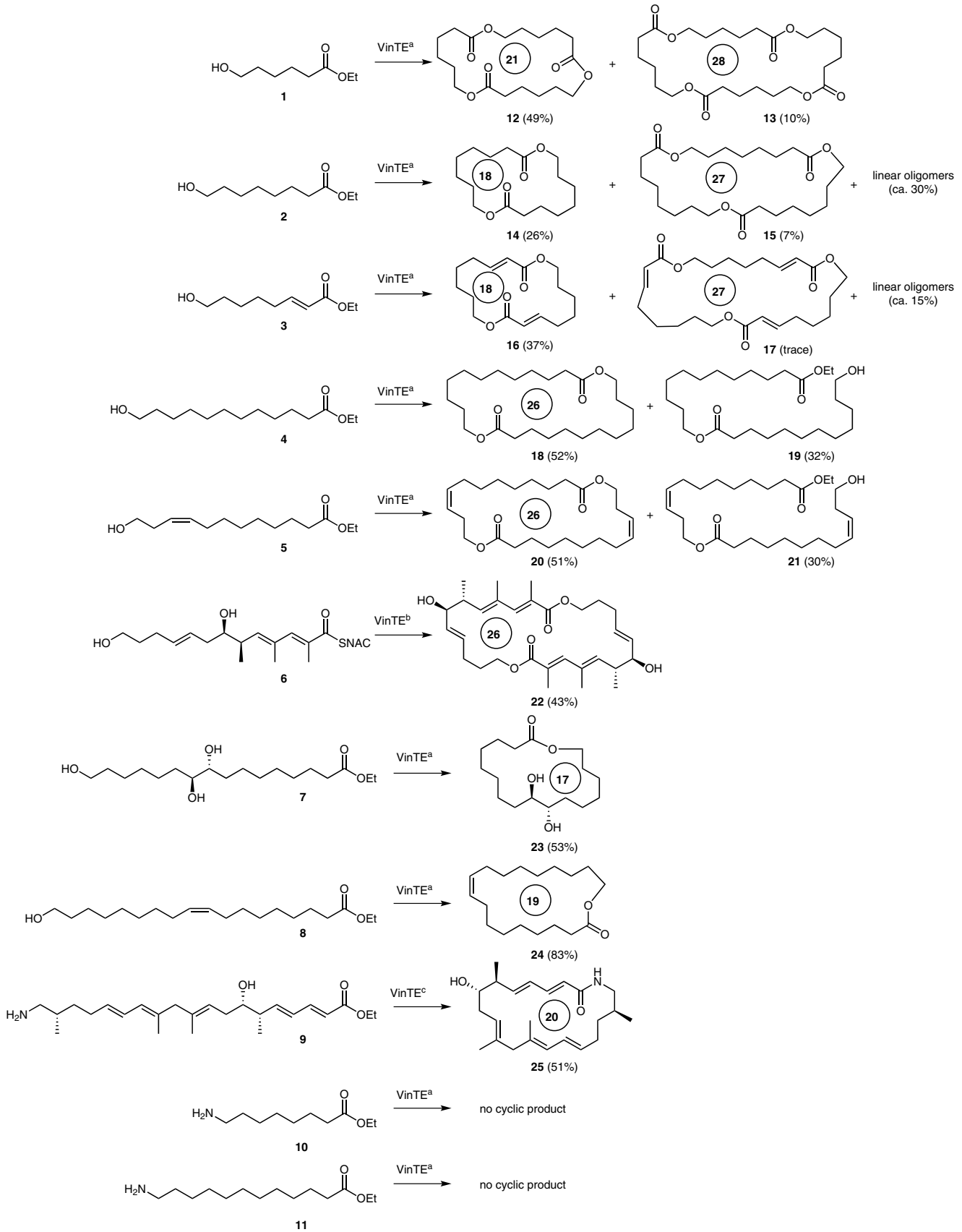


Scheme 2 VinTE reaction with ω-hydroxy fatty acid ethyl esters. [a] 60 μM of VinTE, 1–3 mM of substrate, 28 °C, 24 h; [b] 300 μM of VinTE, 7 mM of substrate, 28 °C, 24 h. [c] 130 μM of VinTE, 1.25 mM of substrate, 28 °C, 40 min.[6]

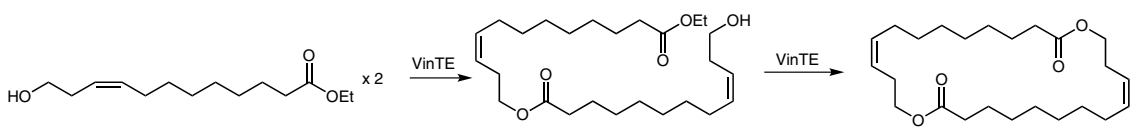
lated. Ethyl 12-hydroxydodecanoate (**4**) and ethyl (*Z*)-12-hydroxy-9-dodecenoate (**5**) were converted into respective linear dimers **19** and **21** and cyclic dimers **18** and **20**. The expected monocyclic compound from **5**, the Yuzu lactone a volatile component of Yuzu fruit,[14] was not formed at all. The *N*-acetylcysteamine thioester (**6**), which is a synthetic intermediate of the macrolide antibiotic FD-892,[15] was converted into cyclic dimer **22** in 43% yield. Finally, the aleuritic acid ethyl ester (**7**) and ethyl (*Z*)-18-hydroxy-9-octadecenoate (**8**) were converted into monocyclic molecules **23** and **24**, respectively. The formed 19-membered macrocycle **23** is a known musk compound in tobacco.[16] As we previously reported, the ethyl ester of the natural secovicenilactam was efficiently cyclized by VinTE for 40 minutes.[6] However, the ω-amino fatty acid ethyl esters **10** and **11** were not cyclized at all.

Consequently, it was found that VinTE catalyzes the oligomerization and macrocyclization of ω-hydroxy fatty acid ethyl esters. The ring sizes of the formed macrocycles were limited to 17–28-membered rings (Scheme 2), presumably due to the cavity size of the active site pocket of VinTE. The structural model of VinTE (Figure S3 in the Supporting Information) based on the crystal structure of the picromycin PKS-TE domain (PicTE or PikTE)[17–19] revealed that VinTE has a slightly larger cavity against 20-membered vicenilactam. Therefore, VinTE appear to accept slightly larger ω-hydroxy fatty acid polyesters for macrocyclization. On the other hand, with the longer length of ω-hydroxy fatty acid esters that are not accommodated in the active site of enzyme, VinTE just catalyzes the polymerization to afford linear polymers. It should be noted that, in the vicenistatin biosynthesis, the *N*-alanyl-secovicenilactam biosynthetic intermediate must be deprotected by a peptidase VinJ to secovicenilactam thioester before macrocyclization by VinTE.[20]

To understand the reaction mechanism of oligomerization and macrocyclization catalyzed by VinTE, the isolated linear dimer **21** was reacted again with VinTE (Scheme 3). As a result, the expected cyclized dimer **20** was produced in high yield. Furthermore, the presumed active site amino acid residue serine 136 (S136) of VinTE (Figures S3 and S4 in the Supporting Information) was mutated to alanine resulting in no activity. Therefore, S136 of VinTE was found to be a critical catalytic residue. Considering these results, the dimerization and macrocyclization mechanism is proposed as follows. The active site of VinTE accommodates two ω-hydroxy fatty acid ester substrates, and the dimerization is triggered by nucleophilic attack of the hydroxy group of the active site S136 to one substrate and the subsequent nucleophilic attack from the hydroxy group of another substrate leading to a dimeric linear ester. The formed linear dimer is then released from the active site and is recognized again by VinTE, and the subsequent intramolecular nucleophilic attack gives the cyclic dimer. When an additional ω-hydroxy ester enters the active site in the presence of a dimeric linear ester, the subsequent polymerization occurs to form a linear trimer. Then, the longer ω-hydroxy fatty acid ester can no longer be cyclized because of the space limitation of the active site of VinTE. A structural model of VinTE (Figure S3 in the Supporting Information) showed the appropriate size of the hydrophobic pocket to recognize the lipophilic 20-membered vicenilactam. Indeed, the well-conserved amino acid residues including the critical serine residue seem to comprise a catalytic triad. The enterobactin NRPS EntF TE domain[21] and gramicidin S NRPS TE GrsB TE domain[22] are known to catalyze both oligomerization and cyclization of elongated polypeptides. The TE domain of a hybrid NRPS/PKS was also hypothesized to be responsible for both the dimerization and cyclization in the biosynthesis of disorazole.[23] Therefore, the observed oligomerization–macrocyclization activity of the PKS TE domain VinTE appeared to be a general feature in PKS/NRPS assembly lines. Cyclic polyketides such as tartolon D[24] could be constructed by the use of thioesterase chemistry in a producer strain.

To investigate the generality of the macrocyclization activity of PKS TEs with ω-hydroxy fatty acid ethyl esters, we examined the catalytic activity of HlsTE in halstoctacosanolide PKS[25] derived from the same producer as vicenistatin and GfsTE in FD-891 PKS[26] derived from *Streptomyces graminofaciens*. However, no macrocyclic products were detected in the presence of HlsTE or GfsTE. Thus, the oligomerization–macrocyclization activity against ω-hydroxy fatty acid esters seemed to be a specific property of VinTE, probably due to the high hydrophobicity in its active site.

Macrocyclization is a featured transformation in organic synthesis, because numerous substances including bioactive natural products often possess macrocyclic structures. Therefore, many synthetic methods[27] including macrolactonization,[28] Prins-type reaction,[29] and ring-closing metathesis,[30] have been developed. In addition, enzymatic polymerization and macrocyclization of carboxylic acids, esters, and monomeric lactones have been developed using lipase.[31] Thus, chemoenzymatic approach[32] with the reported VinTE should provide a new useful method for synthetic biological chemistry. If one can control the length of polymers and ring sizes in the VinTE-mediated reactions, VinTE would become a powerful enzymatic tool.



Scheme 3 Mechanism of VinTE reaction

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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