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Specific enrichment of nonribosomal peptide synthetase module by an affinity probe for adenylation domains

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ABSTRACT

We targeted the development of an affinity probe for adenylation (A) domains that can facilitate enrichment, identification, and quantification of A domain-containing modules in nonribosomal peptide synthetase (NRPS)-polyketide synthase (PKS) hybrids and NRPSs. A 5′-*O*-sulfamoyladenosine (AMS) non-hydrolyzable analogue of adenosine monophosphate (AMP) has been reported as a scaffold for the design of inhibitors exhibiting tight binding of adenylation enzymes. Here we describe the application of an affinity probe for A domains. Our synthetic probe, a biotinylated L-Phe-AMS (L-Phe-AMS-biotin) specifically targets the A domains in NRPS modules that activates L-Phe to an aminoacyladenylate intermediate in both recombinant NRPS enzyme systems and whole proteomes.

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A number of small molecule natural products with significant biological activity such as antimicrobial, anticancer, and immunosuppressant activities belong to a large class of natural products known as polyketide (PK)–nonribosomal peptide (NRP) hybrid molecules and NRPs.[1] Originating from bacteria and fungi, these peptide-containing bioactive natural products consist not only of the 20 proteinogenic amino acids, but also non-proteinogenic amino acids, aryl acids, and other acids, thus generating highly complex chemical diversity.[2] The biosynthesis of these small molecules is performed by highly versatile and large multimodular enzymes called nonribosomal peptide synthetase (NRPS)–polyketide synthase (PKS) hybrids and NRPSs.[3–5] There has been much progress in the understanding biochemical programming and molecular basis of adenylation (A) domain substrate specificity in NRPS and NRPS–PKS hybrid systems. This has facilitated the prediction of structural features of newly discovered NRP and NRP–PK natural products assembled by these systems uncovered by genomic information. Large portions of these compounds often readily correlate to the amino acid specificity of A domains found on their associated modular enzymes.[6–8] Genome sequencing has revealed that secondary metabolite gene clusters encoding these enzymes are widely dispersed and largely uncharacterized.[9] In addition, complicated organisms containing symbiotic bacteria are particularly resistant to most genetic methods that rely on pure, culturable strains. Direct detection of biosynthetic enzymes from bacterial proteomes compliments genetic approaches in understanding the activity and dynamics of these enzymes in their native proteomes. By taking advantage of the A domains of NRPS and PKS–NRPS hybrid systems, this work aims to specifically enrich A domain-containing modules from bacterial proteomes with sequenced genomes using small-molecule probes and directly link the chemotypes of expressed peptide-containing natural products to their biosynthetic enzymes. Chemical probes for A domains using amino, aryl, or other acid building blocks that are found in NRP and NRP–PK hybrid molecules would facilitate probe-guided selective enrichment and identification of A domain-containing modules from proteomic samples by LC–MS/MS analysis (Fig. 1). In sequenced producers, such approaches would have applications in monitoring the expression dynamics of NRPS modules and optimizing bacterial culture conditions. In unsequenced organisms, such studies should facilitate discovery of the expressed NRPS/PKS–NRPS gene clusters.[10,11]

The affinity probe design for the A domains is based on the reaction mechanism for amino acid loading, which is catalyzed by the A domains as depicted in Figure 2a. The A domain recognizes a specific amino, acyl, or another acid from the cellular pool, and catalyzes the formation of a tightly bound acyladenylate intermediate. This in turn transfers the acyl group onto the thiol group of a 4′-phosphopantethein present on a downstream carrier protein (CP) of NRPS assembly line.[12,13] A 5′-*O*-sulfamoyladenosine (AMS), a non-hydrolyzable analogue of adenosine monophosphate (AMP), has been applied widely in the design of inhibitors that

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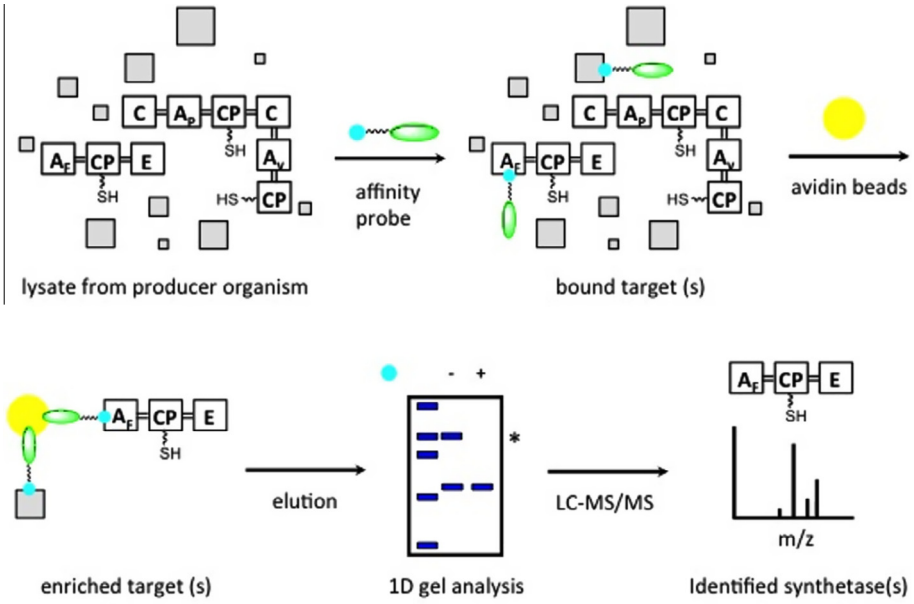

Figure 1. Methods for proteomic analysis of NRPSs using an affinity probe. Modules comprise carrier protein (CP), adenylation (A) [A_F: L-Phe specific; A_P: L-Pro specific; A_V: L-Val specific], epimerase (E), and condensation (C) domains.

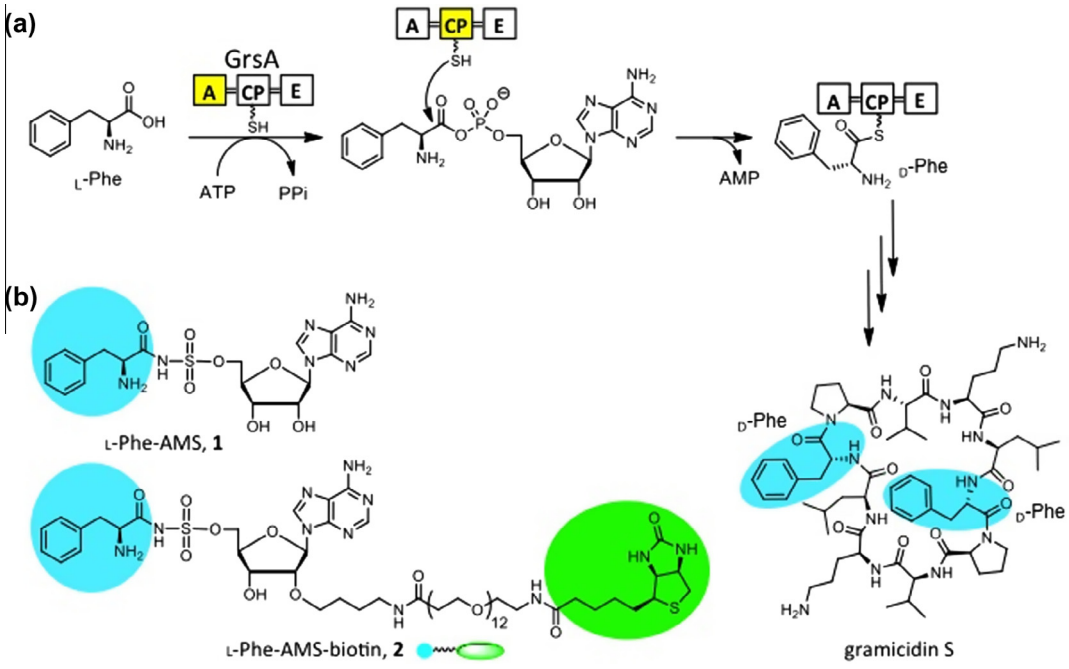

Figure 2. (a) Biosynthesis of the gramicidin S. (b) Structures of L-Phe-AMS **1** and L-Phe-AMS-biotin **2**.

display tight binding of adenylation enzymes and functionally related aminoacyl tRNA synthetases.[14–16] A 5′-*O*-[*N*-(phenylalanyl)-sulfamoyl] adenosine (L-Phe-AMS, 1, Fig. 2b) has been developed by Marahiel and coworkers wherein the reactive acylphosphate linkage has been replaced by a bioisosteric and non-hydrolyzable acylsulfamate group.[15] Recently, an acylsulfamate-based probe installed a photoreactive group has been reported for profiling of the stand-alone aryl-acid adenylation enzyme, MbtA.[17] A more complete set of probes would be crucial, however, for full module identification. Here, we describe the design, synthesis, and characterization of a biotinylated variant of L-Phe-AMS, referred to as L-Phe-AMS-biotin and highlight its utility as an agent for the specific enrichment, identification, and quantification of A domain-containing modules.

Table 1
The apparent K_i values of **1** and **2** for A domain of the tridomain GrsA

Inhibitors	K_i^{app} (nM)
1	54.9 ± 1.2
2*	339 ± 26

* The assays were performed in the presence of 0.0025% Igepal CA-630.

To expand the acylsulfamate scaffold to identify A domains of modular NRPSs and NRPS-PKS hybrids from proteomic samples,

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