

Biomimetic Catalysis of Intermodular Aminoacyl Transfer

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Intermodular aminoacyl transfer is the fundamental bond forming reaction in the biosynthesis of polypeptides by ribosomes and nonribosomal peptide synthetases (NRPS).¹ Here we report the design and functional characterizations of short α -helical peptides that mimic the aminoacyl loading and intermodular aminoacyl transfer steps of NRPS with aminolysis rate enhancements in neutral aqueous solutions of up to 5400-fold (k_{cat}/k_{uncat}). The catalysts operate as noncovalently associated assemblies with composite active sites fashioned at the interface between helical subunits. Following substrate loading at the active site cysteine, the juxtaposition of the resulting aminoacyl thiolester and the amine of the acyl acceptor moiety gives rise to high effective concentrations (up to 54 M) that facilitate interhelical aminoacyl transfer. Moreover, studies based on homo- and heteromeric assemblies, active site substitutions, kinetic analysis, and reaction modeling indicate that the supramolecular catalysts exhibit some basic characteristics of natural enzymes, including precise positioning and pK_a modulation of active site residues, covalent catalysis, and product turnover.^{2,3}

In the present study, our primary objective was to mimic the two fundamental chemical steps of NRPS: aminoacyl substrate anchoring and intermodular aminoacyl transfer. To bring about efficient acyl transfer, we sought to exploit primarily principles of catalysis by approximation⁴ using 26-residue coiled-coil peptides⁵ to assemble noncovalently the aminoacyl donor and acceptor moieties into productive complexes (Figure 1 and Figure S1, Supporting Information). Because parallel coiled-coil homotetramers are pseudo-4-fold symmetric, each complex contains four putative active sites (Figure 1a). We designed and evaluated three active site variations consisting of Cys for substrate loading and one of the following acyl acceptors: a lysine residue (type-I), an aminoacyl ester tethered at a serine residue (type-II), and a second aminoacyl thiolester tethered at a cysteine residue (type-III) (Figure 1b).

We initially evaluated type-I active sites (peptides 1–4) using 100-fold excess Cbz-protected *N*-acetylcysteamine glycyll thiolester as substrate. Under the neutral aqueous conditions, **1** underwent substrate loading at the active site Cys to form **1a**, subsequent interhelical aminoacyl transfer to produce **1b**, and reloading to yield product **1c** (Figures 2, S3, Table 1). Increases in the aminolysis rate of 5400-fold were observed for **1** relative to the background aminoacylation of Lys in control tripeptide **7**. The interhelical mode of acyl transfer was supported by mixing purified **1c** with **1**, which resulted in rapid aminoacyl transfer to yield approximately 2 equiv of **1b** (see Figure S2 for details and an additional proof using labeled peptides). The rate of aminoacyl transfer for **1** was reduced by >300-fold in 6 M Gnd·HCl as would be expected owing to the partial unfolding of the coiled coil. Intermolecular rates of lysine aminoacylation in the context of the folded scaffold were determined by assaying **8** and **9**, which lack an active site Cys residue. Interestingly, the Lys residues in folded peptides **8** and **9** were acylated 50- and 10-fold faster than Lys in unstructured tripeptide **7**, respectively. Preliminary results suggest that this enhanced

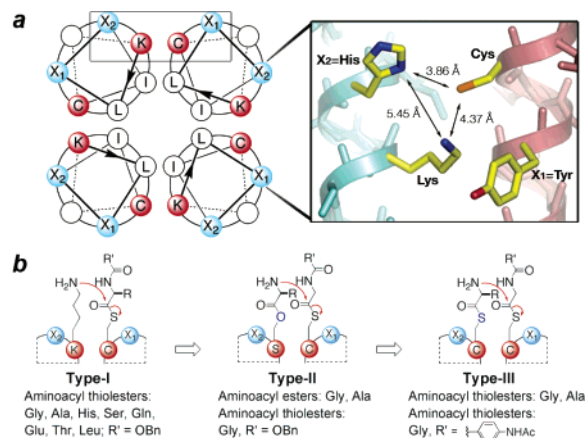


Figure 1. Representations of the coiled-coil scaffold and the composite aminoacyl transfer active sites. (a) (left) Helical wheel diagram of the homotetrameric coiled-coil illustrating the four symmetry-related active sites juxtaposing an aminoacyl donor (cysteine for covalent substrate anchoring via transthiolesterification), an aminoacyl acceptor (amine from lysine or a covalently tethered amino acid), and X_1 and X_2 residues potentially providing electrostatic or general acid–base catalysis. (right) The 2.17 Å crystal structure of a designed homotetramer⁶ illustrating the juxtaposition of the putative active site residues. (b) Schematic illustration of the three active site designs, highlighting aminoacyl transfer from an aminoacyl-donor to the -acceptor moiety located on an adjacent helix. For clarity, only one of the four symmetry-related active sites is shown (boxed region in the helical wheel diagram). For peptide sequences see Table S1.

reactivity is due to a depression of the Lys active site pK_a .^{2a,f} Substrate generality was assessed using a representative set of L-aminoacyl thioesters (Table S2). Although substitutions of the α -substituent had little effect on the aminoacyl transfer rates (k_2 varied by \sim 3-fold), side chain β -substitution resulted in 7 to 13-fold slower aminoacyl loading rates. To probe the influence of active site histidines (X_1 and X_2) on the aminoacyl loading and transfer steps, **2**–**4** were employed in which either or both residues were substituted with alanine. While reductions in the acyl transfer rate of up to 13-fold resulted, the observed acyl transfer efficiency of **4** discounts the possibility of acylimidazolyl intermediates in the reaction.^{2c,3}

The potential for aminoacyl transfer between two proximally tethered amino acids was assessed using type-II and type-III active site designs (Figure 1b). Encouragingly, **5** and **6** having a serine-anchored aminoacyl ester as the acyl acceptor (type-II) underwent substrate loading and aminoacyl transfer with rates similar to the analogous reaction for **1** (Figure S5, Table 1), although the reduced transfer rate for **6** likely results from the increased steric influence of the acyl acceptor α -substituent. The possibility of aminoacyl transfer in the type-III active site was studied using preloaded aminoacyl donor (**10a**) and acceptor (**11a**, R = H, Me) modules (Figure 2). In this system Cys8 in **10a** and Cys13 in **11a** are disabled by Ac side-chain protection. However, combining **10a** and **11a** allows the formation of heterotetrameric assemblies which brings

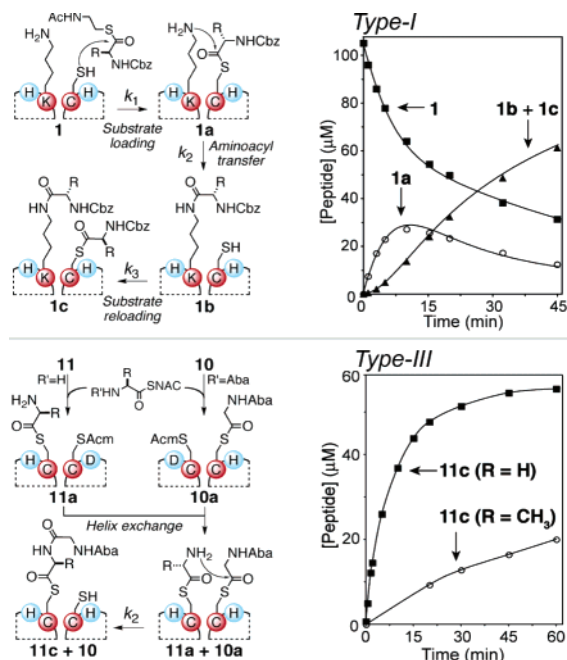


Figure 2. Reaction schemes and product formation in time for type-I and -III active sites. For type-I, reactions contained $\sim 100 \mu\text{M}$ peptide, 10 mM Cbz-Gly-SNAC, 10 mM triscarboxyethyl phosphine (TCEP) as reducing agent, 285 mM Hepes pH 7.0, and $\sim 200 \mu\text{M}$ acetamidobenzoic acid (Aba) as internal concentration standard. Curve fits shown are from reaction modeling using SIMFIT.⁷ Type-III reactions were initiated under similar conditions by combining **10a** ($\sim 70 \text{ mM}$) with **11a** ($\sim 560 \text{ mM}$).

Table 1. Aminoacyl Loading and Transfer Rate Constants^a

peptide ^b	active site residues	k_1 (10^{-3} sec^{-1})	k_2 (10^{-4} sec^{-1})
1	K_HH_C	1.3	9.2
2	K_AH_C	1.0	1.3
3	K_HA_C	1.3	0.7
4	K_AA_C	3.4	0.5
5	S ^{Gly} _HH_C ^c	1.3	9.1
6	S ^{Ala} _HH_C ^c	0.9	0.4
1	K_HH_C ^d	0.5	0.03
7	Aba-SKL-CO ₂ H	<i>e</i>	0.0017
8	K_HH_S	<i>e</i>	0.09
9	K_AH_S	<i>e</i>	0.02

^a Reaction conditions are as described in Figure 2. ^b See Table S1 for peptide sequences. ^c Aminoacyl-esterified Ser residue (type-II). ^d In 6 M Gnd-HCl, pH 7.0. ^e Transthioesterification not possible.

together aminoacyl-donor and -acceptor moieties to create competent active sites (Figure 2c, S4). Indeed, reaction mixtures containing **10a** and **11a** resulted in aminoacyl transfer with rates similar to those observed for type-I and -II active sites (Figure 2), suggesting fast helix exchange followed by efficient intermolecular aminoacyl transfer.

We next examined the viability of catalytic turnover by exploiting heterotetrameric peptide assemblies. Combining **9**, which contains an active site aminoacyl-acceptor (Lys) but no -donor (Cys), with **12**, having an aminoacyl-donor but no -acceptor, makes possible the formation of heterotetrameric assemblies in which Cys and Lys residues are brought into proximity to form composite type-I active sites (Figure 3a). As expected, the observed rates of aminoacylation depended markedly on the concentration of **12** present (Figure 3b), reflecting catalyst participation in the reaction. Multiple product turnovers were observed, suggesting that helix subunit exchange rates are faster than the rate of intermolecular aminoacyl transfer.

The studies reported here establish that two fundamental steps of NRPS can be effectively mimicked by appropriately designed

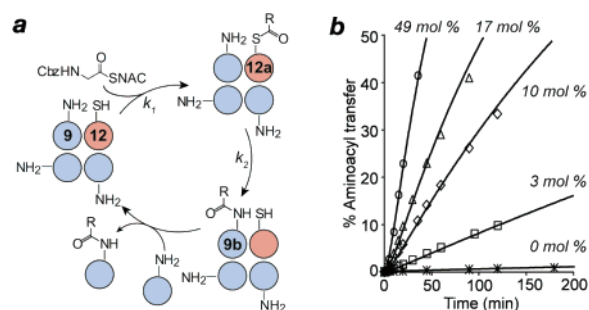


Figure 3. (a) Schematic representation of catalytic aminoacyl loading and transfer cycles based on heterotetrameric assemblies (type-I active site). Colored circles represent α -helices for the donor peptide **12** (pink) and acceptor peptide **9** (blue). For clarity, we depict only the statistically most predominant catalytically competent heterotetrameric coiled-coils. (b) Aminoacylation of **9** ($\sim 360 \mu\text{M}$) in reactions containing catalyst **12** at varying concentrations. The curves shown represent best fits of the data to a minimal reaction model, yielding rate constants of $k_1 = 1.4 \times 10^{-3} \text{ sec}^{-1}$ and $k_2 = 11.0 \times 10^{-4} \text{ sec}^{-1}$. The catalyst shows multiple turnovers (~ 1 turnover per hour for the first 20 h in the 3 mol % reaction).

self-assembling peptides. However, the hallmark of NRPS lies in their ability based on the logic of their domain organization to instruct the formation of specific peptide sequences.¹ It remains to be seen whether the supramolecular approach described here can be further advanced toward programmed peptide synthesis by exploiting the sequence-dependent selective coiled-coil assembly recently demonstrated in the design of complex networks.⁸

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Supporting Information Available: Experimental details, biophysical characterizations, Tables S1–S2, and Figures S1–S5. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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