Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Helix-Forming αβγ-Chimeric Peptide with Catalytic Activity: A Hybrid Peptide Ligase

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Materials

Fmoc-protected (S)-β^3-Homolysine, (S)-β^3-Homoleucine, were purchased from Fluka and (R)-γ^4-Homoaspartic acid and (S)-γ^4- Homolysine and (R)-γ^4-Homoleucine from RareChemicals (Gettorf, Germany). Fmoc-Glu (OtBu)- and Fmoc-Lys (Boc)-NovaSyn®-TGA resins (0.16 mmol g-1and 0.21 mmol g-1, respectively) and 2-chlorotrityl resin were purchased from Novabiochem. Fmoc-L-amino acids, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetra ethyluronium (TBTU), and 1-hydroxybenzotriazole (HOBt) were purchased from Fa. Gerhardt (Wolfhagen, Germany). The following chemicals were used as purchased: acetonitrile (HPLC gradient grade, Acros), dimethylformamide (DMF, p.a., Acros), dichloromethane (DCM, Fisher), 1- Hydroxy-7 azabenzotriazole (HOAt, Iris Biotech), N,N-diisopropylethylamine (DIEA, 98%, Acros), (2-carboxyethyl)-phosphine (TCEP, Fluka), benzyl mercaptan (Fluka), N,N diisoporopylcarbodiimide (DIC 99%, Acros), Triisopropylsilane (TIS 99%, Acros), piperidine (99% extra pure, Acros), 1,8 diazabicyclo[5.4.0]undec-7-ene (Merck), disodium hydrogenphosphate dihydrate (DBU, p.a., Merck), and sodium dihydrogenphosphate dihydrate (ultra >99%, Fluka), acetic acid (p.a. 100%, Roth), trifluoroethanol (99.8%, Acros), TFA (Uvasol, Merck). Acetic anhydride (99%, Acros) was distilled prior to use.

Peptide synthesis, purification, and characterization

All full length peptides as well as nucleophilic peptide fragment were synthesized following standard automated Fmoc solid phase synthesis using a SyroXP-I peptide synthesizer (MultiSyn Tech GmbH, Witten, Germany) on 0.05 mmol scale using HOBT/TBTU activation. All couplings were performed two times with four fold excess of amino acids and coupling reagents. Manual coupling of β- and γ-amino acids was carried out by HOAT/DIC activation. The molar excess of amino acid and coupling reagents was reduced for β- and γ-residues to two fold for the first and one fold for the second coupling. Prior to each deprotection step capping of the possibly non-acylated N-termini was carried out by treatment with 10 % acetic anhydride and 10 % DIEA in DMF (3×10 min). To determine the concentration by UV/Vis spectroscopic analysis, the peptide were N-terminally labeled with aminobenzoic acid (Abz). Finally, the resin was treated with a cleavage cocktail composed of 1 % (v/v) of water, 5 % (v/v) of triisopropylsilane (TIS) and 95 % (v/v) of trifluoroacetic acid (TFA). The peptides were precipitated by addition of ice-cold ether. Purification was carried out by RP-HPLC (Phenomenex Luna C8, 10 mm, 250

mm_21.2 mm) and the purity was confirmed by analytical HPLC (Phenomenex Luna C8, 5 mm, 250 mm_4.6 mm). To identify the products high resolution mass spectra were recorded (see Table S1) on the Agilent 6210 ESI-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA.). Peptide concentrations were determined using the absorbance of aminobenzoic acid (Abz), (λmax=320 nm at pH 7.4) attached to the N-terminus of Acid-pp, Base-pp, B3β2γ, and thioester-peptide. The concentration of cystein peptide has been checked by HPLC, according to an internal Abz-labeled reference (Gly-Abz).

Synthesis of electrophilic peptide fragment[1]

The peptide fragment was synthesized by Fmoc solid-phase strategy using the automated peptide synthesizer on the Alanine-preloaded 2-chlorotrityl (Cl-Trt) resin. The protected peptide was cleaved from the resin with acetic acid/ trifluorethanol/ DCM (1:1:8 v/v/v) for 2 hours at room temperature. To remove acetic acid, the cleavage solution was diluted three times with *n*-hexan; evaporated, redissolved in dioxane and lyophilized. The thioester peptide was obtained by adding PyBOP (3 eq), benzyl mercaptane (3 eq), and DIEA (3 eq) to the crude protected peptide. After 2 hours reaction at room temperature the solvent was removed and the residues were deprotected by a solution of 90:10:1 TFA/ TIS/ H_2O. The product was purified by reverse-phase HPLC (Phenomenex® Luna C8, 10 μM, 250 mm × 21.2 mm), solvent A: H_2O, 0.1 % (v/v) CF_3CO_2H; solvent B: CH_3CN, 0.1 % (v/v) CF_3CO_2H, And further characterized by analytical HPLC (Phenomenex® Luna C8, 5 μM, 250 mm × 4.6 mm) and ESI-TOF mass spectrometry.

Assays of the cross-catalytic efficiencies

Reaction was carried out at equimolar amount of nucleophilic and electrophilic fragment (E and N fragment = [150μM]) at 25°C in 100 mM phosphate buffer, pH 7.4. The reaction performed at final concentration of 50 μM of different templates (Base-pp, B3β2γ and Base7G). Prior to each reaction nucleophilic fragment was incubated for 15 minutes in tris(2-carboxyethyl)-phosphine (TCEP) at final concentration of 250 μM to prevent disulfide bond formation. The reaction was initiated by addition of electrophilic fragment. At the indicated time interval, the 30 μl of reaction mixture was removed from the reaction vessel and diluted by 70 μl of quenching solution (2% TFA in water). The product growth was monitored by HPLC at 320 nm using a CH_3CN/H_2O gradient containing 0.1% TFA. The identities of all peaks in the chromatogram were determined

by ESI-TOF mass spectrometry (see Table S1). The obtained data represent the results of three independent runs.

The concentration-dependance experiments were carried out at equimolar amount of nucleophilic and electrophilic fragment (E and N fragments = [150μM]) at 25°C in 100mM phosphate buffer, pH 7.4. B3β2γ was added at different final concentrations of 10 μM, 30 μM, 50 μM, and 100 μM. The obtained data are the results of two independent runs.

Peptide fragment condensation in the presence of GndHCl were performed in the same way as the reaction described above, except the 100 mM phosphate buffer solution containing GndHCl was partially used to gain the final concentrations of denaturant as mentioned in the Figure 2C. The presented data are the results of two independent runs.

The competition experiments in the presence of 50 μM of applied templates were performed at equimolar amount of nucleophilic and electrophilic fragments (E and N fragments = [150 μM]) and at 25°C in 100 mM phosphate buffer, pH 7.4. Prior to addition of E-fargment in order to initiate the reaction, the reaction mixture was incubated with Acid-pp at the desired concentration for 15 minutes. The experiments were repeated three times and the data were fitted by sigmoidal function and the I50 values were reported where 50% product is formed.

Supplementary discussion

As described in the main text, the peptide bond between residues b16 and c17 in the central heptad of Acid-pp was selected as the ligation site. Therefore, these residues in Acid-pp were substituted by Ala in the electrophilic thioester fragment (E) and Cys in the nucleophilic fragment (N), respectively. This peptide bond is located on the solvent exposed face of the helix, therefore these substitutions permit the ligation without distruption of the critical interfaces in the corresponding coiled coil. In order to confirm this fact, the structure of the 1:1 mixture of B3β2γ/modified acidic peptide (Acid-AC) was compared to that of B3β2γ/Acidpp by regular and temperature-dependent CD spectroscopy. Our data illustrate the retention of conformation and stability of the fold in the equimolar mixture of B3β2γ/Acid-AC (Figure S1).

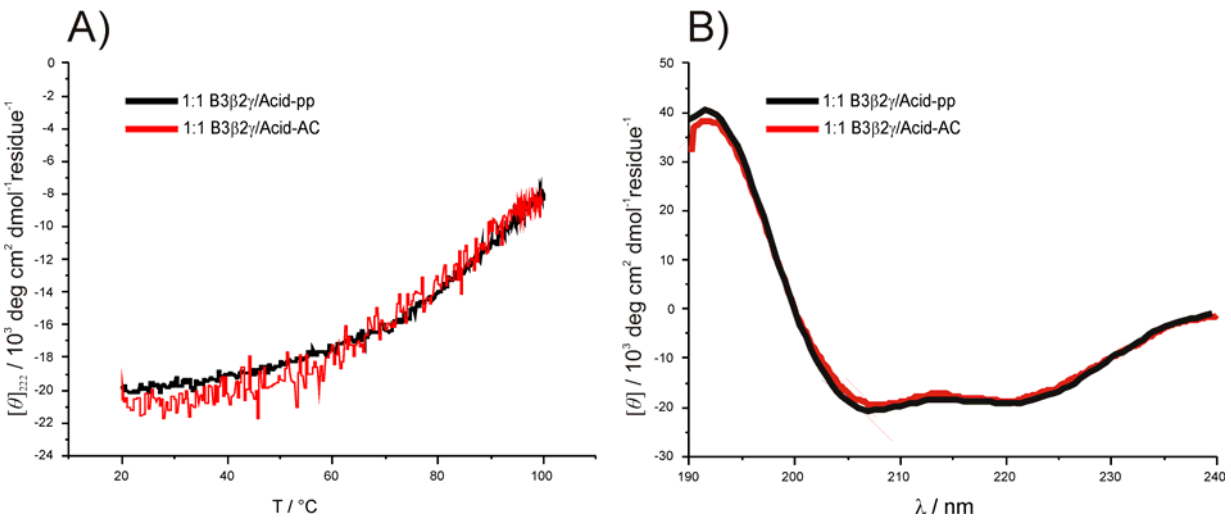

Figure. S1 (A) Thermal denaturation of the equimolar mixtures of B3β2γ with Acid-pp and Acid-AC at 20μM and pH 7.4. (B) CD spectra of the equimolar mixtures of B3β2γ with Acid-pp and Acid-AC at 20μM and pH 7.4.

Table S1. Identification of the peptides by ESI-TOF mass spectrometry.

Peptide	Calc. $[M+H]^+$	Obs. $[M+H]^+$
Acid-pp	3990.14	3990.15
Acid-AC (Product)	4006.08	4006.10
Base-pp	3985.40	3985.10
B3β2γ	3911.31	3911.38
Base-7G	3496.04	3496.10
Thioester-peptide (E)	1969.01	1969.04
Cystein-peptide (N)	2161.12	2161.18

With the exception of the Cystein-peptide (N), all the peptides contain an N terminal Abz label.

[1] Von Eggelkraut-Gottanka, R.; Klose, A.; Beck-Sickinger, A. G.; Beyermann, M. *Tetrahedron Letters* **2003**, *44*, 3551.