Supporting Information

Soluble-Support-Assisted Electrochemical Reactions: An Application to Anodic Disulfide Bond Formation

Shingo Kitada, Masahito Takahashi, Yusuke Yamaguchi, Yohei Okada, Kazuhiro Chiba*

Department of Applied Biological Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

Tel: (+81)-42-367-5667

Fax: (+81)-42-367-5667

E-mail: chiba@cc.tuat.ac.jp

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1. Additional Schemes and Figure

Scheme S1. Preparation of the hydrophobic tags (HO-TAGa and HO-TAGb) used in this work

Reagents and conditions: (a) $C_{22}H_{45}Br$, K_2CO_3 , DMI. (b) $LiAlH_4$, THF.

Scheme S2. Preparation of the model peptide (1)

Reagents and conditions: (a) (i) Fmoc-Cys(Acm)-OH, DIC, DMAP, CH_2Cl_2 ; (ii) DBU, piperidine, THF, quant. over 2 steps. (b) (i) Fmoc-Ala-OH, HBTU, HOBT, CH_2Cl_2 ; (ii) DBU, piperidine, THF. (c) Boc-Cys(Acm)-OH, HBTU, HOBT, DIPEA, CH_2Cl_2 , 93% over 3 steps.

Scheme S3. Preparation of the model peptide (3)

Reagents and conditions: (a) (i) Fmoc-Cys(Trt)-OH, DIC, DMAP, CH_2Cl_2 ; (ii) DBU, piperidine, THF. (b) (i) Fmoc-Ala-OH, HBTU, HOBT, CH_2Cl_2 ; (ii) DBU, piperidine, THF. (c) Fmoc-Cys(Trt)-OH, HBTU, HOBT, DIPEA, CH_2Cl_2 , 75% over 5 steps. (d) TFA, TIS, CH_2Cl_2 , 96%.

Fig. S1. Cyclic voltammogram of the peptide (**1**) (1.0 mM) using Et₄NBr as a supporting electrolyte (0.10 M).

2. General information

^1H and ^{13}C NMR spectra were recorded in CDCl_3 with TMS as the internal standard on JEOL alpha 600 (600 MHz). The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; sept, septet; and m, multiplet and coupling constants, J , were reported in Hz. MS spectra were recorded by TOF-ESI. TLC analysis was performed with Merck Silica Gel 60 F254 plates, and detection was achieved by UV absorption (254 nm) and by charring the plates after spraying them with 12 molybdo(VI) phosphoric acid n-hydrate in 95% ethanol and ceric ammonium sulfate in 10% aqueous H_2SO_4 . Column chromatography was performed with Silica Gel (0.04–0.063 mm). All reagents and solvents were purchased from Kanto chemical, Tokyo Chemical Industry, Sigma-Aldrich, Wako Pure Chemical Industries, and Watanabe Chemical Industries used as received.

3. Experimental information

3.1. General method for the introduction of amino acid in to the hydrophobic tag

The hydrophobic tag (1.00 mmol) was dissolved in CH_2Cl_2 (20 mL). Amino acid (1.50 mmol), DIC (1.50 mmol), and DMAP (1.2 mg, 0.0100 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction completed. After the completion, MeCN was added to the reaction mixture to give the product as a precipitate.

3.2. General method for the deprotection of Fmoc group of the tagged peptide

The tagged peptide was dissolved in 1% DBU and 1% piperidine in THF (20 mL). The reaction mixture was stirred at room temperature until the reaction completed. After the completion, 12 M HCl was added to the reaction mixture to neutralize (pH 7.0), and then MeCN was added to give the deprotected product as a precipitate.

3.3. General method for the coupling of amino acid to the tagged peptide

The tagged peptide was dissolved in CH_2Cl_2 (20 mL). Amino acid (1.2 mol equiv.), HBTU (1.2 mol equiv.), HOBT (1.2 mol equiv.), and DIPEA (2.4 mol equiv.) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction completed. After the completion, MeCN was added to the reaction mixture to give the coupled product as a precipitate.

3.4. General method for the anodic disulfide bond formation

Tetraethylammonium salt (0.10 M) was dissolved in THF/MeOH (1/1). The tagged peptide was then added to the electrolyte solution. The reaction cell was capped with a septum equipped with the platinum anode (20 mm x 20 mm), platinum cathode (20 mm x 20 mm), and the Ag/AgCl reference electrode. The electrolysis was then performed at 1.1 V vs. Ag/AgCl. After the completion, MeCN was added to the reaction mixture to give the cyclized product as a precipitate.

3.5. Acidic deprotection of all protective groups

Tagged peptide was dissolved in 2.5% TIS and 2.5% H_2O in TFA (5 mL). The reaction mixture was

stirred at room temperature until the reaction completed. After the completion, the reaction mixture was filtrated by hydrophilic PTFE filter, and then DIPE was added to the filtrate to give the product as a precipitate.

4. Spectra Information

Boc-Cys(Acm)-Ala-Cys(Acm)-O-TAGa (1)

¹H NMR (CDCl₃, 600 MHz) δ 7.44-7.34 (1H, m), 7.19 (1H, d, *J* = 8.1 Hz), 7.02 (1H, m), 6.80-6.68 (1H, m), 6.42 (2H, d, *J* = 8.1 Hz), 5.54 (1H, m), 5.22-5.09 (2H, m), 4.84-4.75 (1H, m), 4.64-4.55 (1H, m), 4.54-4.41 (2H, m), 4.35-4.17 (3H, m), 3.96-3.87 (4H, m), 3.03 (1H, dd, *J* = 13.9, 3.7 Hz), 2.90 (2H, dd, *J* = 13.9, 6.6 Hz), 2.86-2.77 (1H, m), 2.03 (3H, s), 2.00 (3H, s), 1.84-1.68 (4H, m), 1.45-1.38 (4H, m), 1.44 (12H, s), 1.37-1.19 (76H, m), 0.88 (6H, t, *J* = 6.6 Hz) ¹³C NMR (CDCl₃, 150 MHz) δ 172.0, 172.0, 171.1, 170.8, 170.7, 170.3, 161.1, 158.4, 131.4, 115.5, 104.6, 99.7, 80.3, 68.2, 63.4, 53.3, 53.1, 49.3, 42.3, 40.8, 33.9, 33.5, 31.9, 30.3, 29.7, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 29.3, 29.1, 28.3, 26.0, 26.0, 23.3, 23.2, 22.7, 17.5, 14.1 HRMS calc. for C₇₁H₁₂₉N₅O₁₀S₂ 1275.9181 (M+Na 1298.9079), found 1298.9074

Tagged cyclic peptide (2)

¹H NMR (CDCl₃, 600 MHz) δ 7.44 (1H, s), 7.18 (1H, d, *J* = 8.1 Hz), 6.46 (1H, s), 6.41 (2H, d, *J* = 8.1 Hz), 5.52 (1H, s), 5.25-5.09 (2H, m), 4.82 (1H, s), 4.30 (1H, s), 3.93 (4H, m), 3.47-2.75 (4H, m), 1.83-1.71 (4H, m), 1.67 (1H, s), 1.44 (12H, s), 1.52-1.01 (80H, m), 0.88 (6H, t, *J* = 6.6 Hz) ¹³C NMR (CDCl₃, 150 MHz) δ 171.0, 169.4, 161.2, 158.7, 154.8, 131.7, 115.5, 104.5, 99.7, 68.2, 68.1, 63.5, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.4, 29.3, 29.2, 28.3, 26.0, 22.7, 14.1 HRMS calc. for C₆₅H₁₁₇N₃O₈S₂ 1131.8282 (M+Na 1154.8180), found 1154.8177

Fmoc-Cys-Ala-Cys-O-TAGb (3)

¹H NMR (CDCl₃, 600 MHz) δ 7.75 (2H, d, *J* = 7.6 Hz), 7.58 (2H, d, *J* = 6.9 Hz), 7.39 (2H, t, *J* = 7.6 Hz), 7.31 (2H, d, *J* = 7.6 Hz), 7.01 (1H, s), 6.80 (1H, s), 6.45-6.42 (2H, m), 6.40-6.38 (1H, m), 5.76 (1H, s), 5.13 (1H, d, *J* = 12.4 Hz), 5.05 (1H, d, *J* = 12.4 Hz), 4.83-4.89 (1H, m), 4.60-4.33 (4H, m), 4.21 (1H, t, *J* = 6.9 Hz), 3.90 (4H, t, *J* = 6.2 Hz), 3.00 (3H, s), 2.75 (1H, s), 1.77-1.71 (4H, m), 1.64 (2H, s), 1.45-1.38 (7H, m), 1.35-1.19 (72H, m), 0.87 (6H, t, *J* = 6.9 Hz) ¹³C NMR (CDCl₃, 150 MHz) δ 172.3, 170.5, 170.4, 144.5, 144.4, 142.2, 137.6, 128.6, 127.9, 125.8, 120.9, 107.4, 102.2, 69.0, 68.6, 57.0, 54.6, 50.2, 47.9, 32.7, 30.5, 30.4, 30.4, 30.2, 30.1, 30.0, 27.8, 27.5, 26.9, 26.9, 23.5, 18.8, 14.9 HRMS calc. for C₇₅H₁₂₁N₃O₈S₂ 1255.8595 (M+Na 1278.8493), found 1278.8492

Tagged cyclic peptide (4)

¹H NMR (CDCl₃, 600 MHz) δ 7.82-7.66 (2H, m), 7.66-7.44 (3H, m), 7.44-7.32 (2H, m), 7.32-7.27 (2H, m), 7.23-6.94 (1H, m), 6.60-6.26 (3H, m), 6.10-5.59 (1H, m), 5.49-5.01 (2H, m), 5.01-4.61 (2H, m), 4.61-4.09 (4H, m), 4.09-3.71 (4H, m), 3.71-3.18 (2H, m), 3.18-2.53 (2H, m), 2.11-1.59 (4H, m), 1.55-1.02 (79H, m), 0.88 (6H, t, *J* = 6.9 Hz) ¹³C NMR (CDCl₃, 150 MHz) δ 171.3, 170.2, 169.3, 160.6, 156.6, 155.5, 143.7, 141.4, 136.9, 136.7, 131.1, 127.9, 127.2, 125.2, 120.2, 106.8, 106.7,

1 101.5, 101.3, 70.0, 68.6, 68.3, 68.2, 68.0, 53.7, 52.6, 47.2, 45.9, 32.1, 29.9, 29.2, 26.2, 22.9, 14.3,
2 14.1 HRMS calc. for $C_{75}H_{119}N_3O_8S_2$ 1253.8439 (M+Na 1276.8336), found 1276.8335
3
4 Tagged linear peptide (**5**)
5 HRMS calc. for $C_{184}H_{280}N_{20}O_{31}S_2$ 3329.9661 (M+2+Na 3355.0346), found 3354.9626
6
7 Tagged cyclic peptide (**6**)
8 HRMS calc. for $C_{178}H_{268}N_{18}O_{29}S_2$ 3185.9482 (M+2+Na 3210.9447), found 3210.8705
9
10 Somatostatin (**7**)
11 HRMS calc. for $C_{76}H_{104}N_{18}O_{19}S_2$ 1636.7167 (M+H 1637.7245), found 1637.6945
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