Electronic Supplementary Information

Stimuli-Responsive Conformational Transformation of Antimicrobial Peptides Stapled by Azobenzene Unit

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Materials. Trifluoroacetic acid (TFA), trifluoroethanol (TFE), ninhydrin, piperidine, tris(2-carboxyethyl)phosphine (TCEP), and acetic anhydride were obtained from Aldrich and used as received. N,N′-dimethylformamide (DMF), triisopropylsilane (TIS), and HPLC solvents were purchased from Acros Organics. Diisopropylethyl amine (DIPEA) and 3,6-dioxa-1,8-octanedithiol were purchased from TCI. Rink Amide MBHA resins were purchased from Advanced Chem. Tech. O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU), N-α-Fmoc protected amino acids and 1-hydroxybenzotriazole (HOBt) were obtained from Novabiochem and were used as received.

Peptide synthesis. All peptides were synthesized using Fmoc-chemistry in solid-phase peptide synthesis.[1] Fmoc-protected amino acids were assembled on Rink Amide MBHA resin. The coupling reaction for each amino acid was performed using a 3-molar excess of the corresponding Fmoc amino acid and coupling reagents in DMF. HBTU (0.3 mmol), HOBt (0.3 mmol), DIPEA (0.6 mmol), and Fmoc-protected amino acid (0.3 mmol) in DMF (3 mL) were added to Rink amide resin (200 mg. 0.1 mmol), and the resulting solution was stirred for 2 h at room temperature. After filtration, the resin was washed three times with DMF (3 mL) and methanol (3 mL), respectively. The coupling reaction was repeated until no color change was observed in the ninhydrin test. The Fmoc protection group on the resin was removed by addition of 25% piperidine in DMF. After a 15 min stirring, the resin was washed three times with DMF (3 mL) and methanol (3 mL), respectively. After completion of the solid-phase synthesis, the peptide was deprotected and cleaved from the resin by treatment with a mixture of TFA/TIS/3,6-dioxa-1,8-octane-dithiol/H_2O (94:1:2.5:2.5, v/v) at room temperature for 4 h. After cleavage of the peptide from the resin, the resin was filtered, and excess of TFA was removed in solution. The peptides were then obtained by precipitation into cold diethylether at -20 ºC. The crude peptide was purified by reverse-phase HPLC (YL9100, Yonung Lin Instrumnets, Korea) using a C18 column (Sunfire C18, 4.6 × 150 mm) as the stationary phase and buffer A (water with 0.1%, v/v TFA) and buffer B (acetonitrile with 0.1%, v/v TFA) as the mobile phase. The gradient conditions of the mobile phase included 5 min at 100% A followed by a linear gradient of 0−100% B over 50 min. After freeze drying of the collected fraction, a white solid of peptide was obtained. Successful synthesis of KLA-4-Cys and KLA-15-Cys was confirmed by HPLC and mass spectrometry as shown in Fig. 2a and 2b, respectively. KLA-4-Cys: m/z calculated for $[M+H]^+$ 1514.93, found 1514.86, m/z calculated for $[M+2H]^{2+}$ 757.97, found 757.93. KLA-15-Cys: m/z calculated for $[M+2H]^{2+}$ 886.07, found 886.16, m/z calculated for $[M+3H]^{3+}$ 591.04, found 591.11.

Synthesis of Azo-I. An azobenzene derivative with two iodide units (Azo-I) was synthesized using reported procedure.[2]

Peptide stapling with azobenzene unit. The peptide stapling with azobenzene unit was performed based on the reported procedure.[2] A DMF solution (7.9 mL) of Azo-I (6 μmol) was slowly added using syringe pump (0.8 mL/hr) into the solution of KLA-4-Cys or KLA-15-Cys (4 μmol) with TCEP (12 μmol) in Tris buffer (10 mM, pH 7.5, 7.9 mL). The crude peptides were purified by reverse-phase HPLC (YL9100, Yonung Lin Instrumnets, Korea) using a C18 column (Sunfire C18, 4.6 × 150 mm) as the stationary phase and buffer A (water with 0.1%, v/v TFA) and buffer B (acetonitrile with 0.1%, v/v TFA) as the mobile phase. The gradient conditions of the

mobile phase included 5 min at 100% A followed by a linear gradient of 0−100% B over 50 min. After freeze drying of the collected fraction, KLA-4-Azo and KLA-15-Azo were obtained. Successful synthesis of peptides was confirmed by HPLC and mass spectrometry as shown in Fig. 2c and 2d, respectively. KLA-4-Azo: m/z calculated for $[M+2H]^{2+}$ 904.02, found 904.16, m/z calculated for $[M+3H]^{3+}$ 603.01, found 603.10. KLA-15-Azo: m/z calculated for $[M+H]^{+}$ 2064.22, found 2064.21, m/z calculated for $[M+2H]^{2+}$ 1032.62, found 1032.61.

Circular dichroism. CD spectra were recorded on a J-815 spectropolarimeter (Jasco, Tokyo, Japan) using a quartz cell with a 1 mm path length between 200 and 250 nm at room temperature. The concentration of peptide was 40 μM in in TFE/PBS (10 mM, pH 7.4) mixed solvent (1:1, v/v). Three scans with a scan speed of 50 nm/min were averaged for each measurement. CD spectra were expressed as the mean residue ellipticity. The percent helicity was calculated from the mean residue ellipticity at 222 nm using the following equation:

$$\%\text{helicity} = [\theta]_{222} / [\theta]_{max}$$
$$[\theta]_{max} = -40{,}000 \times (1 - 4 / n)$$

where $[\theta]_{222}$ is the mean residue ellipticity, $[\theta]_{max}$ is the maximal mean residue ellipticity, and n is the number of amino acids.[3] UV irradiation was performed using handheld UV lamp (4 W) with 365 nm wavelength. After 5 min of UV irradiation, CD spectra were obtained. CD spectra with $Na_2S_2O_4$ were obtained after 5 min of $Na_2S_2O_4$ addition (10 eq., 400 μM).

UV/vis spectra measurements. UV/vis spectrum measurements were performed using an Agilent 8453E UV/vis spectrophotometer in a quartz cuvette with a path length of 1 cm. UV irradiation was performed using handheld UV lamp (4 W) with 365 nm wavelength. Immediately after UV irradiation, UV/vis spectra were obtained.

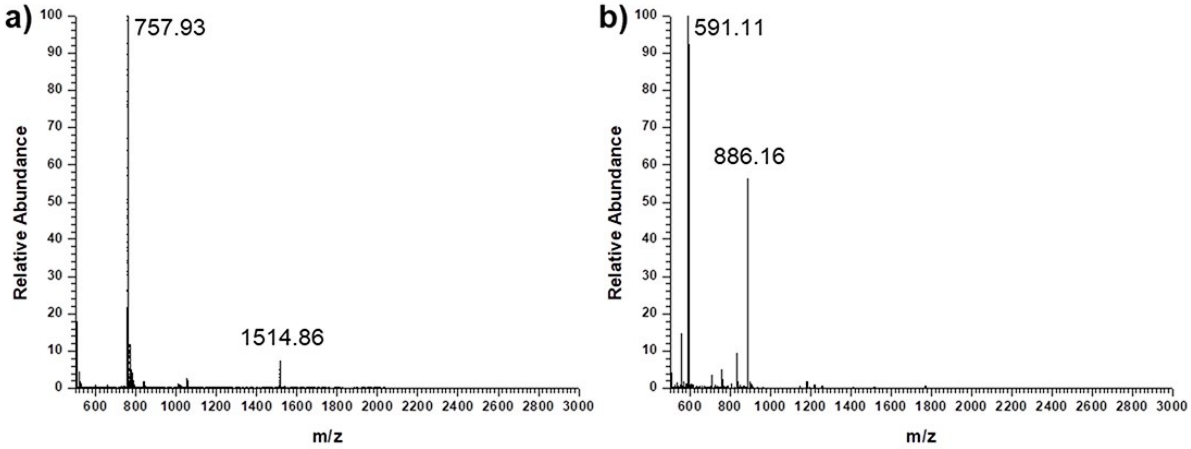

Fig. S1. Mass spectra of KLA-4-Cys (a) and KLA-15-Cys.

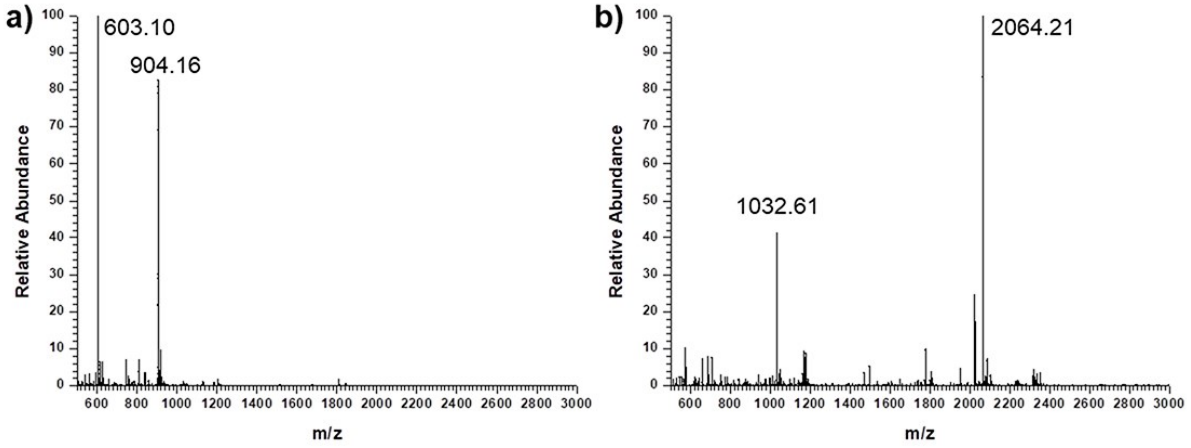

Fig. S2. Mass spectra of KLA-4-Azo (a) and KLA-15-Azo.

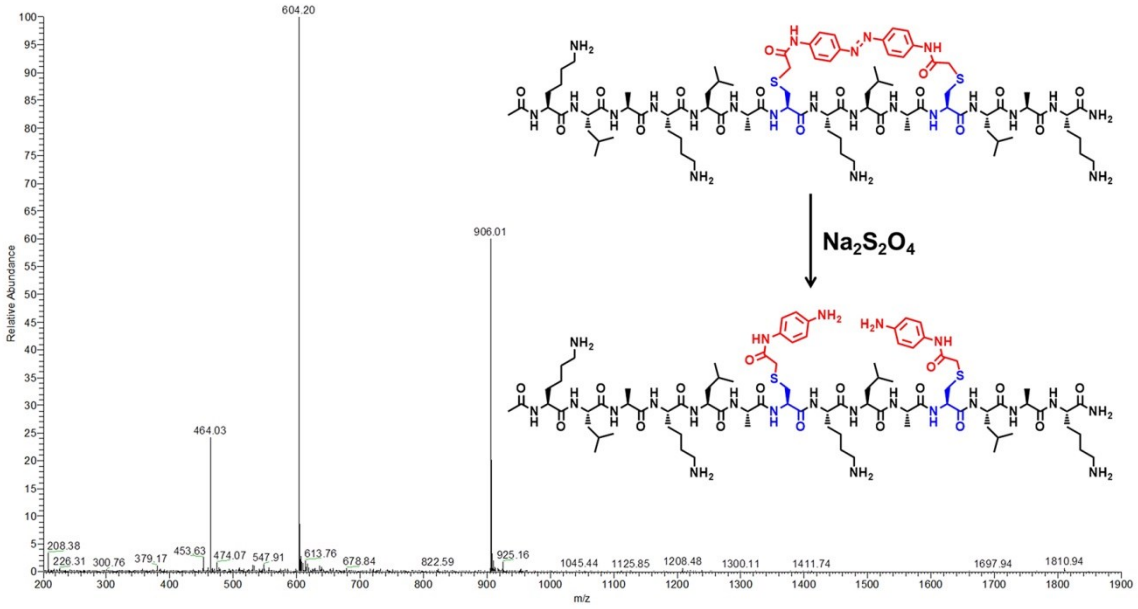

Fig. S3. Mass spectra of KLA-4-Azo after addition of $Na_2S_2O_4$. m/z calculated for $[M+2H]^{2+}$ 906.03, found 906.01, m/z calculated for $[M+3H]^{3+}$ 604.36, found 604.20.

References

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