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FULL PAPER

Rapid photolysis-mediated folding of disulfide-rich peptides

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Abstract: Structure-activity relationship studies are a highly time-consuming aspect of peptide-based drug development, particularly in the assembly of disulfide-rich peptides which often requires multiple synthetic steps and purifications. Therefore, it is vital to develop rapid and efficient chemical methods to readily access the desired peptides. We have developed a photolysis-mediated "one-pot" strategy for regioselective disulfide bond formation. The new pairing system utilises two *ortho*-nitroveratryl protected cysteines to generate two disulfide bridges in less than one hour in good yield. This strategy was applied to the synthesis of complex disulfide-rich peptides such as Rho-conotoxin ρ-TIA and native human insulin.

Introduction

Peptide drugs continue to be attractive targets for developing new therapeutic agents due to their high potency, target selectivity and low toxicity.[1] Disulfide-rich peptides such as insulin-like peptides, cyclotides, conotoxins and defensins are excellent leads for the development of next-generation therapeutics due to their specific physiological roles.[2] Many *in vivo* stability studies also show that disulfide-rich peptides are more stable than linear peptides and are thus better candidates for developing new mimetics.[3] However, generating a library of such peptides for structure-activity relationship (SAR) studies in order to investigate their therapeutic potential is always challenging.[4] Libraries can be achieved biochemically using cellular and/or recombinant protein expression[5] or can be obtained via solid phase peptide synthesis[6, 7] followed by either oxidative folding[7] or regioselective disulfide bond formation.[8] However, both these strategies are highly time consuming and consequently, slow the synthesis of new analogues and the screening process. This limitation has been partially addressed in peptide chemistry by applying a combinatorial chemistry approach,[6] using microwave-assisted synthesis[8] or more recently, ultra-fast flow peptide synthesis that can generate linear peptide libraries rapidly.[7-10]

Cyclization via oxidative folding is often used because of its simplicity but is often the rate-limiting step during production due to slow kinetics during folding to the most thermodynamically stable isomer. In addition, point mutations and other modifications can sometimes result in mis-folded peptides.[7] Consequently, regioselective disulfide bond formation is utilized to ensure the integrity of the cystine connectivity. However, this approach is even more time consuming than oxidative folding since multiple synthetic steps and purifications are required. To address this, several strategies have been developed whereby disulfide bridges are formed sequentially in a "one-pot" fashion[11] whereby a pair of free cysteine residues are in combination with enzyme-cleavable[11a] or oxidatively-cleavable protecting groups.[7b,11b-c] These strategies typically lead to the correctly folded peptide after 2-3 hours although the enzymolysis reaction can take 1-3 days. Note that in the case of the DMSO-mediated oxidative cleavage, sensitive residues such as Tryptophan (Trp) are susceptible to oxidation.[12] In addition to these strategies, "on-resin" oxidation has been achieved prior to acidolytic global deprotection and cleavage of the peptide from the solid support.[13] However, the formation of disulfide bridges can be low yielding due to steric hindrance, "on-resin" hydrophobicity and disulfide shuffling during oxidation and/or cleavage from the resin.[13c] In the case of 3 disulfide bridges or more, a semi-regioselective strategy can be applied whereby one cysteine pair is orthogonally protected whilst the remainder are randomly oxidized.[14] Despite the availability of all these strategies, there is still a need for a fully regioselective methodology to rapidly obtain libraries of disulfide-rich peptides.

Recently, cysteine S-protected with the photocleavable protecting group *ortho*-nitroveratryl (Cys(*o*Nv)) was developed and applied to the formation of a disulfide bridge in combination with S-pyridinesulfenyl protection (Cys(SPy)).[15] This heterogeneous pairing strategy requires the preliminary transformation of a S-*tert.*-butyl cysteinyl residue (Cys(*t*Bu)) into Cys(SPy) which necessitates an additional synthetic step prior to photolysis. Nevertheless, the yields obtained via this strategy encouraged us to exploit the use of two Cys(*o*Nv) residues as an orthogonal pairing system for regioselective cystine formation.[16] Consequently, we have developed a method for the "one-pot" formation of two disulfide bridges to the enable rapid synthesis of several disulfide rich peptides including native human insulin for early stage structure-activity relationship studies.

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Electronic Supplementary Information (ESI) available: Complete synthetic details and compound characterisation.

Results and Discussion

Optimization of the photolysis-mediated oxidation

Peptides bearing two Cys(*o*Nv) residues were assembled via conventional 9-fluorenylmethyloxycarbonyl solid-phase peptide synthesis (Fmoc SPPS) in which Fmoc-Cys(*o*Nv)-OH was activated with DIC/HOBt to minimize epimerization.[15] Our study of the photolysis-mediated folding began with the synthesis of oxytocin (**1**), from the corresponding protected linear analogue **1a** (Figure 1A), to determine the reaction kinetics during photoirradiation/oxidation and the best scavengers for the 2-nitrosobenzaldehyde adduct that is formed.[17] Parameters such as pH and time were also evaluated in the pilot study consisting of the photolysis-mediated oxidation of 200 μg of **1a**, while the concentration of the reaction was set to 0.1mM for the all the experiments. The optimal reaction conditions for *o*Nv removal and concomitant oxidation was found to be pH 7.4 for 30 minutes at 30°C with 2,2'-dithiodipyridine (DPDS)[18] as the oxidizing reagent (0.8 eq.) and methoxyamine as the scavenger (3 eq./*o*Nv). For the UV source, two 40 W spiral lamps with a wavelength range of 300 nm to 400 nm were fixed a couple of centimeters above the glass reaction vessel.

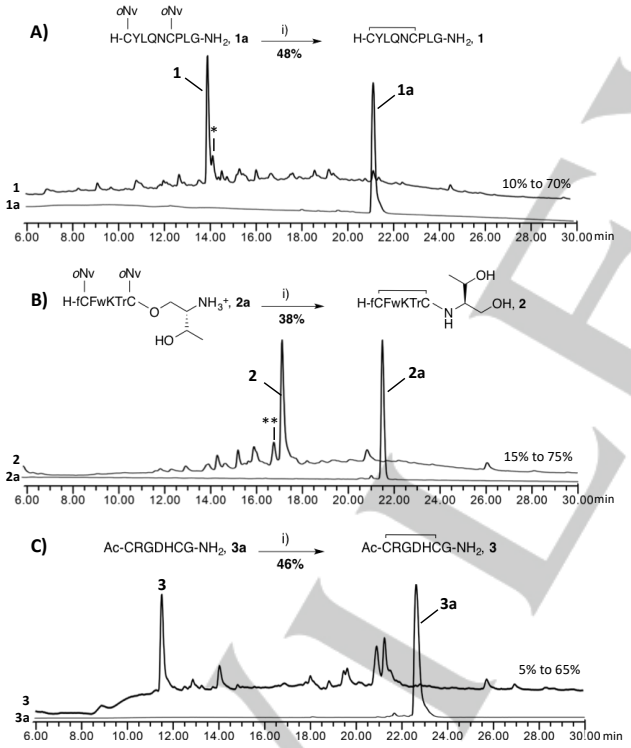

Figure 1. Photolysis-mediated oxidation study: RP-HPLC chromatograms (214nm) of starting materials **1a-3a** and final peptides **1-3**. A) Oxytocin **1a-1**; B) Octreotide **2a-2**; C) *cyclo*-[RGDH] **3a-3**. i) Photolysis of **1a** or **2a** or **3a** with DPDS, methoxyamine, PBS/acetonitrile (ACN) (70:30, v/v), pH 7.4, 30 min, 30°C. *oxytocin dimer; **octreotide with tryptophan oxidation.

Our previous results with the heterogeneous pairing system Cys(SPy)/Cys(*o*Nv)[15] found that the disulfide bond could be generated at pH 5.5; however, the purity of the photolysis-mediated oxidation with Cys(*o*Nv)/Cys(*o*Nv) was lower at this pH value. Following this optimization, the reaction was scaled up to 2 mg to calculate reaction yields and determine whether sensitive amino acids such as Tyrosine (Tyr), Tryptopan (Trp), Histidine (His) or Arginine (Arg) are susceptible to modification during photolysis. From our results with oxytocin (**1**), octreotide (**2**), prepared after O→N acyl transfer[19]) and a *de novo* designed *cyclo*-[RGDH] (**3**), side reactions such as peptide dimerization (Figure 1B, entry **b**) and Trp oxidation (Figure 1, entry **c**) were identified during the reaction (15% of **2**), although this compares favourably to the TFA/DMSO-mediated oxidative cleavage, whereby Trp oxidation is above 30%.[12] This side reaction might be due to broad wavelength UV lamp that is overlapping on the Trp absorption. The yields obtained for cystine formation *via* photolysis were 48% for **1** (+14% compared to our previous report[15]), 38% for **2** and 46% for **3** as calculated from purified **1a**, **2a** and **3a** (peptide characterization Figure SI1 to SI6). The optimization study showed that Trp was the only amino acid that showed sensitivity to photolysis. Based on these encouraging results, a "one-pot" strategy was devised for forming two disulfide bonds in Rho-conotoxin (ρ-TIA, **4**) and native human insulin (**5**). Both peptides provide unique challenges, namely, the formation of two disulfide bonds within a single peptide chain for **4** and within heterodimeric peptide chains for **5**.

Intra-chain disulfide bond formation

Conotoxin ρ-TIA (**4**) is a selective antagonist of the α_1-adrenoreceptor,[20] and has been utilized as a control in experiments relating to cellular high-throughput encapsulation, solubilization and screening (CHESS).[21] It contains two disulfide bonds and is therefore an ideal model system for this study.

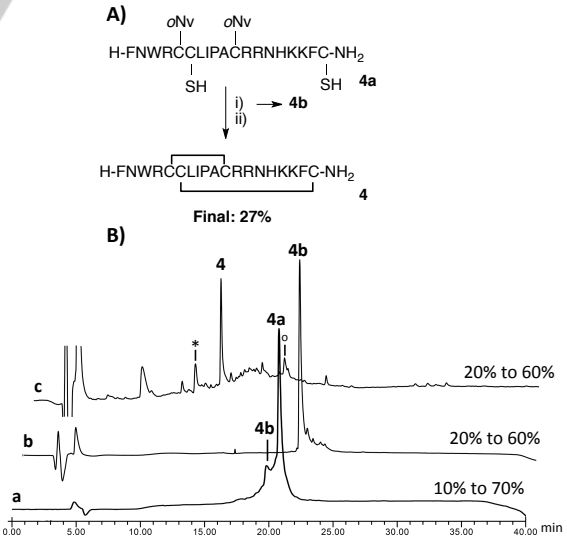

Figure 2. "One-pot" formation of two disulfide bonds in Rho-conotoxin ρ-TIA (**4**). A) Synthetic scheme of **4**, i) **4a**, methoxyamine, PBS/ACN (70:30, v/v) buffer (pH 7.4); then DPDS in acetonitrile, 15 minutes, RT leading to **4b**; ii) DPDS in methanol; then photolysis of crude **4b**, 30 minutes, 30°C. B) RP-HPLC chromatograms (ACN gradient) at 214nm. Entry (a) crude linear peptide; (b) first oxidation leading to **4b**; entry (c) second oxidation through photolysis leading to **4**. *Tryptophan oxidation; °DPDS.

The linear peptide, bearing two Cys(*o*Nv) residues (**4a**) was assembled via Fmoc SPPS and folded in a stepwise manner as depicted in Figure 2A. The first oxidation product **4b** formed after 10 minutes with DPDS as the oxidant (0.8 eq.) at 0.1 mM (Figure 2B, entry **b**). After the addition of a second aliquot of DPDS (0.8 eq.), photolysis was initiated and the temperature maintained at 30°C to form **4** after 45 minutes. As previously observed with octreotide (**2**), a significant proportion of Trp oxidized (17% of **4**), thus reducing the overall yield to 27% (Figure 2B, entry **c**). The broad, asymmetrical peak eluting after the target peptide was attributed to either peptide aggregation and/or peptide misfolding. Despite these side reactions, the yield of the photolysis-mediated folding was still comparable to our previous report for α-conotoxin (34% over 3 steps).[15] This result demonstrates that viability of this method for producing correctly folded disulfide rich peptides rapidly (peptide characterization Figure SI7 to SI9).

Inter-chain disulfide bond formation

Native human insulin (**5**) remains a protein of major interest in terms of its synthesis,[15, 22] artificial C-peptide folding,[23] pharmacokinetics[24] and delivery.[25] The overall yield of chemically synthesized insulins analogues and other insulin-like peptides depends on its amino acid sequence. Until recently, the highest yield using a semi-regioselective method was 5.4%.[14a] We recently reported a regioselective method that utilizing Cys(*o*Nv) and photolysis that resulted in a 4.6% overall yield (calculated from the purified A-chain).[15] But these methods also require several synthetic steps and multiple purifications to obtain the final product. A time efficient and high yielding regioselective method for chemically synthesized insulins is therefore highly desirable for SAR studies, therefore, our "one-pot" strategy evaluated for its production. The two peptide chains were assembled via Fmoc SPPS, with S-*o*Nv protection at residues A7 and B7 and S-acetamidomethyl (S-Acm) protection at A6 and A11 (Figure 3A). For the A-chain (**5a**), the cysteine at A20 was transformed to Cys(SPy) during acidolytic cleavage with DPDS. In addition, an *iso*-dipeptide was incorporated to mitigate insolubility and aggregation issues in aqueous buffers during purification. The combination of the purified pre-activated A-chain (**5a**) and B-chain (**5b**) was achieved in 5 minutes in 6 M guanidine hydrochloride (Gn.HCl) at room temperature, leading to *iso*-acyl **5c** (Figure 3B, entry **a**). The reaction was then diluted with a buffer containing methoxyamine, acetonitrile and PBS at (pH 7.4, 3 eq./*o*Nv) to give a peptide concentration of 0.1 mM, which led to conversion of the *iso*-dipeptide bond to a native peptide bond *via* an O→N acyl transfer.[17b] After the addition of DPDS, the photolysis was initiated and the temperature maintained at 30°C to enable the photoirradiation/oxidation to form **5d**. Over the three steps, the "one-pot" strategy enabled the synthesis of **5d** with a yield of 12% as calculated from the B chain in 35 minutes. After the subsequent iodine oxidation, native human insulin (**5**) was obtained with a final yield of 4% as calculated from the crude A-chain, which is comparable to previously reported yields (Table 1). The biggest improvement afforded by our methodology was enabling the generation of native human insulin after just 2 synthetic steps post-assembly in 2 days from purified A- and B- chain precursors, instead of 4-5 days as reported previously (peptide characterization Figure SI10 to SI15).[14, 15, 25a]

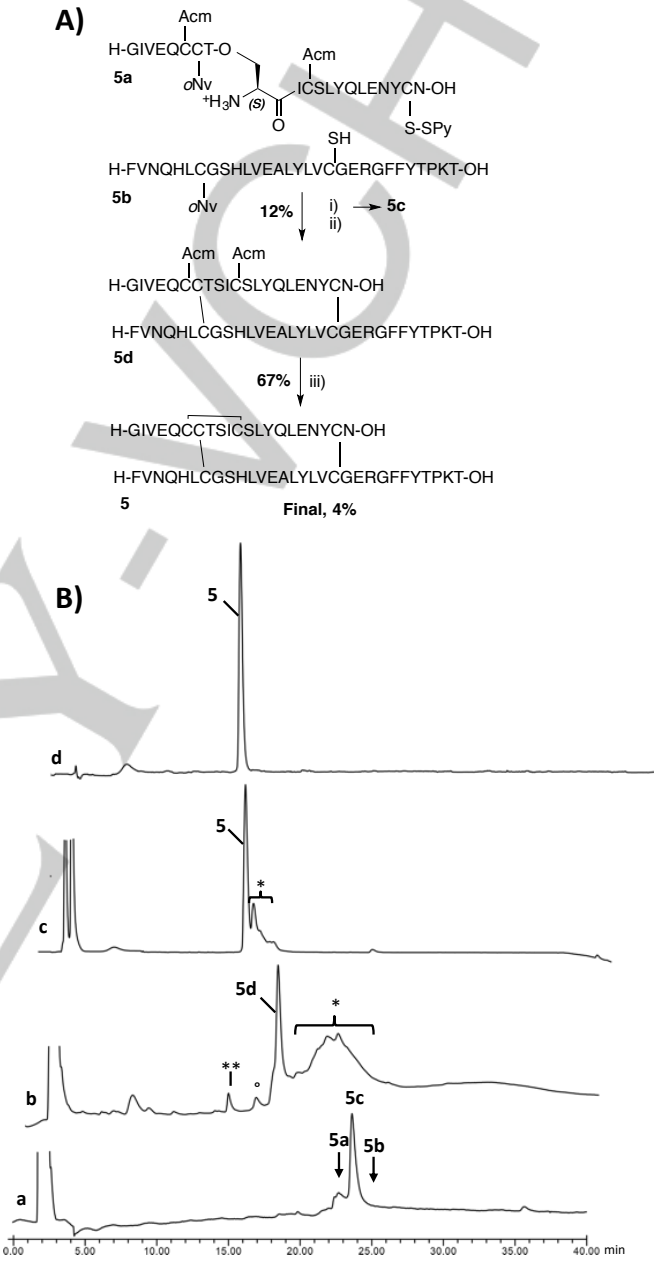

Figure 3. "One-pot" formation of two disulfide bonds in native human insulin (**5**). A) Synthetic scheme of **5**, i) Addition of purified **5a** in Gn.HCl (pH 8.5) to purified **5b** in Gn.HCl (pH 5.5), 15 min, RT; ii) Dilution with methoxyamine, PBS/ACN (70:30), pH 7.4; addition of DPDS in acetonitrile then photolysis of crude **5c**, 30 minutes, 30°C; iii) I_2/AcOH, 60 min, RT. B) RP-HPLC chromatograms (20%-60% ACN gradient in 40 minutes) of photolysis at 214nm. Entry (a) combination of **5a** and **5b** leading to **5c**; entry (b) photolysis-mediated oxidation leading to **5d**; entry (c) iodine oxidation; entry (d) native human insulin.
*Peptide aggregation and/or misfolding, ** **5a** dimer, ° DPDS.

The HPLC retention times of our chemically synthesized insulin (**5**) and commercially available recombinant insulin were compared to confirm that the correctly folded isomer was obtained (Figure SI15).

Table 1. Summary of regioselective disulfide bridge 2 chains strategies applied to native human insulin*:

Strategy for the synthesis of human insulin from 2 chains	Yield**	Reaction/ Purification Steps	Ref
Semi-regioselective (No purification between steps)	5.4%	2/1	14a
Regioselective (No purification between steps)	2.7%	3/1	14a
Regioselective (Silyl chloride-sulfoxide method)	3.8%	3/3	25a
Regioselective (Our previous work)	4.6%	5/5	15
Regioselective (This work)	4.0%	2/2	#

* Selection based on publications reporting yields
** Yield calculated from the purified A chain

Conclusions

In conclusion, an effective and time-efficient synthetic method was developed for the regioselective formation of multiple disulfide bonds in peptides. The optimized conditions were successfully applied to the rapid synthesis of peptides bearing both intra- and inter-chain cystines. As a result, conotoxin ρ-TIA with 2 disulfide bonds was prepared in less than 1 hour using our "one-pot" methodology. Additionally, our strategy was utilized for synthesizing native human insulin. This methodology will be used in tandem with our previously developed strategies such as a thioether linkage within the A-chain[23] or by tethering both the A- and B-chains via an artificial C-peptide to quickly access to insulin-like peptides.[22a,b] It represents an important addition to the available methods for producing these complex peptides thus enabling rapid SAR studies.

Experimental Section

Fmoc SPPS: Peptide chain assembly was carried out on a Gyros Protein Technologies Tribute batch-wise peptide synthesizer or a CEM Liberty™ microwave peptide synthesizer. Fmoc removal was carried out with a 20% piperidine solution in DMF. Coupling was performed *via* activation of Fmoc-protected amino acids (4 eq.) with HCTU (4 eq.) and DIEA (6 eq.). Fmoc-Cys(*o*Nv)-OH (2 eq.) was manually coupled with DIC/HOBt activation. Cleavage and global side-chain deprotection was accomplished with a solution of TFA/TIS/DODT/H_2O (96/2/1/1, v/v'/v''/v''') for 2 h at room temperature. The resin was then removed by filtration and the filtrate was concentrated under a stream of nitrogen. The peptides were precipitated, washed with ice cold Et_2O and centrifuged three times.

General photolysis-mediated assisted reaction for disulfide bond formation: The crude peptide (0.92 µmol of **1a, 2b** or **3a)** was dissolved in PBS/ACN (pH 7.4, ratio-70:30) for a final concentration of 0.1mM; then, a solution of DPDS (29 µL, 1.15 µmol) in acetonitrile was added and subjected to UV light light (40W, 300-400 nm) for 30 minutes to yield desired oxidised peptide (**1, 2 or 3).** The peptide was identified by ESI-MS as a single species and the purity (<95%) was determined by analytical RP-HPLC at 214nm (Figure SI1, SI2 and SI3, respectively)

Photolysis-mediated "one-pot" reaction for the synthesis of **4**: Crude peptide **4a** (5 mg, 1.44 µmol) was dissolved in 7.0 mL of PBS/ACN (70:30 v/v, pH 7.4) for a final concentration of 0.1mM; then, a solution of DPDS (29 µL, 1.15 µmol) was added to form the first disulfide bridge (**4b**) at room temperature. After 15 minutes, solutions of methoxyamine (86 µL, 8.6 µmol) and DPDS (29 µL , 1.15 µmol) were added and the reaction mixture was subjected to UV light (40W, 300-400 nm) for 30 minutes to yield desired peptide **4** (1.2 mg, 0.39 µmol). The peptide was identified by ESI-MS as a single species (observed mass 2390.16, theoretical = 2389.14). The purity (<95%) was determined by analytical RP-HPLC at 214nm (Figure SI4).

Photolysis-mediated "one-pot" reaction for the synthesis of **5d:** Purified peptide **5a** (5 mg, 1.63 µmol) was dissolved in 1 mL of 6 M Gn.HCl buffer (pH 8.5) and added to a solution of purified **5b** (6 mg, 1.5 µmol) in 6 M Gn.HCl buffer (1 mL, pH 5.5). The reaction was stirred for 15 minutes to form peptide **5c**. The solution was then further diluted with the addition of PBS/ACN (70:30 v/v, pH 7.4) for a final concentration of 0.1mM. Solutions of methoxyamine (98 µL , 9.8 µmol) and DPDS (30 µL, 1.2 µmol) were added and subjected to UV light (40W, 300-400 nm) for 30 minutes to yield desired peptide **5d** (1.2 mg, 0.18 µmol). The peptide was identified by MALDI-TOF MS as a single species (observed mass = 5948.99, theoretical = 5947.73). The purity (<95%) was determined via analytical RP-HPLC at 214nm (Figure SI5) and the calculated yield over the 3 steps (chain combination, O-to-N acyl transfer and the photolysis reaction) was 12%.

Synthesis of peptide **5** (native human insulin): Purified peptide **5d** (1 mg, 0.16 µmol) was dissolved in glacial acetic acid (800 µL) and HCl (60 mM, 74 µL). Iodine/acetic acid (20 mM, 620 µL) was then added to the solution, which was stirred for 40 minutes and then quenched by direct addition of ice-cold diethyl ether. The final product was purified via preparative HPLC and lyophilized to give 0.64 mg (0.11 µmol). The peptide was identified by ESI-MS as a single species (observed mass = 5806.65, theoretical = 5807.64, Figure SI6). The calculated yield for this step was 69% and the overall yield as calculated from purified A-chain was 4.0%.

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Keywords: photolysis • disulfide bridge • peptide folding • conotoxin • insulin • acyl transfer

(1) (a) Fosgerau, K.; Hoffmann, T., *Drug Discovery Today* **2015,** *20* (1), 122-128. (b) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D., *Chemical Biology & Drug Design* **2013,** *81* (1), 136-147. (c) Otvos, L.; Wade, J. D., *Front. Chem.* **2014,** *2* (62).
(2) (a) Akondi, K. B.; Muttenthaler, M.; Dutertre, S.; Kaas, Q.; Craik, D. J.; Lewis, R. J.; Alewood, P. F., *Chem. Rev.* **2014,** *114* (11), 5815-5847. (b) Craik, D. J.; Conibear, A. C., *J. Org. Chem.* **2011,** *76* (12), 4805-4817.
(3) (a) Chakraborty, K.; Thakurela, S.; Prajapati, R. S.; Indu, S.; Ali, P. S. S.; Ramakrishnan, C.; Varadarajan, R., *Biochemistry* **2005,** *44* (44), 14638-14646. (b) Bogdanowich-Knipp, S. J.; Chakrabarti, S.; Siahaan, T. J.; Williams, T. D.; Dillman, R. K., *J. Pept. Res.* **1999,** *53* (5), 530-541.
(4) (a) Shabanpoor, F.; Akhter Hossain, M.; Ryan, P. J.; Belgi, A.; Layfield, S.; Kocan, M.; Zhang, S.; Samuel, C. S.; Gundlach, A. L.; Bathgate, R. A. D.; Separovic, F.; Wade, J. D., *J. Med. Chem.* **2012,** *55* (4), 1671-1681. (b) Patil, N. A.; Hughes, R. A.; Rosengren, K. J.; Kocan, M.; Ang, S. Y.; Tailhades, J.; Separovic, F.; Summers, R. J.; Grosse, J.; Wade, J. D.; Bathgate, R. A. D.; Hossain, M. A., *J. Med. Chem.* **2016,** *59* (5), 2118-2125. (c) Hossain, M. A.; Wade, J. D., *Acc. Chem. Res.* **2017,** *50* (9), 2116-2127.
(5) Patil, N. A.; Tailhades, J.; Hughes, R. A.; Separovic, F.; Wade, J. D.; Hossain, M. A., *Int. J. Mol. Sci.* **2015,** *16* (1), 1791.
(6) (a) Peng, L.; Liu, R.; Marik, J.; Wang, X.; Takada, Y.; Lam, K. S., *Nat. Chem. Biol.* **2006,** *2*, 381. (b) Ronga, L.; Jamieson, A. G.; Beauregard, K.; Quiniou, C.; Chemtob, S.; Lubell, W. D., *Biopolymers* (*Pept. Sci.)* **2010,** *94* (2), 183-191.
(7) (a) Simon, M. D.; Heider, P. L.; Adamo, A.; Vinogradov, A. A.; Mong, S. K.; Li, X.; Berger, T.; Policarpo, R. L.; Zhang, C.; Zou, Y.; Liao, X.; Spokoyny, A. M.; Jensen, K. F.; Pentelute, B. L., *ChemBioChem* **2014,** *15* (5), 713-720. (b) Townsend, A.; Livett, B. G.; Bingham, J.-P.; Truong, H.-T.; Karas, J. A.; O'Donnell, P.; Williamson, N. A.; Purcell, A. W.; Scanlon, D., *Int. J. Pept. Res. Thera.* **2009**, 15, 195-203.
(8) (a) Tailhades, J.; Sethi, A.; Petrie, E. J.; Gooley, P. R.; Bathgate, R. A.; Wade, J. D.; Hossain, M. A., *Chem. Eur. J.* **2016,** *22* (3), 1146-1151. (b) Lin, F.; Tailhades, J.; Chan, L. J.; Bathgate, R. A.; Hossain, M. A.; Wade, J. D., *Biochemical Compounds* **2013,** *1* (1). (c) Lin, F.; Hossain, M. A.; Post, S.; Karashchuk, G.; Tatar, M.; De Meyts, P.; Wade, J. D., *Aust. J. Chem.* **2017,** *70* (2), 208-212. (d) Hossain, M. A.; Bathgate, R. A. D.; Kong, C. K.; Shabanpoor, F.; Zhang, S.; Haugaard-Jönsson, L. M.; Rosengren, K. J.; Tregear, G. W.; Wade, J. D., *ChemBioChem* **2008,** *9* (11), 1816-1822.
(9) Pedersen, S. L.; Tofteng, A. P.; Malik, L.; Jensen, K. J., *Chem. Soc. Rev.* **2012,** *41* (5), 1826-1844.
(10) Mijalis, A. J.; Thomas Iii, D. A.; Simon, M. D.; Adamo, A.; Beaumont, R.; Jensen, K. F.; Pentelute, B. L., *Nat. Chem. Biol.* **2017,** *13*, 464.
(11) (a) Góngora-Benítez, M.; Basso, A.; Bruckdorfer, T.; Royo, M.; Tulla-Puche, J.; Albericio, F., *Chem. Eur. J.* **2012,** *18* (50), 16166-16176. (b) Cuthbertson, A.; Indrevoll, B., *Org. Lett.* **2003,** *5* (16), 2955-2957. (c) Cuthbertson, A.; Indrevoll, B., *Tetrahedron Lett.* **2000,** *41* (19), 3661-3663.
(12) Otaka, A.; Koide, T.; Shide, A.; Fujii, N., *Tetrahedron Lett.* **1991,** *32* (9), 1223-1226.
(13) (a) Hargittai, B.; Barany, G., *J. Pept. Res.* **1999,** *54* (6), 468-479. (b) Postma, T. M.; Albericio, F., *Org. Lett.* **2013,** *15* (3), 616-619. (c) Galanis, A. S.; Albericio, F.; Grøtli, M., *Biopolymers* (*Pept. Sci.)* **2009,** *92* (1), 23-34.
(14) (a) Liu, F.; Luo, E. Y.; Flora, D. B.; Mayer, J. P., *Org. Lett.* **2013,** *15* (4), 960-963. b) Yang, Y.; Sweeney, W. V.; Schneider, K.; Chait, B. T.; Tam, J. P., *Protein Sci.* **1994,** *3* (8), 1267-1275.
(15) Karas, J. A.; Scanlon, D. B.; Forbes, B. E.; Vetter, I.; Lewis, R. J.; Gardiner, J.; Separovic, F.; Wade, J. D.; Hossain, M. A., *Chem. Eur. J.* **2014,** *20* (31), 9549-9552.
(16) (a) Isidro-Llobet, A.; Álvarez, M.; Albericio, F., *Chem. Rev.* **2009,** *109* (6), 2455-2504. (b) Góngora-Benítez, M.; Mendive-Tapia, L.; Ramos-Tomillero, I.; Breman, A. C.; Tulla-Puche, J.; Albericio, F., *Org. Lett.* **2012,** *14* (21), 5472-5475.
(17) Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J., *Chem. Rev.* **2013,** *113* (1), 119-191.
(18) Maruyama, K.; Nagasawa, H.; Suzuki, A., *Peptides* **1999,** *20* (7), 881-884.
(19) (a) Tailhades, J.; Gidel, M.-A.; Grossi, B.; Lécaillon, J.; Brunel, L.; Subra, G.; Martinez, J.; Amblard, M., n. *Angew. Chem. Int. Ed.* **2010,** *49* (1), 117-120. (b) Tailhades, J.; Patil, N. A.; Hossain, M. A.; Wade, J. D., *J. Pept. Sci.* **2015,** *21* (3), 139-147.
(20) Sharpe, I. A.; Gehrmann, J.; Loughnan, M. L.; Thomas, L.; Adams, D. A.; Atkins, A.; Palant, E.; Craik, D. J.; Adams, D. J.; Alewood, P. F.; Lewis, R. J., *Nat. Neurosci.* **2001,** *4*, 902.
(21) Scott, D. J.; Kummer, L.; Egloff, P.; Bathgate, R. A. D.; Plückthun, A., *BBA - Biomembranes* **2014,** *1838* (11), 2817-2824.
(22) (a) Karas, J. A.; Patil, N. A.; Tailhades, J.; Sani, M.-A.; Scanlon, D. B.; Forbes, B. E.; Gardiner, J.; Separovic, F.; Wade, J. D.; Hossain, M. A. *Angew. Chem. Int. Ed.* **2016**, *55*, 14743-14747. (b) Patil, N. A.; Tailhades, J.; Karas, J. A.; Separovic, F.; Wade, J. D.; Hossain, M. A. *Angew. Chem. Int. Ed.* 2016, *55*, 14552-14556. (c) Moroder, L.; Musiol, H.-J., *Angew. Chem. Int. Ed.* **2017,** *56* (36), 10656-10669.
(23) (a) Boross, G. N.; Shimura, S.; Besenius, M.; Tennagels, N.; Rossen, K.; Wagner, M.; Bode, J. W., *Chem. Sci.* **2018,** *9* (44), 8388-8395. (b) Thalluri, K.; Kou, B.; Gelfanov, V.; Mayer, J. P.; Liu, F.; DiMarchi, R. D., *Org. Lett.* **2017,** *19* (3), 706-709. (c) Avital-Shmilovici, M.; Mandal, K.; Gates, Z. P.; Phillips, N. B.; Weiss, M. A.; Kent, S. B. H., *J. Am. Chem. Soc.* **2013,** *135* (8), 3173-3185.
(24) Chen, M.-C.; Sonaje, K.; Chen, K.-J.; Sung, H.-W., *Biomaterials* **2011,** *32* (36), 9826-9838.
(25) (a) Akaji, K.; Fujino, K.; Tatsumi, T.; Kiso, Y., *J. Am. Chem. Soc.* **1993,** *115* (24), 11384-11392. (b) Wu, F.; Chabenne, J. R.; Gelfanov, V. M.; Mayer, J. P.; DiMarchi, R. D., *J. Pept. Sci.* **2015,** *21* (3), 223-230.

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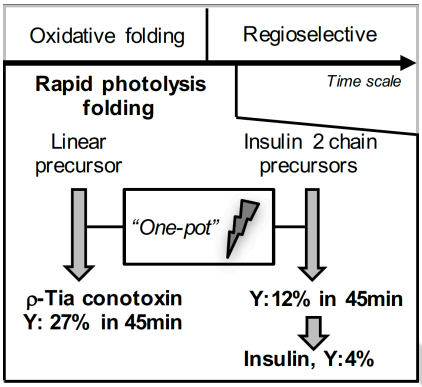

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Rapid photolysis-mediated folding of disulfide-rich peptides