Synthesis of Peptides in Parallel at Elevated Temperatures, Using the Heating Blocks for Syro Automated Parallel Peptide Synthesizers

This application demonstrates the reproducible multiple peptide synthesis on a Syro parallel peptide synthesizer equipped with Syro heating blocks. Effective heat transfer combined with efficient vortex mixing make this system a powerful tool for the synthesis of multiple peptides with increased throughput capability.

The Syro I automated peptide synthesizer (left) and a Syro heating block with heating plate (right).

Introduction

The application of heating at elevated temperatures using conventional heating methods or microwave heating is well known in solid phase peptide synthesis (SPPS) to give peptides in greater purity with reduced synthesis time.[1,2] The new Syro heating blocks for the Syro parallel peptide synthesizers are available with heating plates which can heat up to 24 reactor vials (of the same size) on one heating block simultaneously in parallel. Heating plates are available for the three different reactor vial sizes of 2 mL, 5 mL and 10 mL.

The well-known acyl carrier protein fragment, ACP (65–74), H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2 (VQAAIDYING-NH_2) **(1)** and the decapeptide derived from the MuLV CTL epitope H-Trp-Phe-Thr-Thr-Leu-Iso-Ser-Thr-Iso-Met-NH_2 (WFTTLISTIM-NH_2) **(2)** peptide were selected as the test sequences to evaluate peptide synthesis with the Syro heating blocks. The latter sequence has previously been reported by Jung and Redemann.[3] The majority of deletion peptides arise from the first nine *C*-terminal amino acids.

Here we demonstrate the parallel synthesis of these peptides at elevated temperatures on the Syro I fully automated parallel peptide synthesizer equipped with the heating block option and compare with the results obtained at room temperature.

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Experimental

Materials

All materials were obtained from commercial suppliers; Senn Chemicals (Fmoc-amino acids), Biosolve (NMP, piperidine, DIPEA and 2-propanol), Merck (trifluoroacetic acid (TFA), triisopropylsilane (TIS), and diethyl ether), Sigma-Aldrich (acetonitrile and formic acid), Iris Biotech GmbH (Fmoc-amino acids, HBTU and HOBt), and Biotage AB (Rink amide ChemMatrix® resin, 0.52 mmol/g). Milli-Q (Merck Millipore) water was used for LC-MS analysis.

N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) amino acids contained the following side-chain protecting groups: Asn(Trt), Asp(OtBu), Gln(Trt), Tyr(tBu), Trp(Boc), Thr(tBu) and Ser(tBu).

Peptide Synthesis and Analysis

The peptides were synthesized using a Syro I fully automated parallel peptide synthesizer, equipped with a heating block and 24 x 2 mL heating plate, on Rink amide ChemMatrix® resin (loading 0.52 mmol/g) in 0.025 mmol scale (2 mL reactor vial). N^{α}-Fmoc deprotection was performed at room temperature (RT) in two stages by treating the resin with 40% piperidine/DMF for 3 min followed by 20% piperidine/DMF for 12 min. The resin was then washed with DMF (6 x). The peptides were synthesized using N^{α} Fmoc amino acids (4.0 eq., 0.5 M), employing HBTU (3.9 eq., 0.48 M), HOBt (4.0 eq., 0.5 M) and DIPEA (7.8 eq., 2.0 M in NMP) in DMF and one of the following heating protocols:

1. RT, 10 min
2. RT, 40 min
3. 75 °C, 5 min
4. 75 °C, 10 min

After each coupling step, the resin was washed with DMF (3×). After the synthesis of the peptide sequence and final N^{α}-Fmoc deprotection was completed, the resin was successively washed with DMF (6×), 2-propanol (5×) and dried thoroughly. The peptides were cleaved from the solid support by treatment with TFA-TIS-H2O (95:2.5:2.5) for 30 min and with additional cleavage cocktail for 150 min. The resin was separated by filtration and the cleavage cocktail was collected. The peptide was isolated by precipitation with cold diethyl ether (3 x). Analytical HPLC was performed on a Dionex Ultimate 3000 with Chromeleon 6.80SP3 software coupled to an ESI-MS (MSQ Plus Mass Spectrometer, Thermo). The peptide was analyzed on a Biotage® Resolux™ 300 Å C18 column (5 µm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min. The following solvent system was used: solvent A, water containing 0.1% formic acid; solvent B, acetonitrile containing 0.1% formic acid. The column was eluted using a linear gradient from 5% buffer B to 40% buffer B over 5 min then from 40% buffer B to 100% buffer B over 11 min.

The ACP sequence **(1)** was synthesized and compared using coupling protocols 2 (RT, 40 min) and 3 (75 °C, 5 min,) and in 12 positions for each protocol on the reactor block as shown (Figure 1), to demonstrate the heating reproducibility across the heating block.

The Jung and Redmann (JR) decapeptide sequence **(2)** was synthesized and compared using coupling protocols 1 (RT, 10 min), 2 (RT, 40 min) and 4 (75 °C, 10 min) and in 2 positions for each protocol on the reactor block.

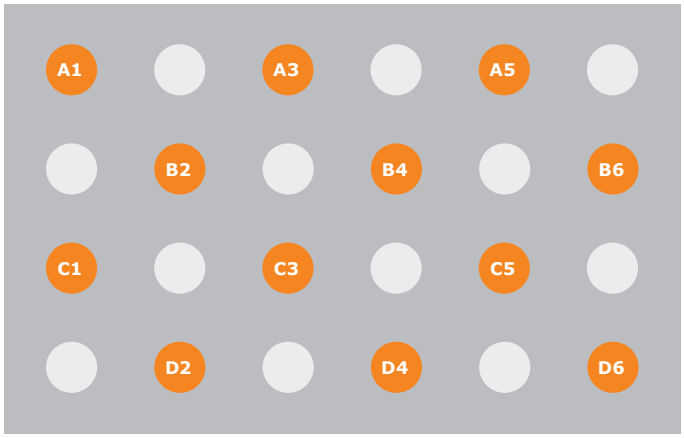

Figure 1. Reactor block positions used for synthesis comparison

Results & Discussion

The ACP Sequence (1)

The ACP sequence **(1)** was successfully synthesized using the Syro heating block with 5 min couplings at 75 °C with average crude purities of 89% (over 12 positions). A similar crude purity was achieved with a 40 min coupling time at room temperature (Figure 2).

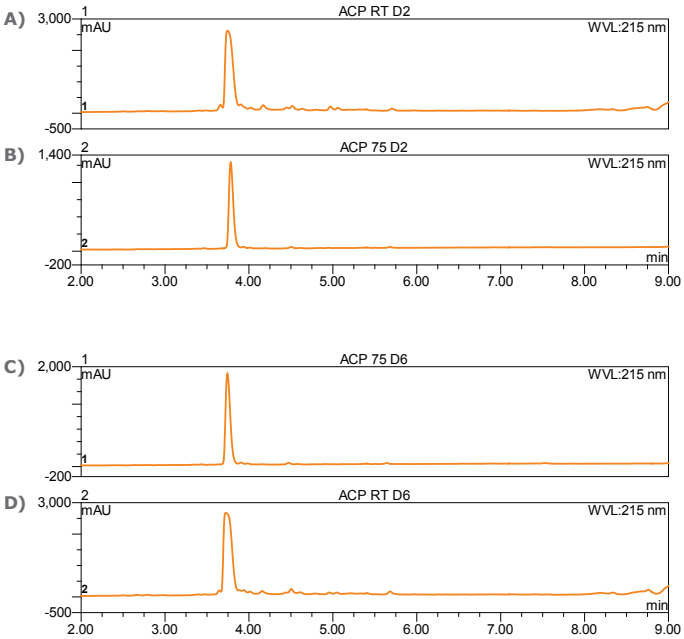

Figure 2. Representative examples of RP-HPLC chromatograms of ACP **(1)** synthesis at 75 °C (B, C) and RT (A, D) at positions D2 (A, B) and D6 (C, D).

The JR Peptide Sequence (2)

The JR peptide sequence **(2)** was successfully synthesized using the Syro heating block which enabled heating of the coupling step at 75 °C to afford the desired peptide with an average crude purity of 59% and confirmed by ESI-MS (Figure 3), calculated average isotopic composition for $C_{58}H_{90}N_{12}O_{14}S$, 1211.472 Da. Found: m/z 1211.70 $[M+H]^+$.

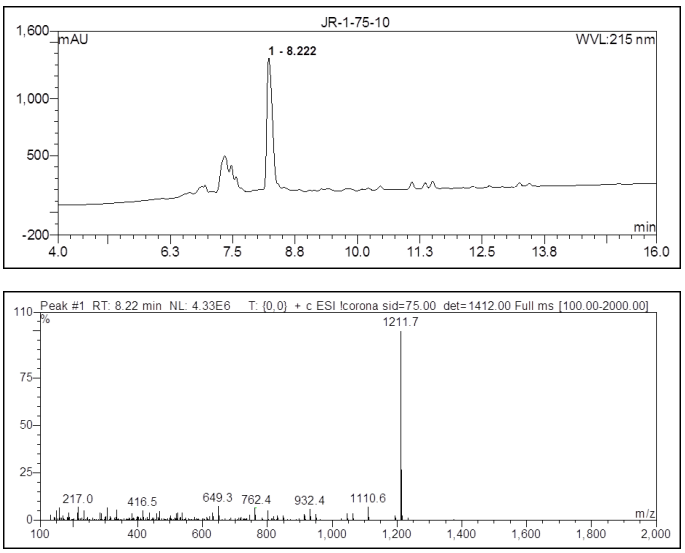

Figure 3. RP-HPLC chromatogram and ESI-MS of the synthesis of JR peptide sequence **(2)** at 75 °C.

The comparative room temperature syntheses with 10 min coupling and 40 min coupling steps afforded the desired peptide **(2)** with crude purities of approximately 40% and 55% respectively (Table 1). The crude purities of the syntheses comparing protocol 2 (RT, 40 min) and protocol 4 (75 °C, 10 min) are very similar, however, the heated synthesis achieved the results with approximately 40% reduction in total synthesis time.

Conclusion

The ACP (65–74) sequence **(1)** and JR peptide sequence **(2)**, were successfully synthesized in 25 µmol scale (2 mL reactor vials) on a Syro I fully automated parallel peptide synthesizer, equipped with the Syro heating block and a 24 x 2 mL heating plate, to afford the desired peptides in excellent crude purities and shorter synthesis time.

We have demonstrated the benefits of using conventional heating for multiple peptide synthesis by synthesizing sequences in parallel with reproducible results across the different positions on the reactor block. We have also shown that the Syro heating blocks provide effective heat transfer across the reactor block and the elevated temperatures combined with the efficient vortex mixing enable the synthesis of peptides in parallel with increased throughput capability.

The Syro parallel peptide synthesis systems are now an even more powerful tool for the synthesis of multiple peptides using the benefits of elevated temperature, namely a large reduction in total synthesis time and improved crude purity.

References

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2. Pedersen, S. L.; Tofteng, A. P.; Malik, L.; Jensen, K. J. *Chem. Soc. Rev.* **2012**, 41, 1826-1844.
3. Redemann, T.; Jung, G. *Peptides (Proceedings of the 24th European Peptide Symposium).* **1996**, 749–750.

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Scale (µmol)	Vial size (mL)	Position	Resin	Coupling time (min)	Temp (°C)	Cycle time (min)	Total synthesis time (min)	Crude purity (%)
25	2	A1	Rink amide ChemMatrix	10	RT	46	460	41
25	2	A2	Rink amide ChemMatrix	10	RT	46	460	40
25	2	A1	Rink amide ChemMatrix	40	RT	76	760	55
25	2	A2	Rink amide ChemMatrix	40	RT	76	760	56
25	2	A1	Rink amide ChemMatrix	10	75	46	460	58
25	2	A2	Rink amide ChemMatrix	10	75	46	460	60

Table 1. Summary of synthesis results of JR peptide sequence **(2)**.

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