Hydrophilic Resin Study with Next Generation Microwave SPPS

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Introduction

The properties of a resin can have a significant impact on the quality of a peptide assembled by SPPS[1]. It is known that proper solvation of the peptide-resin complex during peptide chain assembly is essential for maintaining high levels of reactivity at each step. Traditional resins employ a hydrophobic polystyrene core that is typically cross-linked with 1-2% divinylbenzene providing a good compromise of both mechanical stability and open access for facilitating chemical synthesis. However, it has been shown that use of a more hydrophilic based resin can be advantageous during SPPS particularly for longer and more difficult peptides.

Improving reaction kinetics at any specific temperature can have specific benefits during SPPS beyond facilitating a more complete reaction at each step. An easier deblocking step can reduce the level of basicity or temperature required which can reduce undesirable aspartimide formation for susceptible sequences. Similarly, a faster coupling reaction can limit the necessary lifetime of an activated amino acid thereby reducing the opportunity for epimerization or δ-lactam formation (Arginine) to occur[2].

We chose to investigate the impact of resin on peptide quality under both conventional and microwave SPPS for the $^{65\text{-}74}$ACP, Thymosin, and $^{1\text{-}42}$β-amyloid peptide sequences.

Materials and Methods

All peptides in Tables 1,2, and 3 were synthesized using a CEM Liberty system. The $^{1\text{-}42}$β-amyloid synthesis was performed on a Liberty Blue synthesizer. The conventional results were obtained without the use of microwaves for either the deprotection or coupling steps. Cleavage was performed in all cases with TFA/TIS/H_20 /DODT for 30 min at 38°C using microwave irradiation. Analysis was performed with a Waters Aquity UPLC system using a 3100 Mass Detector system. The following sequences were synthesized in this study: (1) $^{65\text{-}74}$ACP (VQAAIDYING), Thymosin (SDAAVDTSSEITTKDLKEKKEVVEEAEN), and $^{1\text{-}42}$β-amyloid (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA). The PS resin used for the $^{65\text{-}74}$ACP synthesis was 0.79 mmol/g Fmoc-Gly-Wang Resin and the PEG resin used was 0.44 mmol/g Fmoc-Gly-Wang-ChemMatrix resin. For the Thymosin synthesis the PS resin used was 0.59 Rink Amide MBHA PS resin and the PEG resin used was 0.52 mmol/g Rink Amide ChemMatrix resin. The high loading (HL) PEG Resin used was 0.89 mmol/g Rink Amide ChemMatrix resin.

Results and Discussion

The $^{65\text{-}74}$ACP sequence is a well described peptide with known aggregation tendencies. We chose to investigate the synthesis of this peptide using rapid conventional deprotection and coupling times with DIC/Oxyma using a PS and PEG based resin. The purity was substantially improved through use of the PEG based resin. This demonstrates that a hydrophilic based resin can substantially accelerate reaction rates.

Table 1. Effect of Resin on Conventional Synthesis of $^{65\text{-}74}$ACP

Resin	Deprotection		Coupling			UPLC Analysis
	Time (min)	Reagent	Reagent	Time (min)	Excess	Purity
PS Resin	5, 10	A	DIC/Oxyma	60	5	37
PEG Resin	5, 10	A	DIC/Oxyma	30	5	92

A = 20% Piperidine w/ 0.1M Oxyma

We next investigated the effect of resin selection on the synthesis of the longer Thymosin sequence. The use of a PEG resin was effective in increasing the purity of this peptide from 25% to 39% under identical synthesis conditions. Attempts to increase overall purity with larger excesses of coupling reagents were unsuccessful.

Table 2. Effect of Resin on Conventional Synthesis of Thymosin

Resin	Deprotection		Coupling			UPLC Analysis
	Time (min)	Reagent	Reagent	Time (min)	Excess	Purity
PS Resin[a]	1	A	HCTU/DIEA	2	5	<5%
PS Resin[a]	0.5, 3	A	HCTU/DIEA	5	5	25
PEG Resin[b]	0.5, 3	A	HCTU/DIEA	5	5	39
PEG Resin[b]	0.5, 3	A	HCTU/DIEA	5	10	36
PS Resin[a]	5, 10	A	DIC/Oxyma	30	5	37
PS Resin[a]	5, 10	A	HCTU/DIEA	30	5	34

[a]PS Resin = 0.60mmol/g Rink Amide MBHA PS Resin; [b]0.52mmol/g Rink Amide ChemMatrix Resin; A = 20% Piperidine w/ 0.1M Oxyma

The use of microwave irradiation with either a PS or PEG based resin led to a substantial improvement in the synthesis quality of the Thymosin peptide that was above the best case obtained from Table 2. Interestingly, the best combination was obtained through the use of microwave with a high loading PEG resin which resulted in the highest purity obtained (65%) of all synthesis attempts.

Table 3. Effect of Resin on MW Synthesis of Thymosin

Resin	Deprotection		Coupling			UPLC Analysis
	Time (min)	Reagent	Reagent	Time (min)	Excess	Purity
PS Resin	1	A	DIC/HOBt	2	5	60
PS Resin	0.5, 3	A	DIC/HOBt	5	5	58
Lysine Resin	0.5, 3	A	DIC/HOBt	5	5	60
HL PEG Resin (0.89)	0.5, 3	A	DIC/HOBt	5	5	65

A = 20% Piperidine w/ 0.1M Oxyma

An alternative resin strategy is the use of a hydrophilic spacer that can provide a hydrophilic environment for the peptide away from a hydrophobic core resin. A well-known resin that utilizes this feature is the PAL-PEG-PS resin. We investigated the effect of this resin strategy on the synthesis of the long and difficult $^{1\text{-}42}$β-amyloid synthesis, utilizing rapid microwave 1 minute deprotections and 2 minute couplings (6 minute couplings at max T = 50°C were used for histidine residues). This resin (0.19 mmol/g) provided a major improvement in the synthesis of this peptide (68% crude purity) compared to a traditional 0.33 mol/g Ala-Wang PS resin (> 5%). The downside of this strategy is that the overall substitution level is low, due to the additional molecular weight of the spacer. However, the benefits to the overall synthesis quality can far outweigh this disadvantage.

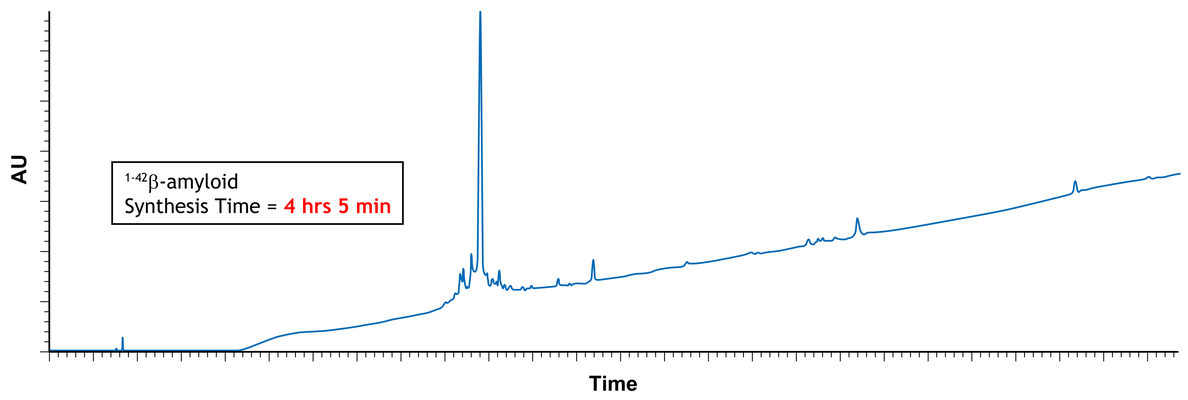

Figure 1. UPLC Profile of Crude Product from $^{1\text{-}42}$β-amyloid Synthesis on Liberty Blue

Conclusions

Use of a hydrophilic based resin can be beneficial during SPPS under both conventional and microwave synthesis conditions. Selectively increasing acylation rates can be especially beneficial when competitive side-reactions can occur such as δ-lactam formation of activated arginine. The biggest advantages were observed under conventional synthesis conditions, although improvements were also observed under microwave conditions as well. Recently, hydrophilic resins and spacers have been utilized in conjunction with microwave SPPS to synthesize 109-mer[3] and 11-mer[4] peptides.

References

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