User Bulletin No. 34

Peptide Synthesizers Fmoc MAP Resins

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SUBJECT: Using MAP Resins on Applied Biosystems Peptide Synthesizers

Introduction

Multiple antigen peptides (MAPs) have demonstrated several advantages in producing anti-peptide antibodies.[1-5] Fmoc MAP resins, now available from Applied Biosystems, are Fmoc-compatible resins connected to a small core matrix of branching lysine residues.

This User Bulletin discusses pre-synthesis considerations, synthesis selections, modifications to cleavage procedures, and post-cleavage considerations in the synthesis of MAP-peptide antigens. A summary of recommendations are provided for easy incorporation of MAPs into your laboratory.

Pre-synthesis Considerations

For automated synthesis, the recommended substitution range for MAP resins is between 0.4 - 0.6 mmol/g (total amino sites). This range minimizes potential steric hindrance of growing peptide chains for high coupling efficiencies.

In addition, the number of branches (peptide synthesis sites) can also affect the total synthesis yield of antigen. When the number of branches exceeds 8, yields drop off dramatically, especially for higher substituted resins. Optimal immunogenicity is found with the 4- and 8-branch MAPs consisting of the same peptide immunogen, compared to either the 2- or 16-branch MAP-peptide.[6]

The orientation of the peptide antigen to the MAP core matrix should mimic the protein structure. Tam[6] has reported improved antigenicity of peptide antigens of 20 or fewer residues when the dominant antigenic site is placed at the most flexible end, distal to the lysine core, of the MAP-peptide. As the dominant antigenic site approaches the proximal end, closest to the lysine core, antigenicity decreases.

Synthesis Selections

In general, a MAP-peptide with a molecular size of approximately 10,000 -11,000 daltons is desirable.[7] Use the 4-branch MAP resin for peptides containing more than 20 residues and the 8-branch MAP resin for peptides 15 residues or shorter. Immunogens containing 16 - 20 residues can be synthesized on either of the 4- or 8-branch MAP resins. Both 4-branch and 8-branch MAP resins are available from Applied Biosystems.

For Applied Biosystems Peptide Synthesizers, the *FastMoc*™ strategy at the 0.25 mmol scale for syntheses using Fmoc chemistry is recommended. No modifications to these standard cycles are necessary.

High performance liquid chromatography (HPLC) profiles have shown improved homogeneity of MAP-peptides when syntheses are performed with a decreased

amount of starting resin. This is due to the innate steric hindrance of the MAP system. We recommend using 0.10 - 0.15 mmol of starting resin with the 0.25 mmol *FastMoc*™ cycles. Figure 1 shows an improved HPLC profile of Dynorphin (1-8) synthesized on an 8-branch Fmoc MAP resin using 0.1 mmol resin with the 0.25 mmol scale *FastMoc* cycles when compared to the same peptide synthesized using 0.25 mmol of the starting resin.

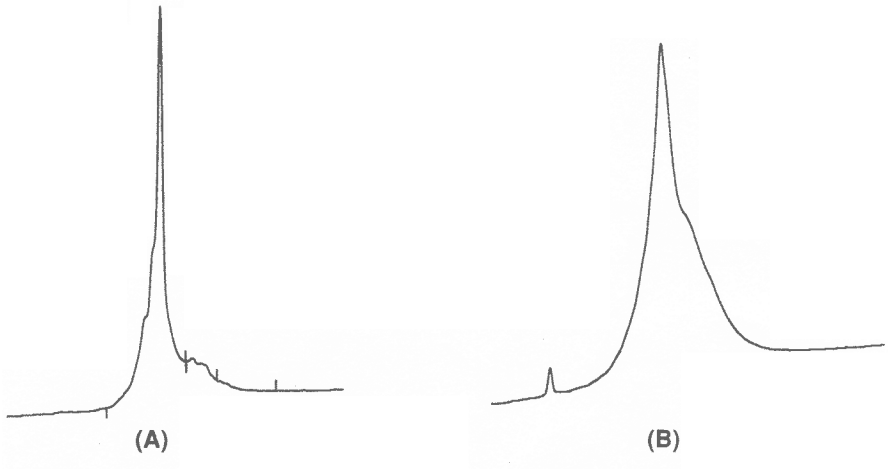

Figure 1. HPLC profiles of an 8-branched unpurified MAP-Dynorphin (1-8) synthesized using 0.25 mmol *FastMoc*™ synthesis cycles, with 0.1 mmol starting resin (A) and 0.25 mmol starting resin (B).

HPLC conditions: Instrument: Applied Biosystems *Model 130A Separation System*
Column: Brownlee Lab Aquapore™ RP-300, 7 µm*, 22 cm x 2.1 mm ID*
Mobile Phase: A: 0.1% TFA in water; B: 0.08% TFA, 100% Ch_3CN
Gradient: 0 to 60% B in 45 min; Flow rate: 0.23 ml/min; Detection: 220 nm

From the syntheses in Figure 1, higher stepwise coupling yields are observed with the lower amount of starting resin (see Table 1). When the amount of starting resin is decreased, higher concentrations of reactants help drive reactions to completion and the need to perform double couple cycles diminishes. However, it is important to remember that MAP-resin based syntheses are subject to the same type of sequence-dependent problems as conventional solid-phase peptide synthesis. These problems can be exaggerated with the multiple branches.

Table 1. Coupling efficiencies for the synthesis of Dynorphin (1-8) using 0.25 mmol *FastMoc*™ synthesis cycles, with 0.1 mmol starting resin and 0.25 mmol starting resin.

Amino Acid	Step Yields 0.1 mmol	Step Yields 0.25 mmol
Ile	98.90%	76.01%
Arg	98.96%	84.09%
Arg	98.68%	85.94%
Leu	98.42%	87.65%
Phe	98.61%	86.78%
Gly	98.73%	90.65%
Gly	98.96%	91.72%
Tyr	98.92%	86.35%

Cleavage Procedure

Peptides are cleaved using standard cleavage protocols. However, cleavage times should be extended to a total of 2 - 3 hours to ensure complete deprotection and recovery of the MAP-peptide. Peptides containing arginine residues (protected with the Pmc or Mtr group) may also require longer cleavage times. Figure 2 below demonstrates the effect of extended cleavage times beyond standard protocol on the HPLC profile of an 8-branched unpurified MAP-Dynorphin (1-8).

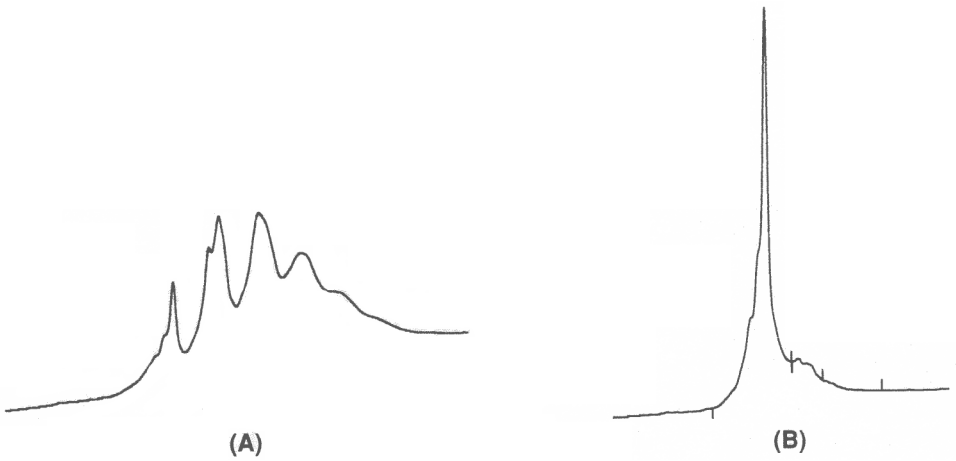

Figure 2. HPLC profiles of an 8-branch unpurified MAP-Dynorphin (1-8) from standard and extended cleavage times. (A) 8-branch Dynorphin (1-8) using 0.25 mmol FastMoc™ synthesis cycles, 0.1 mmol starting resin, and 1.5 hours cleavage time with TFA.[8] (B) Re-cleavage of (A) for a total of 2.5 hours. Same conditions as Figure 1.

Post-cleavage Considerations

The integrity of the MAP-peptide can be analyzed by reverse-phase (RP) HPLC, capillary electrophoresis, sequencing or amino acid analysis. With RP-HPLC, MAP-peptides may not behave like the linear form of the antigen. A 10-residue antigen that has been synthesized on an 8-branch MAP resin is actually composed of 88 amino acids. This MAP-peptide acts like a protein and may generate broad RP-HPLC peaks.

As with linear peptides, the solubility of MAP-peptides is dependent on the solubility characteristics of their component amino acids. When compared to linear peptides, the solubility of MAP-peptides does not appear to be affected by the presence of multiple peptide branches.

Complete homogeneity of the product does not appear to be a determining factor for antibody production. After cleavage, simple MAP-peptides can be used as immunogens without further purification. If by-products are generated in the cleavage step, then they can be removed dialysis[1] or desalted using HPLC.

Summary of Recommendations

- For best results, use 4- and 8-branch MAP resins with a substitution range of 0.4 - 0.6 mmol/g (total amino sites).
- For peptide antigens with 15 or fewer residues, use the 8-branch MAP resin and for peptide antigens over 20 residues, use the 4-branch MAP resin. For immunogens between 16 - 20 residues long, use either the 4- or 8-branch MAP resin.
- Use 0.10 - 0.15 mmol of staring resin with the 0.25 mmol scale *FastMoc*™ cycles.
- Extend cleavage time to a total of 2 - 3 hours for complete deprotection. Peptides containing arginine residues may require longer cleavage times.

Ordering Information

MAP Resins	Part Number
Fmoc MAP Resin 4-Branch	401192
Fmoc MAP Resin 8-Branch	401193

References

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