Simultaneous multiple peptide synthesis: Comparison of T-bags and cotton

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Introduction

The methodology of SPPS has evolved over recent years into several approaches of SMPS. Many techniques of this type have been reported [1]. The increasing interest in cotton [2] as support for SPPS led us to perform a detailed comparison of the peptide synthesis on this carrier with one of the alternative multiple peptide synthesis approaches. We selected the combination of MeBHA resin and T-bags of Houghten [3].

Results and Discussion

In order to compare efficiency of T-bags (containing MeBHA resin) with cotton, we synthesized a set of 50 peptides selected in such a way so as to include the well-known notorious troublemakers like ACP(65–74) and its omission analogs, adipokinetic hormone II, allatostatin I, alytesin, calcitonin, gastrin I, MSH-α, etc. In the Fmoc/Bu^t experiment, N-Fmoc-2,4-dimethoxy-4-(carboxymethyloxy)benzhydrylamine (Bachem) was used as TFA labile linker for both cotton and T-bags. We used bromophenol blue monitoring through the whole Fmoc/Bu^t experiment. With cotton, only a few strips were green to green-blue during any coupling step. With T-bags, nearly all were green to blue and only rarely white or yellow. This suggested, that couplings proceeded better on cotton than on T-bags. In an experiment using Fmoc/Bu^t strategy (Table 1) we found that the purity of peptides prepared on cotton (71%) was better than with T-bags (48%). The pattern of HPLC peaks was in most cases closely analogous for both carriers. The main by-products were

Table 1   *Comparison of T-bags and cotton*[a]

	Fmoc/Bu^t strategy		Boc/Bzl strategy
	T-bags	cotton	T-bags
Gain of weight on resin (%)	82	[b]	95
Yield of crude peptide (%)	52(60)[c]	30(38)[c]	71
HPLC purity at 222 nm	48(46)[c]	71(65)[c]	70

[a] In the Fmoc experiment, 50 peptides were prepared by both T-bag and cotton technology; in the Boc experiment, 21 peptides were prepared by T-bags.

[b] It was not possible to exactly determine these values due to mechanical losses.

[c] These values correspond to peptides which have their counterpart in the Boc experiment.

identified by FABMS and AAA as deletion peptides, peptides containing Met(O) or peptides with an incompletely split Mtr group from Arg. The yield of crude peptide was higher with T-bags (52%) than with cotton (30%). We also studied the effect of prolonged reaction time in the final deprotection step (only for peptides 33–50) on the purity of peptides. For peptides without Arg(Mtr) 1 h treatment with reagent K is sufficient. The prolongation of reaction time to 2 h gave the same results (purity 57%). Peptides with Arg(Mtr) produced 36% purity (3 h) and 46% purity (overnight). With peptides containing Arg(Mtr) it is necessary to optimize the cleavage conditions to get acceptable results.

In the experiment using Boc/Bzl strategy (Table 1), T-bags produced the best results in comparison between T-bags and cotton using Fmoc/Bu^t strategy. The yield of crude peptide was 71% for T-bags (Boc/Bzl strategy) compared with 60% (T-bags, Fmoc/Bu^t strategy) and 38% (cotton, Fmoc/Bu^t strategy). The purity of peptides prepared with T-bags (Boc/Bzl strategy) was also highest, i.e., 70% compared with 46% obtained with T-bags (Fmoc/Bu^t strategy) and 65% for cotton (Fmoc/Bu^t strategy).

The fact that T-bags using Boc/Bzl strategy produced better results in terms of yield and purity than those using Fmoc/Bu^t strategy is surprising, because in the literature in general the Fmoc/Bu^t strategy is claimed to be better. We explain this fact as follows: the published data are for individual syntheses and not for SMPS. The Fmoc/Bu^t strategy can give better results with specific attention with respect to sequence, protecting groups and deprotection used. In SMPS we use 'average' conditions for all peptides and under these conditions Boc/Bzl strategy is more reliable and less sensitive and therefore gives better average results.

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References

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