Rapid Solid-Phase Synthesis of a Calmodulin-Binding Nonapeptide using Thermal and Controlled Microwave Irradiation

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Introduction

Recently, there are a growing number of publications that report the use of microwave heating for solid-phase organic synthesis, either by using a variety of polymeric supports or applying polymer-supported resins and catalysts. Although solid-phase synthesis was originally introduced for the preparation of oligopeptides in 1963 by R.B. Merrifield [1], surprisingly, there are only a few reports so far on the use of microwave irradiation for the preparation of peptides and related species [2,3]. In fact, it is widely accepted that microwave-assisted chemical reactions can be completed in minutes, providing low by-products and higher yields [4]. Moreover, due to the recent progress in microwave technology, a new generation of the microwave oven reached the mark. By using the latest developments in microwave technology, we developed various protocols for the synthesis test peptides under microwave irradiation. The syntheses were performed without temperature control or under temperature monitoring with a fiber optic sensor under pulsed microwave irradiation with intermittent cooling to room temperature or below. Thus, all synthetic steps were performed under very mild conditions, similar to that is used in traditional solid phase peptide synthesis. After the final cleavage step, the crude peptide products were subjected to HPLC/MS analysis. As it was expected, the reaction time of each step decreased to minutes (instead of hours), and the peptides were obtained in better yields and significantly higher purity by the MAPS strategy, as compared to peptides synthesized by classical SPPS protocols.

Instrumentation

CEM Discover Single Mode Reactor

The temperature was monitored by an internal fiber optic probe.

Results

We hereinafter report the microwave-assisted solid-phase synthesis of a nonapeptide (calmodulin-binding peptide, H-Trp-Arg-The-Val-Arg-Ile-Ser-Phi-Lys-COOH) containing amine linker residues which require extensive side chain protection. The syntheses were performed under different conditions including a novel strategy based on the use of pre-cooled reaction vessels to sub-ambient temperature in combination with a pulsed microwave irradiation sequence. For temperature monitoring an internal fiber optic sensor was used. The peptides were synthesized by using standard Fmoc/TOF orthogonal protection (H-Trp(Bu)-Arg(flu)-Oxy-Val-Arg(flu)-Ile(Ser)-Phi-Lys(COOH)) on polystyrene Wang resin. Starting from Fmoc-Lys(tBu), Wang resin was pre-cooled to -10 ºC, loaded with 80% molar excess of the Fmoc-protected amino acid residue. The model nonapeptide was synthesized under five different reaction conditions. Method A: conventional SPPS protocol at room temperature; Method B: under (pulsed) microwave irradiation at room temperature; Method C: under (pulsed) microwave irradiation with intermittent heating of the reaction mixture to sub-ambient temperature (~0 ºC); and Method D under (pulsed) microwave irradiation in MicroKan reactors with intermittent cooling of the reaction mixture to sub-ambient temperature (~0 ºC). Method E: conventional SPPS protocol at elevated temperature (~65 ºC). The peptides were cleaved from the solid support with a cleavage cocktail of TFA/thioanisole/ethanedithioll/thiophenol using vigorous shaking for 3 h at ambient temperature.

Conclusions

We have applied microwave irradiation for the rapid and efficient synthesis of peptides. A test peptide was synthesized under different conditions (Methods A–E) on solid-phase by using an internal fiber optic temperature sensor. The best results were obtained by alternating short pulses of microwave irradiation of constant power with intermittent cooling of the reaction vessel to sub-ambient temperature. The application of MicroKan as microreactors for the microwave bands provided higher purity and better yields in significantly shorter time as compared with that of the conventional SPPS approach or with a standard microwave method not involving exhaustive pre-cooling of the reaction mixture. We are currently investigating the underlying effect of the pre-cooling techniques in more detail and plan to exploit this method for the synthesis of longer peptide sequences on a larger scale in the future.

References


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