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Microwave-assisted solid-phase synthesis of pseudopeptides containing reduced amide bond

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Abstract—Procedures were developed for reducing the reaction time and improving the yield of reductive alkylation in solid phase pseudopeptide synthesis by utilizing microwave irradiation. We chose dipeptides containing the reduced amide bond $\psi[CH_2NH]$ as a model system and optimized the microwave assisted reductive alkylation reaction in solid phase pseudopeptide synthesis using Fmoc chemistry. Under the optimized condition, the reductive alkylation reaction used for incorporating the reduced amide bond into the dipeptides was completed in only 8.5 min, whereas the normal reductive alkylation reaction required a total of 300 min. The purity and yield of the various dipeptides containing the reduced amide bond synthesized in this way are better than those achieved using the reductive alkylation method without microwave irradiation. We chose α helical peptides, which are known as a difficult sequence to synthesize, and incorporated the reduced amide bond by the microwave-assisted reductive alkylation reaction. We successfully synthesized pseudopeptides containing the reduced amide bond as a major product by using the novel microwave-assisted method, whereas the same products were obtained as a minor product when using the reductive alkylation method without microwave irradiation.

Solid phase synthesis has been widely applied and employed for the preparation of peptides, pseudopeptides, and various organic compounds.[1] Although the solid phase synthesis method is advantageous for the facile removal of the reagents and efficient generation of libraries, its use has been hampered by several limitations. The main problems with this method are the difficulties for the reagents to reach the active sites located in the solid phase, resulting in slow reaction and degradation of the resin under long reaction conditions, and the solubility problem of the reagent at high concentrations.[2] Pseudopeptides have received attention as the source for binding agents such as antagonists, agonists, and enzyme inhibitors for proteomics applications, because pseudopeptides have much better bioavailability than peptides and allow for the easy development of non-peptide drugs.[3] Thus, many classes of pseudopeptides have been reported.[3] However, the solid phase synthesis of pseudopeptides requires a longer reaction time and greater amount of reagents than the solid phase synthesis of peptides. To overcome the drawback of solid phase synthesis, the microwave assisted solid phase synthesis method was applied to various solid phase reactions.[4] However, this microwave method has not previously been applied to the solid phase synthesis of pseudopeptides containing a reduced amide bond. Unlike the amide bond, the reduced amide bond, $\psi[CH_2NH]$ (Fig. 1), has great resistance against enzymes, greater flexibility, no H-bonding acceptor, and is protonated under physiological conditions. In order to develop non-peptide drugs and to investigate the importance of the amide bond in their activity, many pseudopeptides containing reduced amide bonds have been synthesized.[5,6] Thus, we focused on pseudopeptides containing reduced amide bonds and developed a novel microwave irradiation procedure for the purpose of reducing the reaction time and improving the yield of reductive alkylation in solid phase synthesis using Fmoc chemistry.

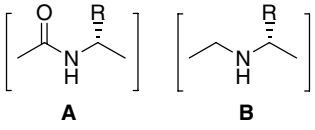

Figure 1. Representation of an amide bond (A) and a reduced amide bond (B).

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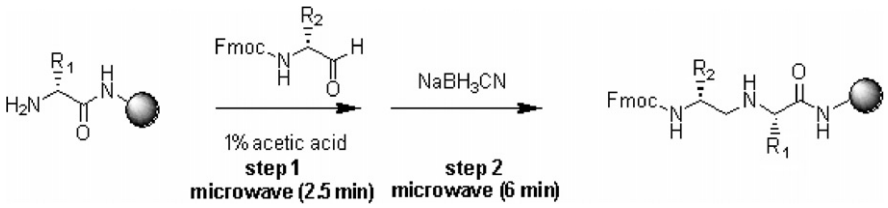
We and other research groups have synthesized several pseudopeptides containing reduced amide bonds in solid phase synthesis by using Fmoc chemistry. According to our previous result,[6] the reductive alkylation method provided a good yield for the synthesis of a model dipeptide containing a reduced amide bond, but gave a low yield for the synthesis of longer pseudopeptides (>5mer). The method required at least 5 h to obtain a considerable yield and, sometimes, the reductive alkylation reaction had to be repeated. This indicates that the synthesis of a pseudopeptide containing two or multiple reduced amide bonds required at least one day, as compared with the synthesis of a peptide of the same size. In the era of proteomics, the time required for the synthesis of the targeted pseudopeptides becomes an important issue. It would be advantageous to increase the yield and the reaction rate of the reductive alkylation in solid phase synthesis. It is well known that microwave irradiation increases the speed of many chemical reactions in solid phase synthesis. Thus, we studied the possibility of using microwave irradiation to improve the yield and to increase the reaction speed in the solid phase synthesis of pseudopeptides containing reduced amide bonds.

We chose dipeptides containing a reduced amide bond as a model system and optimized microwave irradiation procedures for the reductive alkylation reaction in solid phase synthesis, using Fmoc chemistry. Then, this microwave assisted method was applied to the synthesis of pseudopeptides (>5mer) containing a reduced amide bond. Their yield and purity were compared with those obtained by the reductive alkylation method without microwave irradiation.

All of the dipeptides containing a reduced amide bond were synthesized on Rink amide MBHA resin. The coupling reaction of the amino acids to the resin was repeated until no color change of the resin bound peptide was observed by the ninhydrin test. The method (Scheme 1) of reductive alkylation used for introducing the reduced amide bond consists of (a) the preparation of an imine by the reaction of an aldehyde with an amine in the presence of 1% acetic acid, (b) the reduction of the imine to produce the reduced amide bond accomplished by the addition of a mild reducing reagent, $NaBH_3CN$. The corresponding aldehydes were synthesized by the reduction of Fmoc protected *N*,*O*-dimethylhydroxamates with $LiAlH_4$ at 0 °C in THF and were immediately used.[7] For the purpose of comparison, the pseudopeptides were prepared using the microwave-assisted method described below, as well as by the same method without microwave irradiation. In both methods, the reaction solution was stirred by manual agitation in an N_2 atmosphere and the corresponding aldehydes were dissolved in DMF. The reaction was performed under microwave irradiation (150 W) and the temperature of the reaction did not exceed 80 °C, because the partial deprotection of the Fmoc group of the corresponding aldehyde in the presence of 1 M $NaBH_3CN$ was observed under microwave irradiation (200 W, 100 °C) for 10 min. Diglyme (5%, v/v) was added as a cosolvent in the microwave irradiation procedure because the addition of a small volume of diglyme improved the coupling yield of the reductive alkylation reaction assisted by microwave irradiation. However, the addition of a large volume of diglyme (67%, v/v) resulted in a decrease of the coupling yield of the same reaction. This may be due to the low swelling volume of the resin in diglyme solution. Under the optimized conditions,[8] the microwave assisted reductive alkylation reaction provided a considerable yield only in 8.5 min, whereas the same reaction without microwave irradiation required 5 h to obtain a similar yield (Table 1). The coupling yield of the reductive alkylation reaction was monitored by Fmoc titration[9] after the product was cleaved from the resin by treatment of TFA solution and the resulting product was analyzed by using C_{18} reverse phase HPLC and an ESI-Mass spectrometer. HPLC and ESI mass analyses of the final products indicated that both meth-



Scheme 1. Introduction of reduced amide bond into the peptide on the resin assisted by microwave irradiation.

Table 1. Coupling yield of reductive alkylation for various amino acids to the resin bound peptide

Sequence	With microwave		Without microwave	
	Time (min)	Yield (%)	Time (min)	Yield (%)
Fmoc-Lψ[CH_2NH]L-$CONH_2$	8.5	92	300	88
Fmoc-Fψ[CH_2NH]L-$CONH_2$	8.5	98	300	89
Fmoc-Sψ[CH_2NH]L-$CONH_2$	8.5	90	300	85
Fmoc-Kψ[CH_2NH]L-$CONH_2$	8.5	92	300	85
Fmoc-Qψ[CH_2NH]L-$CONH_2$	8.5	90	300	82

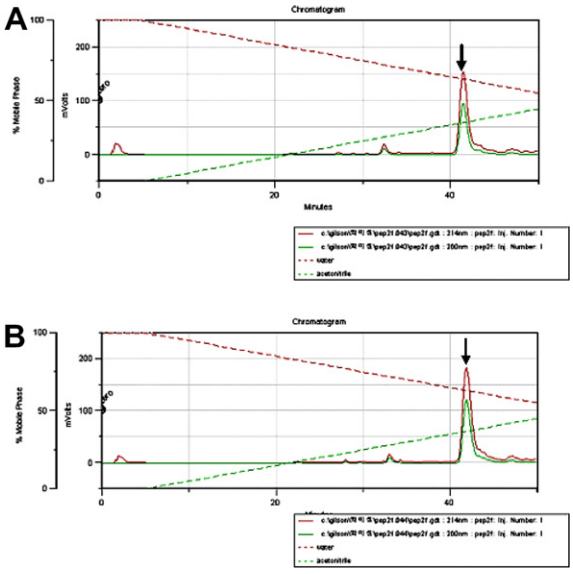


Figure 2. HPLC chromatogram of Fmoc-Phe ψ[CH_2NH]Leu-NH_2 (A) with microwave irradiation, (B) without microwave irradiation.

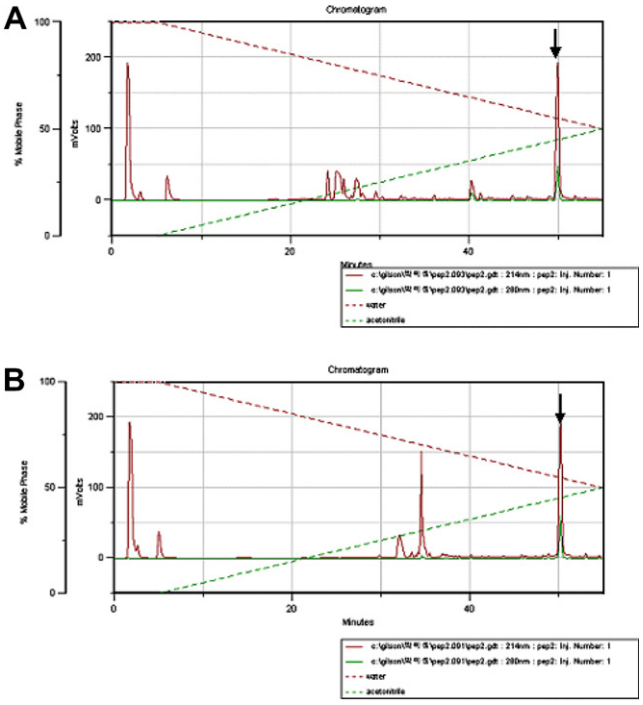


Figure 3. HPLC chromatogram of 5mer pseudopeptide Lψ[CH_2NH]-KLLK-NH_2 (A) microwave-assisted method, (B) without microwave irradiation. The arrow indicates the target pseudopeptide.

ods provided the target compound as a major product and no racemization occurred during the microwave-assisted reaction (Fig. 2, Supplementary data). Table 1 shows that the coupling yields for the various dipeptides containing the reduced amide bond synthesized in this way are better than those achieved using the reductive alkylation methods without microwave irradiation.

After optimizing the microwave-assisted procedure, we chose α helical 5mer and 12mer peptides which are known as a difficult sequence to synthesize in solid phase synthesis as model peptides[10] and synthesized the corresponding pseudopeptides containing the reduced amide bond by using the microwave irradiation procedure. We synthesized the 4mer and 11mer peptides attached to Rink amide resin in Fmoc chemistry and then introduced the reduced amide bond at the N-terminal end of the peptide-resin by microwave assisted reductive alkylation. When we introduced the reduced amide bond at the fifth amide bond position, the coupling yield achieved using the microwave assisted method was better than that obtained by the reductive alkylation method without microwave irradiation (Table 2, Fig. 3, Supplementary data). Both methods provided the target pseudopeptide as a major product with identical retention time and mass, and the purity achieved by using microwave irradiation was comparable or better than that obtained by the method without microwave irradiation, as shown in Figure 3. However, when we introduced the reduced amide bond of the Lys residue at the 11th amide bond position of the peptide attached to the resin, the coupling yield achieved using the microwave method was much better than that obtained by the method without microwave irradiation. Furthermore, the HPLC profile (Fig. 4, Supplementary data) indicated that the microwave-assisted method provided the target pseudopeptide as a major product, while the same reductive alkylation reaction without microwave irradiation provided the target pseudopeptide as a minor product.

The product corresponding to peak 1 in the HPLC chromatogram was the target pseudopeptide, while the side product corresponding to peak 2 in the HPLC chromatogram was analyzed to be LLK-KLWL-KLLK-NH_2 using a MALDI TOF mass spectrometer. We tried increasing the reaction time (12 h) of the reductive alkylation reaction without microwave irradiation, however, the coupling yield was still only about 10%. This result indicates that the microwave irradiation accelerated the reaction rate and improved the coupling yield of the reaction. When we introduced the reduced amide bond of the Leu residue at the 11th amide bond position, the coupling yield and the purity achieved using

Table 2. Coupling yield of reductive alkylation reaction for the synthesis of pseudopeptides

Sequence	With microwave		Without microwave	
	Time (min)	Yield (%)	Time (min)	Yield (%)
Lψ[CH_2NH]-KLLK-NH_2	8.5	98	300	92
Kψ[CH_2NH]LLK-KLWL-KLLK-NH_2	8.5	72	300	10
Lψ[CH_2NH]LLK-KLWL-KLLK-NH_2	8.5	80	300	10

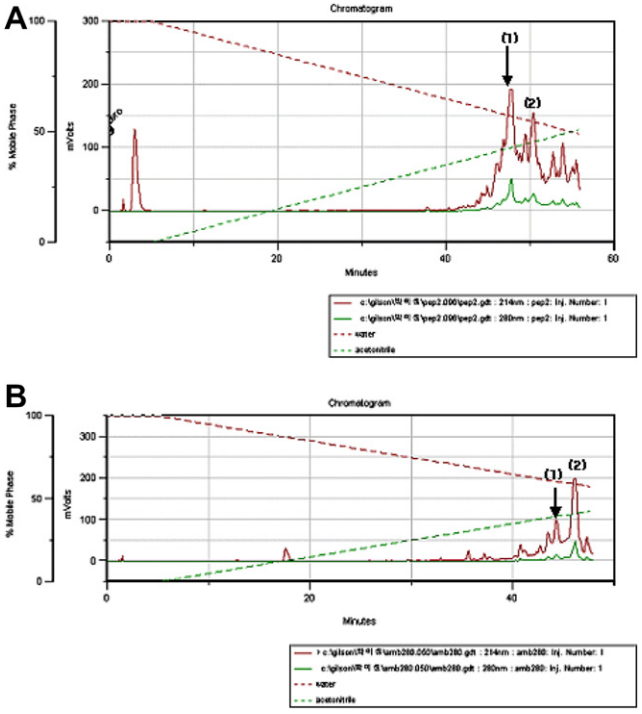


Figure 4. HPLC chromatogram of 12mer pseudopeptide Kψ[CH_2-NH]LLK-KLWL-KLLK-NH_2 (A) microwave-assisted method, (B) without microwave irradiation. The arrow indicates the target pseudopeptide.

the microwave method was much better than that obtained by the method without microwave irradiation.

We successfully developed a fast and efficient procedure to synthesize pseudopeptides containing reduced amide bonds using microwave irradiation. Our newly developed method is highly advantageous for incorporating the reduced amide bond into small dipeptides, as well as long α helical peptides. Considering the recent demand for pseudopeptides in the era of proteomics, the microwave-assisted procedure would be of great utility in the synthesis of pseudopeptides and pseudopeptide libraries.

Acknowledgments

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Supplementary data

HPLC condition and HPLC chromatograms for the pseudopeptides and mass spectra for the pseudopeptides. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.11.151.

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8. The reductive alkylation was performed by dissolving the N-protected aldehyde (4 equiv, 0.4 mmol) in DMF (1 mL) and adding the resulting aldehyde solution to a solution of the resin bounded peptide (1 equiv, 0.1 mmol) in DMF (1 mL) containing 1% acetic acid and 5% diglyme. The reaction was conducted by microwave irradiation (150 W, 80 °C, 2.5 min) generated by a Biotage AB initiator (Sweden). To the mixture, $NaBH_3CN$ (4 equiv, 0.4 mmol) in THF (20 μL) was added and the reaction was continued with microwave irradiation (150 W, 80 °C, for 6 min). After the reaction was completed, the reaction mixture was cooled by N_2 gas and the resin was washed with DMF and then MeOH. Deprotection was achieved by treatment with a mixture of trifluoroacetic acid:water:thioanisole (9:0.5:0.5, v/v/v) at room temperature for 4–5 h. The crude peptide was analyzed by HPLC with a waters C_{18} column using a water (0.1% TFA)–acetonitrile (0.1% TFA) gradient. The products were characterized by ESI mass spectrometry (Mass1200L Quadruple LC/MS system, Varian) and MALDI TOF mass spectrometry (Voyager-DE STR, Applied Biosystem).
9. We weighed the dry resin bound species (3–4 mg) and added 0.5 mL of a solution of piperidine in DMF (50%, v/v)

and shaked the resulting solution for 15 min. 20 μL of the supernatant of the solution containing the resin was diluted to 3 mL by adding DMF using a cuvette. We prepared the reference solution without the supernatant. The absorbance of the test solution at 301 nm was measured in a UV/vis spectrometer (Lambad 40, Perkin Elemer, UK) and the degree of substitution was calculated by the following equation. Calculated degree of substitution (mmol/g) = absorbance at 301 nm × volume (mL)/7800 × weight (mg).

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