Rapid Preparation of a Cerebellin Positional Scan Library

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Introduction

Peptide synthesis often suffers from long reaction times and low yields due to problems such as aggregation. Microwave energy can help break up these aggregates, improving the synthesis quality while speeding up the synthesis rate.

In order to evaluate the role of specific residues in peptide activity and binding interactions, an alanine scan library can be prepared. Alanine scanning is used to identify sites of protein-protein interactions by systematically replacing the functional groups along the peptide with a single methyl group while maintaining the same backbone confirmation.[1]

Cerebellin, a 16-mer peptide, is found in the Purkinje cells of the brain. Cerebellin has been shown to play a role in the maintenance of parallel fiber-Purkinje cell synapses.[2] It has also been shown to increase catecholamine release from the human adrenal gland.[3]

The next generation microwave peptide synthesizers incorporate significant hardware and method improvements allowing for cycle times of only 4 minutes. With these rapid methods, the synthesis of peptide libraries can be performed in an iterative fashion in a timeframe that rivals parallel synthesizers, but with superior peptide quality. Solid-phase peptide synthesis of a fourteen peptide alanine scanning library of cerebellin was performed using the Liberty Blue automated microwave peptide synthesizer.

Materials and Methods

Reagents

All Fmoc amino acids were obtained from Peptides International (Louisville, KY). 1-Hydroxybenzotriazole (HOBt) monohydrate was obtained from Advanced ChemTech (Louisville, KY). Fmoc-Rink Amide MBHA LL resin was obtained from EMD Millipore (Billerica, MA). Diisopropylcarbodiimide(DIC), piperidine, trifluoroacetic acid (TFA), triisopropylsilane (TIS), and 3,6-dioxa-1,8-octanedithiol (DODT) were obtained from Sigma Aldrich (St. Louis, MO). Dichloromethane (DCM), *N,N*-dimethylformamide (DMF), anhydrous diethyl ether, acetic acid, HPLC grade water and acetonitrile were obtained from VWR (West Chester, PA).

Peptide Synthesis

The peptides were prepared using the CEM Liberty Blue automated microwave peptide synthesizer on 0.277 g of Fmoc-Rink Amide MBHA resin (0.36 meq/g substitution). Deprotection (20% piperidine with 0.1 M HOBt in DMF) was performed for 1 minute at 90 °C. Coupling reactions were performed with 5 fold excess Fmoc-AA-OH with 1:1:1 AA/DIC/HOBt for 2 minutes at 90 °C (5 minutes at 50°C for His; 2x2 minutes at 90 °C for Arg). Cleavage was performed using 92.5:2.5:2.5:2.5 TFA/H_2O/TIS/DODT for 30 minutes at 38 °C. Following cleavage, the peptide was precipitated and washed with diethyl ether.

Liberty Blue HT

Peptide Analysis

The peptides were analyzed on a Waters Atlantis C18 column (2.1 ×150 mm) at 214 nm with a gradient of 5 – 70% MeCN (0.1% trifluoroacetic acid), 0 – 20 min. Mass analysis was performed using an LCQ Advantage ion trap mass spectrometer with electrospray ionization (Thermo Electron, Waltham, MA).

Results and Discussion

An Alanine scan library of fourteen peptides based on the neuropeptide cerebellin was synthesized at 0.1 mmol scale. All peptides were obtained at greater than 50% purity (Table 1). Each synthesis was accomplished in less than 70 minutes, and the entire library was generated in less than a day.

Table 1. Cerebellin Alanine scan

Peptide	Sequence	Purity
Cerebellin	SGSAKVAFSAIRSTNH	71%
H16A	SGSAKVAFSAIRSTN**A**	67%
N15A	SGSAKVAFSAIRST**A**H	57%
T14A	SGSAKVAFSAIRS**A**NH	68%
S13A	SGSAKVAFSAIR**A**TNH	78%
R12A	SGSAKVAFSAI**A**STNH	70%
I11A	SGSAKVAFSA**A**RSTNH	66%
S9A	SGSAKVAF**A**AIRSTNH	60%
F8A	SGSAKVA**A**SAIRSTNH	70%
V6A	SGSAK**A**AFSAIRSTNH	60%
K5A	SGSA**A**VAFSAIRSTNH	52%
S3A	SG**A**AKVAFSAIRSTNH	72%
G2A	S**A**SAKVAFSAIRSTNH	62%
S1A	**A**GSAKVAFSAIRSTNH	59%

Conclusions

The speed and efficiency of the next generation microwave peptide synthesizers is changing the way library peptide synthesis is performed. Traditionally, parallel synthesis was required to generate libraries of peptides in a timely fashion, but now with 4 minute cycle times, peptides can be generated sequentially in the same amount of time or faster than the traditional methods. In addition, preparing a library of peptides in a sequential manner provides complete control of the synthesis of each and every peptide, and the peptides are available immediately after synthesis eliminating the cleavage and purification bottlenecks typically experienced with parallel synthesizers.

References

1. Morrison K L, Weiss G A. *Combinatorial alanine-scanning*. Current Opinion in Chemical Biology. 2001; **5**: (3). 302-307.

2. Selimi F, Heintz N. *How neurons keep in touch*. Nature Neuroscience. 2005; **8**: (11). 1417-1418.

3. Mazzocchi G, Andreis PG, De Caro R, Aragona F, Gottardo L, Nussdorfer GG. *Cerebellin enhances in vitro secretory activity of human adrenal gland*. J. Clinical Endocrinology Metabolism. 1999; **84**: (2). 632-635.