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Preparation of peptides and their inhibitory activity effects against BACE1

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Introduction

β-Site amyloid precursor protein cleaving enzyme 1 (BACE1) is an enzyme targeted for drug development in Alzheimer's disease (AD) [1]. BACE1 produces amyloid β-peptides, which may contribute to the onset of AD. We examined BACE1-cleavage peptide sequences through the digestion of synthetic peptides, including part of the Swedish (SW) mutant amyloid precursor protein sequence, using recombinant BACE1 [2, 3]. The results enabled us to design substrate-based enzyme inhibitors. Several sequences were identified for BACE1-targeted drug development. In this study, we synthesized peptide-based inhibitors using predetermined sequences, and their inhibitory effects against recombinant BACE1 were determined.

Results and Discussion

The synthetic scheme and structure of peptide **1** are shown in Figure 1. The design H-Glu-Ile-Thi-Sta-Nva-OH (**1**, Thi: 3-(2-thienyl)-L-alanine, Nva: L-norvaline) was based on the previously determined sequence H-Ser-Glu-Ile-Thi-Thi ↓ Nva-Ala-Glu-Phe-Arg-OH (↓ indicates the cleavage site targeted by BACE1). Peptide **1** was prepared as a pentapeptide with positions P_4–$P_{1'}$. At the P_1 position, statine (Sta: (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid), which is a common unnatural amino acid used in protease inhibitors, was introduced. Similarly, several peptides, H-Glu-Ile-Thi-Xaa-Nva-OH, which included statine-related amino acids (Xaa) at the P_1 position, were synthesized. The SW mutant-based peptide H-Glu-Val-Asn-Sta-Asp-OH was also prepared. The peptides were synthesized by solid-phase peptide synthesis. Typically, Fmoc-amino acids were coupled to a resin in the presence of *N*,*N*'-diisopropylcarbodiimide and 1-hydroxybenzotriazole monohydrate in *N*,*N*-dimethylformamide. Piperidine was used to remove the protecting group. After elongating the whole peptide chain, cleavage from the resin was achieved using a cocktail containing trifluoroacetic acid. The resulting crude products were purified by high-performance liquid chromatography (HPLC).

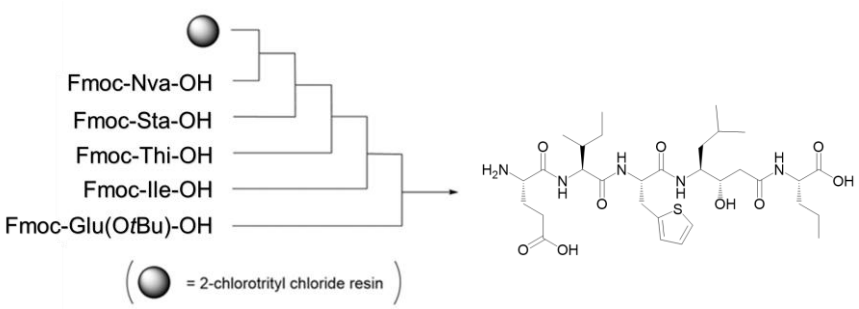


*Figure 1. Synthesis of H-Glu-Ile-Thi-Sta-Nva-OH (**1**). Synthesis was achieved based on a standard solid-phase synthesis method. Piperidine was used to remove the protecting group. Coupling reactions were conducted in the presence of N,N'-diisopropylcarbodiimide and 1-hydroxybenzotriazole monohydrate in N,N-dimethylformamide. The products were purified by high-performance liquid chromatography.*

Inhibitory activity was evaluated using previously described methods with slight modifications [2, 3]. The inhibitors were incubated at 37°C in the presence of recombinant BACE1 (R & D Systems, 931-AS-050) and a fluorescence-quenching substrate (Peptide Institute Inc., code 3212-v) in acetate buffer (pH 4.0) for 1 h. The reaction mixture was subjected to HPLC, and the cleaved fragments were identified by mass spectrometry. The peak areas from the chromatogram were used to evaluate the inhibitory activity. Peptide **1** exhibited activity against BACE1, and its inhibition was higher than that of the SW-based peptide. We subjected the (3R,4S)-derivative of statine to peptide synthesis to generate H-Glu-Ile-Thi-(3R,4S)Sta-Nva-OH. Consistent with previous findings, peptide **1,** including statine, showed higher inhibitory activity compared with the peptide that included the (3R,4S)-derivative at the P_1 position [1]. Peptides containing phenylnorstatine (Pns; (2R, 3S)-3-amino-2-hydroxy-4-phenylbutyric acid) and its stereoisomer allophenylnorstatine (Apns; (2S, 3S)-3-amino-2-hydroxy-4-phenylbutyric acid) were also synthesized. The Pns in the P_1 position showed higher efficiency compared with Apns [4]. Overall, several peptides were prepared based on the BACE1 superior-cleavage sequence, and their inhibitory activities were evaluated. Some peptides showed inhibitory activity against BACE1. A detailed evaluation of inhibitory activity is currently underway.

Acknowledgments

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