Selection of peptomeric inhibitors of bovine α-chymotrypsin and cathepsin G based on trypsin inhibitor SFTI-1 using a combinatorial chemistry approach

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Abstract A peptomeric library consisting of 360 monocyclic analogues of trypsin inhibitor SFTI-1 isolated from sunflower seeds was designed and synthesized by a solid-phase approach in order to select chymotrypsin and cathepsin G inhibitors. All peptomers contained a proteinogenic-Phe-mimicking N-benzylglycine (Nphe) at positions 5 and 12. Into the synthesized library, different peptoid monomers were introduced in the 7–10 segment. Deconvolution of the library against both proteinases through an iterative method in solution revealed that the strongest chymotrypsin inhibitory activity was displayed by two analogues, [Nphe5,12]SFTI-1 (1) and [Nphe5,12, Naem8]SFTI-1 (2), where Naem stands for N-(2-morpholinoethyl)glycine. After deconvolution against a cathepsin G analogue, [Nphe5,12, Npip8,9, Nnle10]SFTI-1 (3) (Npip = N-(3,4-methylenedioxybenzyl)glycine) appeared to be the most potent inhibitor with a high serum stability. It is worth noting that the analogues obtained by a combinatorial approach display high specificity towards one of the experimental enzymes. Another interesting feature is the lack of Pro8 in analogues 2 and 3, the amino acid residue absolutely conserved in the family of Bowman–Birk inhibitors.

Keywords Chymotrypsin · Cathepsin G · Inhibitors · Solid-phase synthesis · Combinatorial library · Peptomers

Introduction

Sunflower trypsin inhibitor SFTI-1 is the smallest peptide related to the Bowman–Birk family of serine proteinase inhibitors [1]. This head-to-tail cyclic 14-amino-acid residue peptide contains one disulfide bridge and a Lys5 residue in the P1 position, which is responsible for inhibitory specificity. Its primary structure is shown in Fig. 1.

Owing to its small size and strong trypsin-inhibitory activity (K= 1.1 × 10¹⁰ M⁻¹) [2], SFTI-1 is considered to be a very attractive template for the design of proteinase inhibitors with potential use as pharmacological agents. However, it is known that peptides are not ideal therapeutic agents because of their high sensitivity to protease degradation and limited cell permeability. Many strategies have been developed to overcome these problems [3]. In recent years, biomimetic oligomers called peptoids have made a significant impact in the area of drug design [4]. These oligomers are composed of N-substituted glycine residues that mimic proteinogenic amino acids. Hybrid combinations of peptides and peptoids, named after Ostergaard and Holm [5] as “peptomers” (peptide-peptoid hybrid polymers), are another interesting class of compounds that combine properties of peptides and peptoids. Recently we have shown [6] that introduction of N-substituted glycine residues (peptoid monomers) mimicking Lys and Phe in position P1 of monocyclic SFTI-1 with a disulfide bridge gave potent trypsin and chymotrypsin inhibitors, respectively. As a result, we obtained two peptomeric analogues of SFTI-1 which displayed strong inhibitory activity and contained a proteolysis-resistant P1–P'1 reactive site. As the introduction of the peptoid monomers into the peptide chain can be easily achieved via solid-phase synthesis, we decided to design, synthesize, and select peptomeric analogues of SFTI-1 using combinatorial chemistry methodology. Based on our previous results, and also on the results of others, a peptomeric library of general formula shown in Fig. 2 was synthesized.

In addition to Nphe present at the position 5 (equivalent to substrate specificity P1 position) in all the peptomers,
we decided to introduce this peptoid monomer in position 12, originally occupied by a Phe residue. In this way we decided to assess how much replacement of further naturally occurring residues with N-substituted glycine derivatives affected the inhibitory activity. We focused our interest on the Ile\textsuperscript{7}–Pro\textsuperscript{8}–Pro\textsuperscript{9}–Ile\textsuperscript{10} region of the sequence that makes an important contribution to the structural integrity and rigidity of SFTI-1 [7]. This is a turn region located near the active site (Lys–Ser peptide bond) and also includes the cis-Pro\textsuperscript{8} residue conserved in the entire Bowman–Birk family. N-Substituted glycine derivatives were introduced in the peptide chain by the submonomer approach [8]. Briefly, the N-terminal amino group of the newly synthesized peptide was acylated by bromoacetic acid followed by nucleophilic substitution using one of the primary amines listed in Fig. 3.

In all the synthesized peptomers, the substrate-specific P\textsubscript{1} position is occupied by Nphe which mimics the proteino- genic Phe residue. Therefore, we decided to deconvolute this library using bovine \(\alpha\)-chymotrypsin and cathepsin G (CG). The latter enzyme, along with proteinase 3 and human neutrophil elastase, are released from activated neutrophils at inflammatory sites. Cathepsin G is involved in several physiological processes such as tissue degradation [9], platelet activation [10], proteolysis of blood coagulation factors [11], and generation of angiotensin II [12]. It also displays a broad spectrum of antiviral, antibacterial, and antifungal activities [13,14]. Its unique substrate pocket structure makes it dual specific (trypsin- and chymotrypsin-like).

Materials and methods

Peptide synthesis

All the peptides were synthesized by solid-phase method using Fmoc chemistry. The following amino acid derivatives were used: Fmoc-Gly, Fmoc-Arg(Pbf), Fmoc-Cys(Trt), Fmoc-Thr(rBu), Fmoc-Ser(rBu), Fmoc-Ile, Fmoc-Pro, Fmoc-Phe, and Fmoc-Asp(OrBu). The C-terminal amino acid residue, Fmoc-Asp(OrBu), was attached to the 2-chlorotrityl chloride resin (loading = 1.46 meq/g) (Calbiochem–Novabiochem AG, Switzerland) in the presence of equimolar amount of \(N\), \(N\)-disopropylethylamine (DIPEA) based on the amino acid in anhydrous condition in dichloromethane (DCM) solution. Peptide chains were elongated in consecutive cycles of deprotection and coupling. Deprotection was performed with 20% piperidine in Dimethylformamide/\(N\)-methyl-2-pyrrolidone (DMF/NMP) (1:1, v/v) with addition of 1% Triton X-100. \(N\)-Substituted glycine derivatives were introduced into the peptide chain by the submonomer approach [8] using bromoacetic acid and primary amines. After completing the syntheses, the peptides were simultaneously deprotected and cleaved from the resin using a trifluoroacetic acid (TFA)/phenol/triisopropylsilane/H\(_2\)O (88:5:2:5, v/v/v/v) mixture [15]. In the last step, the disulfide bridge formation was performed by 0.1 M solution of I\(_2\) in MeOH using the procedure described elsewhere [16]. The progress of the reaction was monitored by high-performance liquid chromatography (HPLC). The crude resynthesized...