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# Design and Synthesis of Cathepsin B Inhibitors by an Affinity Labeling Approach[1)]

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The affinity labeling approach by the use of S-(3-nitro-2-pyridinesulfenyl) group was demonstrated to be effective and versatile to the design and synthesis of thiol protease inhibitors. The inhibitors were found to be highly specific to thiol proteases and to have no effect on serine proteases and acid proteases.

Cathepsin B is an intracellular proteolytic enzyme which belongs to the group of closely related thiol proteinases including cathepsin H and L. The physiological role of this enzyme is thought to be that of the degradation of tissue proteins within the lysosomes and it has been postulated to be involved in proteolytic processing of protein and hormone precursors.[2)]

Many inhibitors with halomethyl ketone functional groups were designed as potential enzyme inhibitor drugs for the treatment of certain diseases and conditions.[3)] However, there are potential problems with using these inhibitors as drugs since halomethyl ketones are relatively strong electrophiles and may indiscriminately alkylate non-target molecules present under in vivo conditions.

To overcome this problem, a hydrolytic transition state analog approach and an affinity labeling approach were considered. We wish to report here the design and synthesis of cathepsin B inhibitors.

One of the authors has shown that the hydrolytic transition state analog of statine (Sta) containing peptides are effective as renin inhibitors.[4)] Thus, H-Arg-Arg-Sta-Phe-OH (mp 247-250 °C (dec), $[\alpha]_D^{22}$ -7.1° (*c* 0.2 MeOH)) was designed and prepared but the inhibitory potency[5)] against rat liver cathepsin B, H, L was rather weak: inhibitory % at 250 μM against cathepsin B, H, L were 4.5%, 23.7%, and 34.5%, respectively.

The weak inhibitory potency may be due to the difference in substrate specificity: renin is an angiotensinogen specific enzyme but cathepsin B, H, L, and papain degrade various kind of peptides as substrates.

To explore a versatile method to develop thiol protease inhibitors, an affinity labeling approach was investigated by the assumption that a functional group bound to substrates or substrate like peptides can selectively modify the active sites of the target enzymes. The functional group for cysteine protease inhibitors by this approach should react selectively with the SH group of cysteine proteases and should not react with the OH group of serine protease or the COOH

group of acid proteases.

One of the authors has recently shown that a peptide containing S-(3-nitro-2-pyridinesulfenyl(Npys))-cysteine residue reacts selectively with free thiol of another cysteine containing peptide to form an unsymmetrical disulfide bond.[7]

The principle of selective labeling of cysteine proteases by the use of S-Npys group is depicted in Fig. 1.

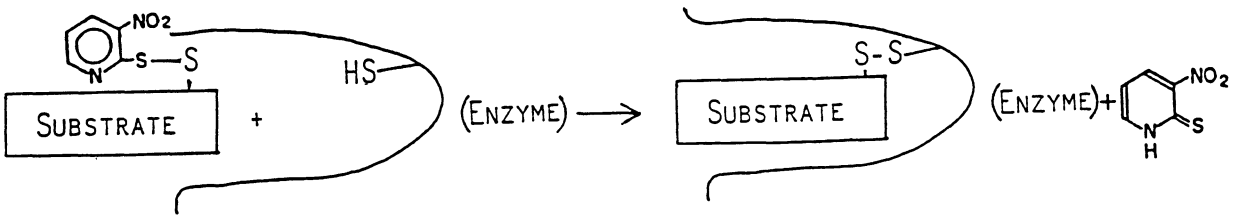


Fig. 1. Principle of selective labeling of cysteine proteases by the use of the S-Npys group.

Thus, various kind of substrate analogs which has the S-Npys-cysteine residue were designed and prepared using the recently demonstrated cathepsin B model.[8] Some of these analogs and their inhibitory potencies against rat liver cathepsin B, H, L and papain are listed in Table 1.

All the peptides were prepared by the solid phase method with using Boc-amino acids according to the general procedures of Stewart and Young.[9] In a typical experiment, starting from Boc-Phe esterified to 2% cross-linked polystyrene-divinylbenzene copolymer (2 g, 1.14 mmol), the Boc derivatives of Cys(Npys), Arg(Tos), Arg(Tos), Ac-Phe were coupled with 3 equiv. of dicyclohexylcarbodiimide and 6 equiv. of 1-hydroxybenzotriazole, using 3 equiv. of each amino acid. The Boc groups were removed with TFA/$CH_2Cl_2$ (1:1 v/v). A coupling of 2 - 5 h was used and the complete coupling were judged by the method of Kaiser.[10] The finished peptide resin was dried and treated with 20 ml of HF containing 2 ml of anisole for 30 min at 0 °C. After removal of the HF, the resin was washed with several portions of ether and the liberated peptide was extracted twice with 30 ml of TFA. The solvent was removed and the residue was treated with ether. The crude product was purified with Sephadex G-25 in 1 M AcOH or HPLC on C-18 column using a linear gradient of 0.1% TFA in water to 50% acetonitrile in water containing 0.1% TFA over 60 min at a flow rate of 7 ml/min. Yellow fractions containing a single spot on TLC were pooled and evaporated. The pure Ac-Phe-Arg-Arg-Cys(Npys)-Phe-OH·2TFA was obtained by precipitation from MeOH-ether-petroleum ether; 642 mg, 49% yield from the initial Boc-Phe-resin: mp 223 - 227 °C (dec), $[\alpha]_D^{22}$-64.8° (*c* 0.2, MeOH). The amino acid ratio: Arg 1.89, $CySO_3H$, 0.94, Phe, 2.

Considering the substrate structures for cathepsin B, H, L, we postulated that the Cys(Npys) residue could replace the leaving 4-methyl-cumaryl-7-amide (MCA) residue of substrates and could modify the SH function of the active site of thiol proteases if inhibitors can totally mimic the substrates.

H-Arg-Arg-Cys(Npys)-OH (2) was found to be a noncompetitive inhibitor ($Ki=2.5\times10^{-4}M$) to cathepsin B and further designs were investigated. To improve

Table 1. Synthetic Peptides with S-Npys Cysteine Residue and Their Inhibitory Potencies[a] to Thiol Proteases

| Compounds[b] | Mp/°C(dec) $([\alpha]_D^{22}$/° (c 0.2,MeOH)) | Inhibitory potencies Inhibition %, $IC_{50}$ | | | |
|---|---|---|---|---|---|
| | | Cathepsin B | Cathepsin H | Cathepsin L | Papain |
| H-Arg-Ser-Cys(Npys)-OH (1) 200 μM | 175-180 (+15.1) | 3.2% | 26.0% | 20.0% | - |
| H-Arg-Arg-Cys(Npys)-OH (2) 200 μM | 205-209 (-44.0) | 55.8% | 51.1% | 41.1% | - |
| H-Arg-Arg-Cys(Npys)-Phe-OH (3) 250 μM | 206-210 (-75.0) | 92.3% | 79.7% | 67.3% | - |
| Ac-Ala-Arg-Cys(Npys)-Phe-$NH_2$ (4) | 231-234 (-19.6) | $5.5\times10^{-4}$M | - | - | - |
| Ac-Ala-Arg-Cys(Npys)-Phe-OH (5) | 204-208 (-70.4) | $4\times10^{-4}$M | $9\times10^{-4}$M | - | - |
| Ac-Ala-Arg-Arg-Cys(Npys)-Phe-OH (6) | 227-230 (-128.5) | $1\times10^{-4}$M | $2.5\times10^{-4}$M | - | $9\times10^{-5}$M |
| Ac-Ala-Arg-Arg-Cys(Npys)-Phe-$NH_2$ (7) | 232-237 (-77.6) | $1.3\times10^{-4}$M | $2\times10^{-4}$M | - | $1.2\times10^{-4}$M |
| Ac-Phe-Arg-Arg-Cys(Npys)-Phe-OH (8) | 223-227 (-64.8) | $5\times10^{-5}$M | $1.2\times10^{-4}$M | - | $1.9\times10^{-4}$M |

a) Net inhibitory potencies may be higher than those observed[5] in the presence of a large amount (0.01 M) of cysteine : for example, 8 showed $IC_{50}= 7\times10^{-6}$M against papain in the absence of cysteine. The thiol group of papain was modified with 8 ($7\times10^{-6}$M) and it completely lost enzymic activity. Full enzymic activity was regenerated with tri-butylphosphine as described in the previous reports (R. Matsueda et al., Chem. Lett.,1981, 737; T. Kimura et al., Anal. Biolchem.,122, 274 (1982)).

b) All the compounds were well characterized by elemental and amino acid analyses.

active site binding affinity, we tried to substitute the neutral amino acids to $NH_2$-terminal and COOH-terminal. Ac-Phe-Arg-Arg-Cys(Npys)-Phe-OH ( 8 ) showed the highest potency and markedly inhibited thiol proteases but it was found that these inhibitors have no effect on serine proteases such as trypsin and acid proteases such as pepsin. $IC_{50}$ of this compound was $5 \times 10^{-5}$M but the net potency may be higher than this value since the assays were carried out in the presence of large amount (0.01 M) of cysteine, which partially decomposes inhibitors.

It is assumed that the Arg-Arg- part of the inhibitor binds ionically to Asp (67) and Glu (241), the N-terminal Phe binds to Tyr (73), and the C-terminal Phe binds to Tyr (173), Leu (179) and Trp (219) of the recently demonstrated rat liver cathepsin B model.[8] Thus, the Cys(Npys) residue of the inhibitor may be able to react with Cys (29) and inactivate the enzyme as shown in Fig. 2.

In conclusion, the affinity labelling approach by the use of the S-Npys group is effective for the design and synthesis of thiol protease inhibitors. This approach is versatile because it is rather easy to design from their sub-

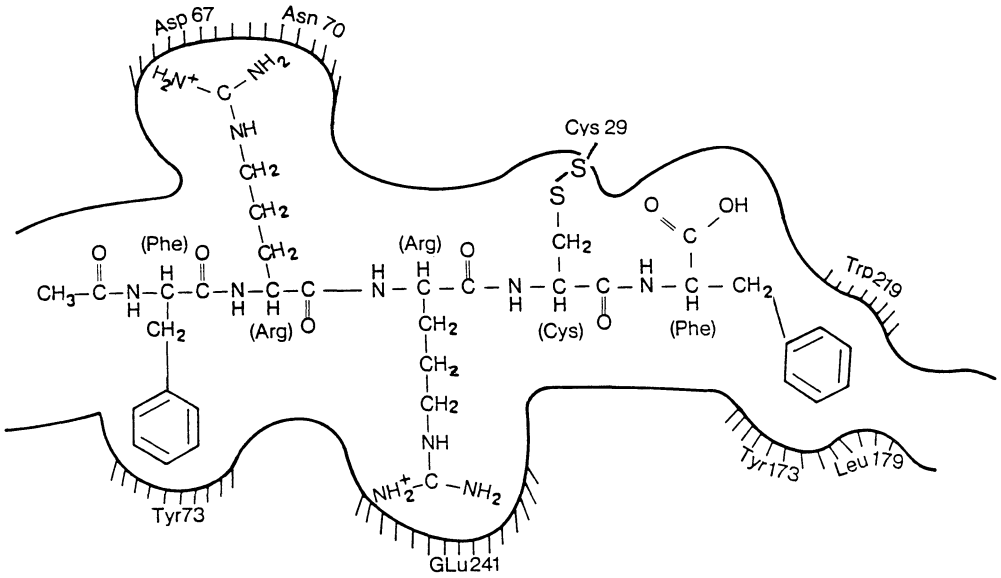


Fig. 2. Postulated binding of Ac-Phe-Arg-Arg-Cys(Npys)-Phe-OH to active site region of cathepsin B model.[8]

strates, and it can produce thiol protease specific inhibitors which do not modify serine proteases or acid proteases. Further application of this approach to other thiol enzymes and the modification of receptors with cysteine residue are under investigation.

References

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