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EFFECT OF YM-47141, A NEW INHIBITOR PRODUCED BY *FLEXIBACTOR SP. Q17897*, ON ELASTASE

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YM-47141, a peptidic compound recently isolated from *Flexibactor sp. Q17897*, strongly inhibited human leukocyte elastase (HLE) with K_i value 2.1×10^{-7} M. Unlike other serine protease inhibitors, YM-47141 exhibited relatively weak effects on cathepsin G and α-chymotrypsin and its inhibitory K_i values were 9.2×10^{-6} M and 1.3×10^{-6} M, respectively. It had little, or no inhibitory effect on plasmin, thrombin, trypsin and kallikrein ($IC_{50} > 10^{-4}$ M). The inhibition of HLE by YM-47141 was reversible and of a mixed type.

Keywords: Elastase, serine protease, protease inhibitor, pulmonary emphysema, YM-47141, *Flexibactor sp. Q17897*

INTRODUCTION

Elastase, a component of extracellular matrix distributed in various tissues, is the protease known to hydrolyse elastin. Pancreas elastase is well-known as a digestive enzyme, while leukocyte elastase has aroused increasing interest for its participation in the pathogenesis of pulmonary diseases such as pulmonary emphysema, adult respiratory distress syndrome (ARDS), lung fibrosis and inflammation and rheumatoid arthritis.[1–3] Among these diseases, pulmonary emphysema has been shown to involve the action of leukocyte elastase particularly strongly.[1] Various hypotheses have been proposed for the etiology of pulmonary emphysema, including respiratory airway constriction due to tissue atrophy, inhalation of irritant gases or dust and protease-antiprotease imbalance. The likeliest among these is the protease-antiprotease imbalance. In addition, disorders in the synthesis

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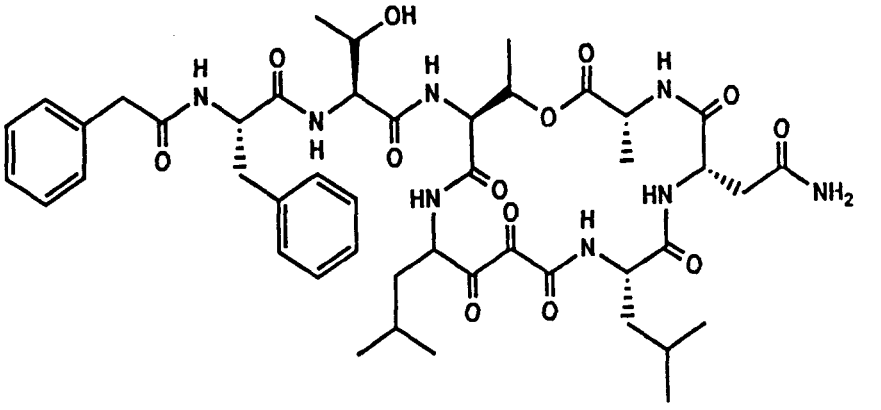

FIGURE 1 Structure of YM-47141. The structure was determined by ^{1}H and ^{13}C-NMR and mass spectrometry, as described in a previous paper.[5]

of pulmonary elastin and elastic fibers may be involved in the pathogenesis of pulmonary diseases and various inflammatory diseases.

We recently isolated a new protease inhibitor, YM-47141, produced by *Flexibactor sp. Q17897*, and described its taxonomy, physico-chemical properties, biological activities and structure (Figure 1).[4,5] In this study, we investigated the inhibitory effect of YM-47141 on elastase and other proteases.

MATERIALS AND METHODS

Materials

YM-47141 was prepared from *Flexibactor sp. Q17897*, as described elsewhere.[5] HLE was purchased from Athens Research and Technology Inc. (USA). Bovine plasma thrombin was obtained from Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan). Human plasma plasmin and synthetic substrates (S2222, S2238, S2251 and S2301) were purchased from Daiichi Kagaku Pharmaceutical Co., Ltd (Tokyo, Japan). Bovine pancreas elastase, bovine pancreas trypsin, human plasma kallikrein, human leukocyte cathepsin G, bovine pancreas α-chymotrypsin, and succinyl-Ala-Ala-Phe-Arg-*p*-nitroanilide were obtained from Sigma Corp. (St. Louis, MO, USA). Succinyl-Ala-Ala-Ala-*p*-nitroanilide was obtained from Peptide Institute, Inc. (Osaka, Japan).

Inhibition Assay for YM-47141 on Various Proteases

The inhibitory effect of YM-47141 on HLE or bovine pancreas elastase was determined according to the method of Schiessler *et al.*[6] The absorbance at 405 nm of *p*-nitroanilide generated from the substrate after reaction for 20 min at 25°C was measured. Inhibitory effects on other proteases were determined as follows: the method of Matsuoka *et al.* for plasmin, thrombin, trypsin and kallikrein,[7] that of Barrett and Kirschke for cathepsin G,[8] and that of Delmar *et al.* for α-chymotrypsin.[9] The K_i value was determined by the following equation fitted for mixed-type inhibition:

$$V_p = V/\{1 + i \cdot K_m)/K_i \cdot K_m\},$$

$$K_p = K_m(1 + i/K_i)/\{(1 + i \cdot K_m)/K_i \cdot K_m\},$$

where K_m is the Michaelis-Menten constant, K_p is the apparent Michaelis-Menten constant, K_i is the inhibition constant, K_m is the dissociation constant determined by the equation: ESI $\rightarrow$ EI + S, V is the maximum reaction rate constant, V_p is the apparent maximum reaction rate and i is the inhibitor concentration.

RESULTS

The new protease inhibitor, YM-47141, proved to inhibit HLE strongly and specifically. The K_i value for YM-47141 was calculated to be 2.1×10^{-7} M from the Lineweaver-Burk plots at the inhibitor concentrations of 0.5×10^{-7}, 1.0×10^{-7} and 2.0×10^{-7} M (Figure 2). The inhibition of HLE by YM-47141 was reversible and of a mixed-type. YM-47141 inhibited porcine pancreas elastase with an IC_{50} of 3.9×10^{-8} M, a similar value to 1.5×10^{-7} M obtained with HLE under the same conditions.

Inhibitory effects of YM-47141 on other serine proteases are summarized in Table I. YM-47141 showed a weak inhibitory effect on human leukocyte cathepsin G and bovine pancreas α-chymotrypsin, the K_i values being 9.2×10^{-6} M and 1.3×10^{-6} M, respectively. YM-47141 showed no inhibitory effect on human plasma plasmin, bovine plasma thrombin, bovine pancreas trypsin or human plasma kallikrein ($IC_{50} > 10^{-4}$ M).

DISCUSSION

The inhibitory effect of the inhibitor, YM-47141, isolated from *Flexibactor sp. Q17897* on leukocyte elastase, has been evaluated. The Lineweaver-Burk plots

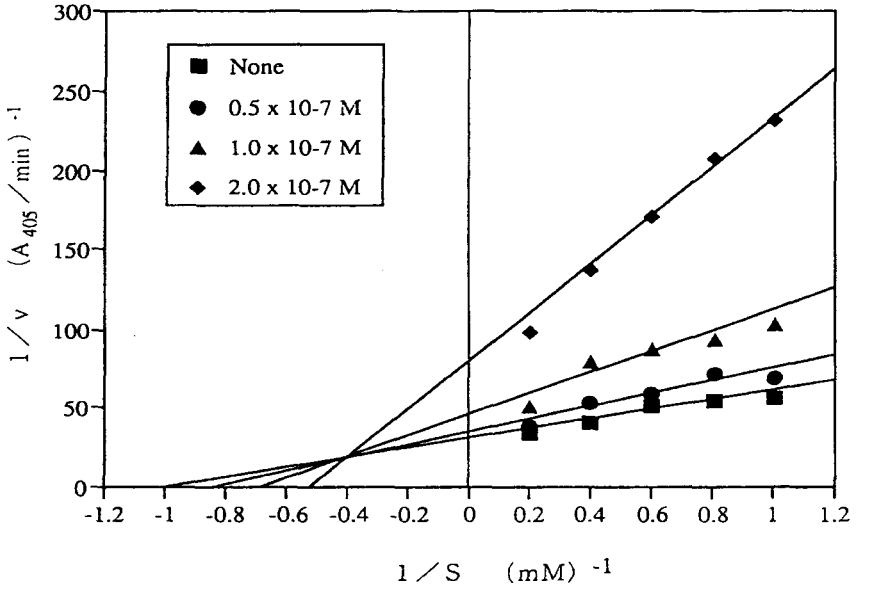

FIGURE 2 Lineweaver-Burk plots of YM-47141. The incubation was carried out at a HLE concentration of 1.1×10^{-7} M. Concentrations of substrate and the inhibitor were varied as indicated. Data represent the means of 3 determinations at each point.

showed that the inhibition was of a mixed type. In contrast, the inhibition by generally known elastase inhibitors such as elastatinal and Q-17897-C-2 produced by *Flexibactor sp. Q17897* was of a competitive type (data not shown). ONO-5046 also showed competitive inhibition.[10] Therefore, the inhibition of HLE by YM-47141 is considered to involve more than two sites; one is the active site of HLE and is the common binding site for these elastase inhibitors, while the other is a non-active site of HLE but is the specific binding site for YM-47141. Another known HLE inhibitor, SC-39026, showed non-competitive inhibition at lower concentrations and mixed type inhibition at higher concentrations.[11]

Among the enzymes tested, elastase was the most sensitive to YM-47141, followed by α-chymotrypsin and cathepsin G. YM-47141 showed little inhibitory effect on serine proteases, including plasmin, thrombin, trypsin and kallikrein. These results support the view of high structural homology between HLE, α-chymotrypsin and cathepsin G.

YM-47141 inhibited porcine pancreas elastase with an IC_{50} value of 3.9×10^{-8} M, a similar value to that for HLE. The potent inhibitory activity of YM-47141 against both these elastases provides evidence for their structural similarity.

TABLE 1 Effects of YM-47141 on various serine proteases

Serine Protease	*Substrate*	*Buffer*	*Protease conc.*	K_i *(or* IC_{50}*)*
Human leukocyte elastase	Suc-AAA-*p*NA	0.2 M triethanolamine (pH 7.8)	1.1×10^{-7} M	2.1×10^{-7} M
Porcine pancreas elastase	Suc-AAA-*p*NA	0.2 M triethanolamine (pH 7.8)	1.1×10^{-7} M	$(3.9 \times 10^{-8}$ M)
Human plasma plasmin	S-2251	0.05 M Tris (pH 7.8)	0.04 U/ml	NI[a]
Bovine plasma thrombin	S-2238	0.05 M Tris (pH 7.8)	1.0 U/ml	NI
Bovine pancreas trypsin	S-2222	0.05 M Tris (pH 7.8)	3.6 U/ml	NI
Human plasma kallikrein	S-2302	0.05 M Tris (pH 7.8)	0.02 U/ml	NI
Human leukocyte cathepsin G	Suc-AAPF-*p*NA	0.1 M HEPES (pH 7.8)	0.025 U/ml	9.2×10^{-6} M
Bovine pancreas α-chymotrypsin	Suc-AAPF-*p*NA	0.1 M Tris (pH 7.8)	0.015 U/ml	1.3×10^{-6} M

S-2251, H-D-Val-Leu-Lys-NH-C_6H_4-NO_2·2HCl; S-2238, H-D-Phe-Pip-Arg-NH-C_6H_4-NO_2·2HCl; S-2222, C_6H_5-CO-Ile-Glu-(γ OR *)-Gly-Arg=NH-C_6H_4-NO_2·HCl; S-2302, H-D-Pro-Phe-Arg-NH-C_6H_4-NO_2·2HCl.
[a]Since the IC_{50} value was higher than 10^{-4} M, no K_i value was obtained.

The finding that α-chymotrypsin and cathepsin G have an elastinolytic activity[12] indicates that *in vivo* administration of YM-47141 might be useful for the treatment of pulmonary diseases such as pulmonary emphysema and rheumatoid arthritis.

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