A new function of kininogens as thiol-proteinase inhibitors: inhibition of papain and cathepsins B, H and L by bovine, rat and human plasma kininogens

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The amidolytic activities of papain and rat liver cathepsins B, H and L were strongly inhibited by high (HMM) and low (LMM) molecular mass kininogens from bovine, human and rat plasmas, and their K_i values were estimated to be in the order of 10^{-10}–10^{-11} M for papain and 10^{-8}–10^{-9} M for cathepsins. The derivatives of bovine kininogens, HMM kinin-free protein, HMM kinin- and fragment 1·2-free protein, and LMM kinin-free protein also showed strong inhibitory activity toward these thiol-proteinases. These results suggest that a reactive site which interacts with thiol-proteinases is contained in the heavy chain portion in kininogens.

Kininogen   *Thiol-proteinase inhibitor*   *Papain*   *Cathepsin B*   *Cathepsin H*   *Cathepsin L*

1. INTRODUCTION

Thiol-proteinase inhibitor in human plasma has recently been isolated by several investigators and its inhibitory activity has been analysed on papain, ficin, muscle calpain (CANP) and human liver cathepsins B, H and L [1–5]. Recently, Ohkubo et al. [6] reported that one of the thiol-proteinase inhibitors (α_2-TPI) in human plasma is identical with LMM kininogen by analysing the base sequence of cDNA for the inhibitor. We purified HMM and LMM kininogens from bovine and rat plasmas and characterized their chemical properties [7–9]. We describe here the inhibitory activities of these kininogens and human HMM kinin-free protein toward papain and cathepsins H, B and L purified from rat liver. The inhibitory activities of these

kininogens and their derivatives have been compared in terms of K_i values. The results demonstrate a new function of HMM and LMM kininogens as thiol-proteinase inhibitor.

2. MATERIALS AND METHODS

Bovine HMM and LMM kininogens and their derivatives were isolated as in [7,10]. Rat HMM kininogen and T-kininogen were purified by the methods of Hayashi et al. [8] and Enjyoji et al. [9]. Human HMM kinin-free protein was kindly supplied by Dr K. Fujikawa, Department of Biochemistry, University of Washington, Seattle, WA. The concentrations of these kininogens and their derivatives were calculated from the absorbance at 280 nm and amino acid analysis, as in [7]. Cathepsins B [11], H [12] and L [13] were purified from rat liver as previously reported. Cathepsin B was also purified from human liver [11]. Papain was a product of Sigma, St. Louis, MO. The activities of thiol-proteinases were measured using

Table 1

K_i values for the inhibition of various thiol-proteinases by HMM and LMM kininogens and their derivatives

	Cathepsin B	Cathepsin L	Cathepsin H	Papain
Bovine				
HMM kininogen	12	0.7	15	0.07
HMM kinin-free protein	1.2	0.4	7.8	0.04
HMM kinin- and fragment 1·2-free protein	2.0	1.8	12	0.01
LMM kininogen	23	1.8	44	0.05
LMM kinin-free protein	11	1.7	9.2	0.02
Rat				
HMM kininogen	45	1.2	>2000	0.11
T-kininogen	170	2.8	470	0.19
Human				
HMM kinin-free protein	170	0.2	1.9	0.02
	>2000[a]			

[a] For cathepsin B purified from human liver

K_i values were calculated by Henderson plot [15] and expressed as nM

fluorogenic peptide substrates, Z-Phe-Arg-MCA for papain, cathepsins B and L, and Arg-MCA for cathepsin H in 0.4 M sodium acetate buffer (pH 5.5), containing 4 mM EDTA and 8 mM cysteine as in [14]. K_i values were calculated by Henderson plot [15].

3. RESULTS

The amidolytic activity of papain was strongly inhibited with increasing amounts of HMM and LMM kininogens from bovine and rat plasmas. The derivatives of bovine kininogens, HMM kinin-free protein, HMM kinin- and fragment 1·2-free protein and LMM kinin-free protein, and human HMM kinin-free protein also inactivated papain, whereas S-alkylated heavy chain, fragment 1·2 and fragment 1·2-light chain derived from bovine HMM kininogen did not inhibit significantly (not shown). From the slope of inhibition curves with the amidase activity vs concentration of kininogens and the derivatives, K_i values were calculated by Henderson plot, as shown in table 1. The inhibition of the amidolytic activity of rat liver cathepsins B, H and L was also analysed in the same way,

using kininogens and their derivatives. Bovine HMM and LMM kininogens and their derivatives and human HMM kinin-free protein inhibited cathepsins B, H and L, with K_i values in the order of 10^{-8}–10^{-9} M (table 1). The inhibitory activity of rat kininogens on cathepsins B and H, except for cathepsin L, was relatively weak, as compared with those of bovine kininogens. All these kininogens inhibited papain more strongly than cathepsins. Although human HMM kinin-free protein inhibited rat cathepsins, the inhibitory activity on human cathepsin B was very weak.

4. DISCUSSION

This paper demonstrates that HMM and LMM kininogens from bovine and rat plasmas have a strong inhibitory activity on papain and rat liver cathepsins B, H and L. Particularly, K_i values for the inhibition of papain by these kininogens and human HMM kinin-free protein are very low, comparable with those of human thiol-proteinase inhibitors previously reported [5]. The K_i value of rat T-kininogen is also comparable with rat thiol-proteinase inhibitor [16]. Ohkubo et al. [6] have

described that human HMM kininogen appears to lack inhibitory activity toward ficin, although the data were not shown. However, the present data provide evidence that not only LMM but also HMM kininogen has the ability to inhibit thiol-proteinases.

Among the derivatives from bovine kininogens, HMM kinin-free protein, HMM kinin- and fragment 1·2-free protein and LMM kinin-free protein show strong inhibitory activity on thiol-proteinases. Since the amino acid sequence of the heavy chain portions of bovine HMM and LMM kininogens are identical [17,18], these results indicate that a reactive site of the kininogens for thiol-proteinase is located in their heavy chain portions.

Although the reason why S-alkylated heavy chain prepared from bovine HMM kinin-free protein did not show any inhibitory activity remains to be established, this may be due to a conformational change induced during the reduction and S-alkylation procedures. It is of interest that K_i values of HMM and LMM kinin-free proteins for the inhibition of cathepsins B and H are significantly lower than those of kininogens. This enhancement of the inhibitory activity of kininogens after kinin liberation awaits further investigation.

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