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Squamous cell carcinoma antigen is a potent inhibitor of cysteine proteinase cathepsin L

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Abstract A squamous cell carcinoma antigen (SSCA), which is a member of the serpin family of proteinase inhibitors, was purified from sera of cancer patients. It did not inhibit serine proteinases. However, it non-competitively inhibited human cathepsin L with a K_i of 0.064 nM, but not cathepsins B and H among cysteine proteinases. These results indicated that SCCA is a non-functional serpin that inhibits cathepsin L in cancer cells.

Key words: Squamous cell carcinoma antigen (SCCA); Serpin; Proteinase inhibitor; Cathepsin L

1. Introduction

Squamous cell carcinoma antigen (SCCA) is a protein isolated from human uterine cervical SCC tissue [1–3] that is widely used for the diagnosis and management of SCC [4–6]. In addition, serum SCCA levels are elevated in inflammatory dermatoses with eczema and psoriasis [7]. The leakage of SCCA into body fluids from SCC tissues is apparently influenced by the infiltrative growth and the degree of histologic differentiation of tumor cells [8]. However, the function of SCCA in tumor cells remains unknown. Proteolytic mechanisms have been implicated in tumor cell metabolism and invasion [9–14]. The cDNA of SCCA has been cloned by Suminami et al. [15] and the deduced amino acid sequence revealed ideal homology with the serpin family of proteinase inhibitors. Takahashi et al. [16] have purified a high molecular mass cysteine proteinase inhibitor (psoriastatin) from psoriatic scale, and demonstrated its strong immunoreactivity to an antibody against SCCA.

Here, we describe that SCCA isolated from sera of cancer patients specifically inhibits lysosomal cysteine proteinase cathepsin L.

2. Materials and methods

2.1. Materials

Cathepsins B, H, and L were prepared from autopsied human liver samples as described [17]. Trypsin, α-chymotrypsin and elastase from porcine pancreas, thrombin, plasmin, plasminogen and kallikrein from human plasma, urokinase from human urine, papain (2× crystallied) from papaya latex were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tissue plasminogen activator was obtained from Cappel Products (USA). Human cathepsin G was from Cosmo Bio Co. (Tokyo, Japan). *t*-Butyloxycarbonyl (Boc)-Phe-Ser-Arg-4-methylcoumaryl-7-amide (MCA), Boc-Val-Pro-Arg-MCA, Boc-Val-Leu-Lys-MCA, succinyl (Suc)-Leu-Leu-Val-Tyr-MCA, carbobenzoxy (Z)-Gly-Gly-Arg-MCA, Z-Arg-Arg-MCA, Z-Phe-Arg-MCA, *N*-methoxysuccinyl(Suc(OMe))-Ala-Ala-Pro-Val-MCA and Arg-MCA were products of the Peptide Institute Inc. (Osaka, Japan). Suc-Ala-Ala-Phe-MCA was from Enzyme System Product Inc. (USA).

2.2. Preparation of SCCA

SCCA positive sera from patients with lung or uterine cervical cancer were obtained from Fujigaoka Hospital Central Laboratory. SCCA was isolated from pooled sera (about 600 ml), dialyzed against 0.5 M NaCl in 20 mM Tris-HCl buffer, pH 7.5, and purified on anti-psoriastatin IgG-Sepharose 4B column (3 × 1 cm) prepared according to the protocol supplied by Pharmacia. The SCCA fraction dialyzed against 25 mM acetate buffer, pH 5.0, was applied to a Mono S column equilibrated with same buffer equipped with a fast protein liquid chromatography (FPLC) System (LKB-Pharmacia, Sweden). The proteins were eluted with a linear gradient of 0 to 0.45 M NaCl. The SCCA fraction was dialyzed against Polybuffer 92, adjusted to pH 8.5, then applied to a Mono P column. The proteins were eluted by Polybuffer pH gradient from pH 8.0 to 4.5. The SCCA fraction was concentrated by ultrafiltration with an Amicon PM-10 membrane. The concentrate was filtered by HPLC on a G3000SW column equilibrated with the starting buffer. The SCCA fraction was further purified by a 2nd FPLC on Mono S as shown in Fig. 1. The preparation was apparently homogenous on SDS-PAGE. SCCA was detected and the concentration was determined using an IMx-SCC test kit (DaiNabot Co., Japan).

2.3. Inhibitory assays

Boc-Phe-Ser-Arg-MCA, Suc-Leu-Leu-Val-Tyr-MCA, Boc-Val-Pro-Arg-MCA, Z-Gly-Gly-Arg-MCA, Suc(OMe)-Ala-Ala-Pro-Val-MCA, Suc-Ala-Ala-Phe-MCA, Z-Arg-Arg-MCA and Arg-MCA were the substrates for trypsin, α-chymotrypsin, thrombin, urokinase, elastase, cathepsin G, cathepsin B and cathepsin H, respectively. Z-Phe-Arg-MCA was the substrate for cathepsin (B + L), papain and kallikrein. Boc-Val-Leu-Lys-MCA was the substrate for plasmin and plasminogen activator. All substrates were dissolved in 0.05% Brij 35. Proteinases were incubated for 5 min at 25°C with increasing amounts of SCCA before the reaction was started by adding 10 μl of substrate. The amount of MCA liberated from the substrates was determined fluorometrically with excitation and emission wavelengths of 360 and 460 nm, respectively, using a Hitachi fluorescence spectrophotometer, model MPF-750. The buffers were (i) 0.1 M Tris-HCl, pH 7.5, containing 10 mM $CaCl_2$ for trypsin, kallikrein, plasmin and plasminogen activator, (ii) 0.1 M Tris-HCl, pH 8.0, containing 10 mM $CaCl_2$ for α-chymotrypsin, (iii) 0.1 M phosphate buffer, pH 6.8, containing 1 mM EDTA and 4 mM dithiothreitol (DTT) for papain, cathepsin B and cathepsin H, (iv) 0.1 sodium acetate buffer, pH 5.5, containing 1 mM EDTA and 4 mM DTT for cathepsin L, and (v) 0.1 M phosphate buffer, pH 7.0 for elastase and cathepsin G. The inhibition constant (K_i) was calculated according to the method of Dixon [18].

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Abbreviations: SCCA, squamous cell carcinoma antigen; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography; Boc, *t*-butyloxycarbonyl; Suc, succinyl; Z, carbobenzoxy; Suc(OMe), *N*-methoxysuccinyl; MCA, 4-methylcoumaryl-7-amide; DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

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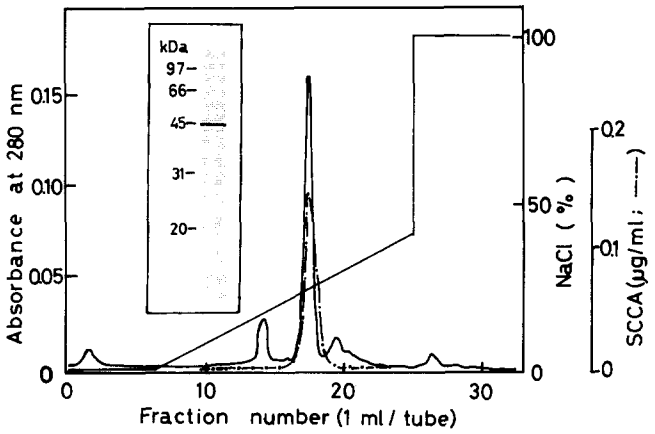

Fig. 1. The 2nd FPLC of SCCA on a Mono S column. The SCCA fraction from HPLC on G3000SW was dialyzed against a 25 mM acetate buffer, pH 5.0, and applied to a Mono S column. The fraction was eluted at a flow rate of 1 ml/min with a linear gradient of 0 to 0.45 M NaCl in the same buffer. (Insert) SDS-PAGE of SCCA. Electrophoresis was performed by Laemmli's method using a 12.5% gel and the protein (0.6 μg) was stained with Coomassie brilliant blue.

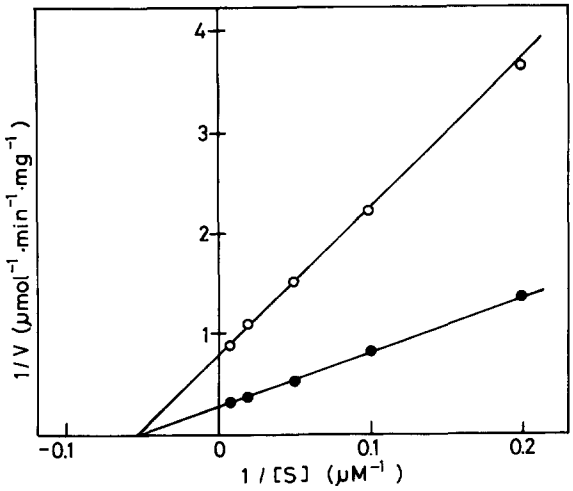

Fig. 2. Kinetic analysis of the reaction of cathepsin L with SCCA. Lineweaver–Burk plot of the substrate (Z-Phe-Arg-MCA) concentration against cathepsin L activity in the presence (○, 0.142 μM) or absence (●) of SCCA.

3. Results and discussion

We used an anti-psoriastatin antibody (IgG) as a ligand for affinity chromatography to isolate SCCA from the sera of cancer patients. The total SCCA in sera was adsorbed onto the column. The final yield of SCCA was about 37%. SCCA migrated as a single protein band with molecular mass of 45 kDa on SDS-PAGE (Fig. 1). The inhibitory ability of this preparation towards serine and cysteine proteinases was measured. The results are summarized in Table 1. Although the amino acid sequences around the reactive site loop of serpins is homologous with that of SCCA [13], the latter did not inhibit serine proteinases. SCCA specifically inhibited cathepsin L and papain but not cathepsins B and H, which are all cysteine proteinases. The inhibition was independent of the preincubation time of the mixture of SCCA and proteinase. A Lineweaver–Burk plot showed that SCCA non-competitively inhibited cathepsin L with a K_i value of 0.064 nM (Fig. 2).

There are at least sixteen families of serine proteinase inhibitors and most of them obey the 'canonical' mechanism of inhibition [19,20]. The target proteinase specificity of inhibitory serpins is determined by the nature of the reactive site peptide bond (P1–P1'). The P1–P1' bond, Ser–Ser, of SCCA, which has yet to be found in other members of the serpin family, was not a reactive site for serine proteinases. There are non-functional serpins, which have structural variations such as the exposed helix in intact ovalubumin [21] and the extreme form of loop insertion in latent plasminogen activator inhibitor-1 [22]. The reason why SCCA was non-functional may be explained by the presence of structural variations that prevent the reactive site peptide bond being susceptible to serine proteinases.

Recently, it has been reported that cytokine response modifier A (CrmA), one of the serpins, inhibited an interleukin-1β converting enzyme, a member of a new family of cysteine proteinases [23]. SCCA significantly inhibited cathepsin L with a K_i value similar to that of two specific protein proteinase inhibitors of the enzyme, cystatins and cathelin [24,25]. Although the active site of SCCA towards cysteine proteinases can not be determined based upon the information of sequence homology available, and its inhibition mechanism remains to be established, the above evidence indicates another example of 'cross-class' interaction between a proteinase and its inhibitor. The role of SCCA is still unknown. Cathepsin L is synthesized in large amounts and secreted by many malignantly transformed cells in vitro [26] and the elevated expression of cathepsin L is associated with malignancy in several types of human cancer [27]. The results reported here suggested that SCCA is an important regulator of cathepsin L activity in the malignant behavior of tumor cells.

Table 1
Inhibitory activities of SCCA on serine and cysteine proteinases

Proteinases	Inhibitory activity (%)
Trypsin	N.I.
Cymotrypsin	N.I.
Thrombin	N.I.
Plasmin	N.I.
Urokinase	N.I.
Plasminogen activator	N.I.
Elastase	N.I.
Cathepsin G	N.I.
Cathepsin B	N.I.
Cathepsin H	N.I.
Cathepsin L	78
Papain	80

Inhibition assays were performed as described in section 2. The reaction mixture was incubated for 5 min at 25°C at a SCCA : proteinase ratio of 3.7 : 1 (mol/mol). N.I., not inhibited.

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