The Squamous Cell Carcinoma Antigen 2 Inhibits the Cysteine Proteinase Activity of a Major Mite Allergen, Der p 1*

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The squamous cell carcinoma antigens 1 (SCCA1) and SCCA2 belong to the ovalbumin-serpin family. Although SCCA1 and SCCA2 are closely homologous, these two molecules have distinct properties; SCCA1 inhibits cysteine proteinases such as cathepsin K, L, and S, whereas SCCA2 inhibits serine proteinases such as carhinspin G and human mast cell chymase. Although several intrinsic target proteinases for SCCA1 and SCCA2 have been found, the biological roles of SCCA1 and SCCA2 remain unknown. A mite allergen, Der p 1, is one of the most immunodominant allergens and also acts as a cysteine proteinase probably involved in the pathogenesis of allergic diseases. We have recently shown that both SCCA1 and SCCA2 are induced by two related Th2-type cytokines, IL-4 and IL-13, in bronchial epithelial cells and that SCCA expression is augmented in bronchial asthma patients. In this study, we explored the possibility that SCCA proteins target Der p 1, and it turned out that SCCA2, but not SCCA1, inhibited the catalytic activities of Der p 1. We furthermore analyzed the inhibitor mechanism of SCCA2 on Der p 1. SCCA2 contributed the suicide substrate-like mechanism without formation of a covalent complex, causing irreversible impairment of the catalytic activity of Der p 1 as SCCA1 does on papain. In addition, resistance to cleavage by Der p 1 also contributed to the inhibitor mechanism of SCCA2. These results suggest that SCCA2 acts as a cross-class serpin targeting an extrinsic cysteine proteinase derived from house dust mites and that it may have a protective role against biological reactions caused by mites.

The squamous cell carcinoma antigens 1 (SCCA1; SERPINB3) and SCCA2 (SERPINB4) belong to the ovalbumin-serpin (serine proteinase inhibitors) family and are 91% identical at the amino acid level (1). Both genes locate at 18q21.3 very closely, suggesting that either gene could arise from the other by gene duplication (2). SCCA1 was originally purified from squamous cell carcinoma of uterine cervix (3), and it turned out that SCCA1 and SCCA2 are co-expressed broadly in normal tissues: the epithelium of tongue, tonsil, esophagus, uterine cervix, vagina, and the conducting airways; Hassall's corpuscles of the thymus; and some areas of the skin (4). Although SCCA1 and SCCA2 are very homologous, these two molecules have distinct properties; SCCA1 inhibits cysteine proteinases such as cathepsin K, L, and S, and papain, whereas SCCA2 inhibits serine proteinases such as carhinspin G and human mast cell chymase (1, 5, 6). The specificities of SCCA1 and SCCA2 are due to the difference in the reactive site loop (RSL) sequences because only 7 amino acid residues among 13 (54%) were identical in the RSL regions (P7 to P6) of these proteins (7). Although target proteinases for most serpins are the chymotrypsin family, the serpin inhibiting cysteine proteinases is defined as a cross-class inhibitor. Cytokine response modifier A (CrmA) derived from cowpox virus and protease inhibitor 9 (PI9, SERPINB9) inhibits both a serine proteinase (granzyme B) and a cysteine proteinase (caspase proteins) (8–11). So thus far, SCCA1, CrmA, and PI9 are all obvious cross-class serpins (12). Although several intrinsic target proteinases for SCCA1 and SCCA2 have been found, the biological roles of SCCA1 and SCCA2 remain unknown. We have recently shown that expression of both SCCA1 and SCCA2 is up-regulated by two related Th2-type cytokines, IL-4 and IL-13, in bronchial epithelial cells and that SCCA expression is augmented in bronchial lesions and in peripheral blood of bronchial asthma patients (13). It is well known that IL-4 and IL-13 are involved in the pathogenesis of bronchial asthma (14, 15), predominately expressed in the lesions of asthma patients (16–18). These findings raise the possibility that SCCA1 and SCCA2 may perform their activities in the lesions of bronchial asthma.

Der p 1 and Der f 1, group I allergens derived from house dust mites, Dermatophagoides pteronyssinus and Dermatophago ides farinae, respectively, are major components of mites (10–20%), and their presence is closely correlated with development of bronchial asthma, atopic dermatitis, and allergic rhinitis (19–22). It has been reported that more than half of anti-mite allergen antibodies and 10–20% of total IgE in aller-

TOF, matrix-associated laser desorption ionization time-of-flight; PEO-M, polyethylene oxide-maleimide.
gic patients are anti-Der p 1 antibodies, which indicates that Der p 1 is one of the most immunodominant allergens (19, 21). Der p 1 is a 25-kDa cysteine proteinase; its amino acid sequence conserves 3 critical amino acids (Cys-34, His-170, and Asn-190) comprising the catalytic triad as other cysteine proteinases (23). The structure of Der p 1 has been modeled based on the crystal structure of papain, suggesting that Der p 1 is composed of two domains separated by a cleft, where the active site with the catalytic triad locates (24). It has been reported that Der p 1 cleaves several proteins such as occludin (25), protease-activated receptor 2 (PAR-2) (26), CD23 (27), CD25 (28), and CD40 (29) in vitro. Although the precise role of the catalytic activity of Der p 1 in vivo has not been elucidated, the following results indicate that the catalytic activity of Der p 1 would be important for the pathogenesis of bronchial asthma, in addition to its antigenicity. 1) Der p 1 disrupts tight junctions by cleaving occludin, increasing the permeability of the bronchial epithelial barrier (25). 2) Der p 1 causes secretion of inflammatory cytokines such as IL-6, IL-8, granulocyte-macrophage colony-stimulating factor, and RANTES in bronchial epithelial cells by activating PAR-2 (26, 30, 31). 3) Der p 1 induces the Th2 subset by cleaving CD25 on T cells (28) or CD40 on dendritic cells (29).

We hypothesized that SCCA proteins induced by IL-4 and IL-13 target extrinsic proteinases derived from house mites in the lesions of bronchial asthma. To explore this possibility, we examined whether SCCA1 and SCCA2 inhibit the catalytic activities of group I mite allergens in this study. It turned out that SCCA2 inhibited the catalytic activities of both Der p 1 and Der f 1 and that SCCA2 may be resistant to the cleavage by Der p 1. These results suggest that SCCA2 acts as a cross-class serpin targeting extrinsic cysteine proteinases, Der p 1 and Der f 1, and that SCCA2 may have a protective role against mite-caused biological reactions.

### EXPERIMENTAL PROCEDURES

**Materials**—Papain, E-64, cathepsin G, cathepsin L, and human mast cell chymase were purchased from Sigma, Peptide Institute Inc. (Osaka, Japan), Calbiochem, Athens Research & Technology (Athens, GA), and Cortex Biochem (San Leandro, CA), respectively.

**Generation of Plasmids and Recombinant Proteins—**SERPINB3 and SERPINB4 cDNA incorporated into pGEX-KG-4T (Amersham Biosciences) were prepared as reported before (32). SCCA2 mutants were generated by oligonucleotide-directed mutagenesis using two complementary primers with mutations. Standard PCR amplification was performed using the SCCA2 cDNA as a template and a mixture of primers. DNA fragments with mutations were ligated into pGEX-KG-SCCA2 plasmid. The RSL-replaced mutants of SCCA1 and SCCA2 were similarly generated by digestion and ligation into the StuXbaI site of them.

GST-fused SCCA1 and SCCA2 proteins were expressed in an Escherichia coli strain, BL21, and isolated by using glutathione-Sepharose 4B beads (Amersham Biosciences). Purity of the generated proteins was greater than 95%, as estimated by Coomassie staining of an SDS-PAGE gel. Concentrations of the proteins were determined by Protein Assay (Bio-Rad).

**Generation of the Der p 1 and Der f 1 Protein—**Recombinant Der p 1 and Der f 1 proteins were generated as described before (33, 34). Briefly, proforms of four recombinant house dust mite group 1 allergens, Der p 1-N52Q, Der p 1-WT, Der f 1-N53Q, and Der f 1-WT, were secreted into the culture supernatant of transfected cells of Pichia pastoris and converted to the mature forms with processes removed by dialysis against an acidic buffer. The mature forms were purified with anion exchange column chromatography. The purity was more than 95%, as estimated by SDS-PAGE. The protein concentration was determined by Protein Assay. Der p 1-N52Q and Der f 1-N53Q were used for most experiments.

**Enzyme Assays—**Enzyme assays of Der p 1 and Der f 1 were performed as described before (33, 35). The substrate used for enzyme assay was butyloxycarbonyl-Gln-Arg-methylcoumarin (Boc-Gln-Ala-Arg-MCA), purchased from Peptide Institute Inc. The indicated concentrations of Der p 1 or Der f 1 preactivated with 2 mM dithiothreitol for 10 min at 25 °C were incubated with the indicated concentrations of GST-fused SCCA proteins for 30 min at 25 °C in activity-measuring buffer (50 mM sodium phosphate, pH 7.0, 1 mM EDTA, 2 mM dithiothreite-

### FIG. 1. Inhibitory effects of SCCA molecules on the catalytic activities of group I house mite allergens. In A, SCCA1 (square) or SCCA2 (circle) or E-64 (rhombus) was incubated with 10 nM preactivated Der p 1 (closed) or Der f 1 (open) at the indicated I0/E0 ratio for 30 min at 25 °C. Residual enzyme activities are depicted. In B, SCCA1 (closed triangles) or SCCA2 (closed circles) or GST alone (closed squares) was incubated with the indicated concentrations of preactivated Der p 1 at the I0/E0 ratio = 1 for 30 min at 25 °C. Inhibitory activities are depicted.
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Fig. 2. Inhibitory effects of SCCA molecules on the cleavage of CD25 by Der p 1. One μM SCCA1 or SCCA2 was incubated with 2 μM preactivated Der p 1 for 30 min at 25 °C. Then, stimulated Jurkat T cells were incubated with a mixture of Der p 1 and SCCA proteins with final concentrations 0.6 and 0.3 μM, respectively, for 2 h at 37 °C. Expression of CD25 on the cell surface (A) and amount of soluble CD25 (B) are depicted.

Fig. 3. Identification of the cleaved sites of SCCA molecules by Der p 1. Ten μM SCCA1 or SCCA2 and 5 μM Der p 1 were incubated for 2 h at 4 °C, and the reactive samples were applied to MALDI-TOF mass spectrometry. The detected peaks (A) and the identified cleavage sites (B) are depicted. The arrows represent the peptides from the indicated residues to the C terminus (A).

RESULTS

Expression and Purification of Functional SCCA1 and SCCA2—To perform functional analyses of SCCA1 and SCCA2, we expressed and purified recombinant proteins of GST-fused SCCA1 and SCCA2. We confirmed that SCCA1 inhibited the cysteine protease activities of papain and cathepsin L but not the serine protease activities of cathepsin G and human mast cell chymase, whereas SCCA2 showed the opposite effects, as reported previously (32). These results demonstrated that purified SCCA1 and SCCA2 proteins were functional.

Inhibition of Catalytic Activities of Der p 1 and Der f 1 by SCCA2—We first analyzed whether SCCA1 or SCCA2 inhibited catalytic activities of Der p 1 and Der f 1. The $k_{cat}$ and $K_m$ values of Der p 1 used in the experiments were estimated as 0.444 ± 0.0173 s⁻¹ and 248 ± 16.2 μM, respectively (Table I). An irreversible inhibitor for cysteine proteinase, E-64, displayed only 39% of inhibition at a 10:1 ratio at 10 nM Der p 1 or
Therefore, active site titration of Der f 1 was impossible, so we analyzed dose-dependent effects of SCCA1 and SCCA2 on the catalytic activity of Der f 1 and Der f 1. When the concentration of Der f 1 or Der f 1 was fixed at 10 μM, SCCA2 inhibited catalytic activities of both Der f 1 and Der f 1 in a dose-dependent manner, whereas SCCA1 showed only 10% inhibition at a 10:1 ratio (Fig. 1A). The inhibitory effects of SCCA2 were independent of glycosylation of Der f 1 and Der f 1 (data not shown). When the ratio of Der f 1 and SCCA1 or SCCA2 was fixed at 1:1, the inhibitory effect of SCCA2 increased dependent of the concentration, reaching almost 100% at 1 μM (Fig. 1B). SCCA1 showed a weaker inhibitory effect when compared with SCCA2. GST alone had no inhibitory effect. These results demonstrated that SCCA2, and to a lesser extent SCCA1, targeted Der f 1 and that SCCA2 is a cross-class serpin that can inhibit both serine and cysteine proteinases as well as CrmA, PI9, and SCCA1.

Inhibitory Effects of SCCA Proteins on CD25 Cleavage by Der f 1—To examine whether SCCA2 shows its inhibitory effect on Der f 1, when Der f 1 targets not only the synthetic peptide, but also an intact protein as a substrate, we analyzed the inhibitory effects of SCCA proteins on CD25 cleavage by Der f 1. Incubation of Der f 1 with activated Jurkat cells caused the cleavage of CD25 (Fig. 2A, 31 ± 2.0%, n = 3). The presence of SCCA2 completely restored expression of CD25, and so did SCCA1 to some extent. Furthermore, existence of the soluble CD25 in the supernatant was observed in parallel with the cleavage of CD25 on Jurkat cells (Fig. 2B). These results demonstrated that SCCA2, and to a lesser extent SCCA1, targeted Der f 1 and that SCCA2 is a cross-class serpin that can inhibit both serine and cysteine proteinases as well as CrmA, PI9, and SCCA1.

Determination of the Cleavage Sites in SCCA1 and SCCA2 by Der f 1—We next identified the cleavage sites in RSLs of SCCA1 and SCCA2 by Der f 1, using MALDI-TOF mass spectrometry. Analysis of Der f 1-cleaved peptides in SCCA1 by MALDI-TOF mass spectrometry showed the existence of four...
peaks corresponding to the peptides from Phe-352, Gly-353, Ser-354, and Ser-355 to the C terminus, whereas the analyses with SCCA2 displayed only one peak, corresponding to the peptide from Leu-354 to the C terminus (Fig. 3). These results indicated that Der p 1 would interact with SCCA2 firmly, generating a serpin-proteinase complex as other serpins do. In contrast, the interaction between Der p 1 and SCCA1 would not be tight, and Der p 1 would cleave non-specifically the RSL of SCCA1.

Non-covalent Binding of SCCA2 with Der p 1—Although it is well known that a serpin and its target proteinase form an acyl-enzyme intermediate linked by an oxy-ester bond, stable for hydrolysis (36), we have recently demonstrated that SCCA2 inhibited the catalytic activity of papain without forming a covalent bond (32). We next analyzed the association manner of SCCA2 and Der p 1. To retain the native association between SCCA2 and Der p 1, we employed a gel-filtration system. We used a mixture of Der p 1 and SCCA2 in which the catalytic activity of Der p 1 was completely inhibited. Subsequently, SCCA2 and Der p 1 were eluted according to their molecular masses (Fig. 4A). These results demonstrated that SCCA2 interacted with Der p 1 by non-covalent binding as well as the interaction between SCCA1 and papain.

Irreversible Inhibition of Der p 1 by SCCA2 Treatment—We next investigated how SCCA2 inhibited the catalytic activity of Der p 1 without forming a covalently bound complex. To study the effects of the interaction between SCCA2 and Der p 1, we analyzed the catalytic activity of the SCCA2-treated Der p 1 eluted by the gel-filtration column. Although the catalytic activity of Der p 1 was completely inhibited in the solution containing 2 μM Der p 1 and SCCA2, which was applied to the column, it turned out that the catalytic activity of fractionated Der p 1 decreased, but still existed, when compared with the solution before fractionation (36 ± 9.4%, n = 3, Fig. 4B). The $k_{\text{cat}}/K_m$ value of SCCA2-treated Der p 1 was significantly less than that of BSA-treated Der p 1 (54.2 ± 4.40 versus 1790 ± 46.3 s$^{-1}$ μM$^{-1}$, $p = 0.0000003$, Table I), which confirmed the impairment of the catalytic activity.

These results raised the possibility that SCCA2 treatment caused a conformational change of Der p 1, down-regulating its catalytic activity. To explore this possibility, we compared chemical modification of SCCA2-treated or BSA-treated Der p 1 by biotin-conjugated maleimide, a cysteine residue-modifying reagent. Modification of SCCA2-treated Der p 1 by biotin-conjugated maleimide was reduced when compared with the level of BSA-treated Der p 1 (56 ± 14%, n = 3, Fig. 4C). These results suggest that down-regulation of the catalytic activity of Der p 1 may be due to its conformational change, although we cannot exclude the possibility that the cysteine residue of the active center was unexpectedly modified by SCCA2.

### Table II

**Alignment of RSLs of SCCA proteins and their inhibitory activities**

| Position | Proximal hinge | Reactive site loop | Distal hinge | Inhibition |
|----------|----------------|-------------------|--------------|------------|
| 1 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 1’ | 2’ | 3’ | 4’ | 5’ | 6’ | 11’ |
| SCCA2 | G V E A A A A A | T A V V V V V E L S S P S T | C | + |
| SCCA1 | V | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 RSL1 | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA1 RSL2 | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 mut1 (V351G) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 mut2 (V352F) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 mut4 (L354S) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 mut3 (E353G) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 E353A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 E353Q | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 mut5 (S356P, P357T) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 tm (E353G, S356P, P357T) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SCCA2 tm P356A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

* a: the same amino acid as SCCA2.

### Resistance of SCCA2 against Cleavage by Der p 1—Although the irreversible conformational change of Der p 1 contributed to the inhibition mechanism of SCCA2, it could not fully explain it because fractionated Der p 1 still showed catalytic activity at the reducing level (Fig. 4B). As only intact serpin sustains its inhibitory activity in the suicide substrate-like mechanism, it would be possible that the difference of susceptibility to cleavage by the target proteinase could affect the inhibitory activity of serpin. To explore this possibility, we analyzed the digestion profile of SCCA2 in the presence of Der p 1 in a time-dependent manner. It turned out that SCCA2 was resistant to digestion by Der p 1 when compared with SCCA1 and that 53% of SCCA2 still existed, intact, after 30 min of incubation (Fig. 5). When SCCA1 was incubated with papain, intact SCCA1 immediately began to decrease and was completely lost within 15 min. These results demonstrated that resistance against the cleavage by the target proteinase was a unique property of SCCA2, and it could at least partially explain why SCCA2 exerted its potent inhibitory activity on Der p 1 when compared with SCCA1.

### Preference of Amino Acids in the RSL Sequences of SCCA2 and SCCA1 for Inhibitory Effect on Der p 1—The distinct properties of SCCA1 and SCCA2 regarding the inhibitory effects on Der p 1 are assumed to be due to the difference of their RSL sequences. Actually, swapping the RSL of SCCA1 for that of SCCA2, or vice versa, revealed that the inhibitory effect on Der p 1 was dependent on the RSL of SCCA2 (Table II, SCCA1 RSL2, SCCA1 RSL1). We then exchanged each amino acid specific for the RSL of SCCA2 with that corresponding to SCCA1 and analyzed its inhibitory effect on Der p 1 (Table II). When Val-351, Val-352, or Leu-354 was replaced with Gly, Phe, or Ser, respectively, all of the mutated types attenuated the inhibitory effect (SCCA2 mut1, SCCA2 mut2, SCCA2 mut4), demonstrating that these residues were critical. Surprisingly, when Glu-353 or both Ser-356 and Pro-357 were exchanged with Gly or Pro and Thr, respectively, the inhibitory effect was augmented when compared with native SCCA2 (SCCA2 mut3, SCCA2 mut5). Furthermore, when Glu-353, Ser-356, and Pro-357 were all replaced with Gly, Pro, and Thr, respectively, the inhibitory action was dramatically up-regulated (Fig. 6, A and B, SCCA2 tm). Although Glu-353 was exchanged with Ala or Gln instead of Gly, the inhibitory effect was more enhanced than native SCCA2 (SCCA2 E353A, SCCA2 E353Q), indicating that removal of the ionic strength of Glu-353 would be important for up-regulation of the inhibitory effect.
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Fig. 6. Inhibitory effects of SCCA2 tm on the catalytic activity of Der p 1. In A, SCCA2 tm (triangle) was incubated with 10 nM Der p 1 (closed) or Der f 1 (open) at the indicated I/E ratio, as shown in Fig. 1. In B, SCCA2 tm (open circles) was incubated with the indicated concentrations of Der p 1 at the I/E ratio = 1, as shown in Fig. 1. The results of Fig. 1, A and B, are superimposed. In C, 10 nM Der p 1 was incubated with a 1:1000 ratio of each synthetic peptide for 30 min at 25 °C. Residual enzyme activities are depicted.

demonstrating the importance of the amino acid sequence of the RSL (SCCA1 RSL2 tm, data not shown).

To validate the importance of the amino acid sequence of the RSL in SCCA2 tm, we generated synthetic peptides corresponding to the RSLs of SCCA1, SCCA2, and SCCA2 tm, and then analyzed their inhibitory effects. At 1:1000 ratio, the peptide corresponding to SCCA2 tm, but not the peptides corresponding to SCCA1 and SCCA2, inhibited the catalytic activity of Der p 1 (Fig. 6C), again demonstrating the critical role of the sequence of the RSL in SCCA proteins.

Characterization of SCCA2 tm—We then compared the biochemical characteristics of SCCA2 and SCCA2 tm. It turned out that the biochemical properties of SCCA2 tm were almost the same as SCCA2. 1) It blocked the cleavage of CD25 by Der p 1 (data not shown). 2) It was cleaved by Der p 1 between Gly-353 and Leu-354 (data not shown). 3) It did not form a covalent complex with Der p 1 (data not shown). 4) SCCA2 tm caused irreversible inhibition on the catalytic activity of Der p 1 (33 ± 17%, n = 3, Fig. 4B), and incorporation of maleimide decreased to the same level as SCCA2 (48 ± 16%, n = 3, Fig. 4C). However, SCCA2 tm was more resistant to cleavage by Der p 1 than SCCA2 (67.5 ± 7.3% versus 53.1 ± 8.8% at 30 min, 68.0 ± 4.1% versus 47.7 ± 13.8% at 60 min, Fig. 5). These results demonstrated that the difference of resistance against cleavage by Der p 1 contributed to the different inhibitory activities of SCCA2 and SCCA2 tm.

DISCUSSION

In this study, we demonstrated that SCCA2 inhibited the cysteine proteinase activity of group I mite allergens, Der p 1 and Der f 1. Although it had been thought that SCCA2 targeted only serine proteinases, SCCA2 inhibits both serine and cysteine proteinases belonging to the cross-class serpin family, as well as CrmA, PI9, and SCCA1. It has been reported that Der p 1 performs various biological activities correlated with allergic reactions such as disruption of tight junction by cleaving occludin (25), secretion of inflammatory cytokines in bronchial epithelial cells by activating PAR-2 (26, 30, 31), and induction of Th2 subset by cleaving CD25 or CD40 (28, 29) in vitro. Although the precise pathological role of the catalytic activity of Der p 1 in vivo remains unclear, it is assumed that the cysteine proteinase activity of Der p 1 acts as a trigger or a worsening factor of bronchial asthma, based on the in vitro data. If this were the case, SCCA2 might play a protective role against group I mite allergens in the lesions of bronchial asthma, although IL-4 and IL-13, which induce expression of SCCA2 in bronchial epithelial cells, are themselves known to be involved in the pathogenesis of bronchial asthma (14, 15). The interaction between SCCA2 and group I mite allergens may help us elucidate the complexity of bronchial asthma. Alternatively, SCCA proteins might play a protective role against cysteine proteinases derived from parasites because it is known that some cysteine proteinases derived from Leishmania mexicana affect its virulence (37) and that IL-4/IL-13 protect against various parasites (38).

We next examined the inhibitory mechanism of SCCA2 on Der p 1, and the following events occurred. 1) SCCA2 was cleaved at the predicted site in its RSL (Fig. 3). 2) SCCA2 and Der p 1 did not form a complex with a covalent binding (Fig. 4A). 3) Interaction with SCCA2 partially impaired the catalytic activity of Der p 1, probably by irreversible conformational change (Fig. 4, B and C). The serpins employ a suicide substrate-like inhibitory mechanism in which the exposed RSL of the serpin is recognized by the proteinase, and then a “bait” peptide bond (P1-P1') that mimics the normal substrate of the proteinase is attacked by the active serine residue of the proteinase (12, 39). Upon the interaction, a standard serpin forms an acyl-enzyme intermediate with a serine proteinase linked by an oxy-ester bond. In its cleaved form, the P side of the RSL inserts into the body of the protein, which dramatically changes the conformations of the serpin and the proteinase, rendering it impossible for the ester bond to hydrolyze (36).
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have recently shown that the inhibitory mechanism of another cross-class serpin, SCCA1, is unique among the serpin superfamily in that SCCA1 performs its inhibitory activity in two ways: contributing the suicide substrate-like mechanism without formation of a covalent complex and causing irreversible impairment of the catalytic activity of papain (32). The biochemical events occurring in SCCA2 and Der p 1 described above indicate that this mechanism would be common among cross-class serpins. It is speculated that the thiol-ester bond between a cross-class serpin and its target cysteine proteinase was unstable or that the distorted ester bond located not so far from catalytic partners, enabling the ester bond to hydrolyze. Concomitantly, the interaction may induce conformational change of the target proteinase, which irreversibly loses its catalytic activity. The reports that other cross-class serpins, CrmA and P19, do not form SDS-resistant complexes with caspase proteins, although they do so with a serine proteinase, granzyme B (8–11), may indicate the same properties.

We also found that SCCA2 was resistant to cleavage by Der p 1 when compared with SCCA1 (Fig. 5). This is a unique property of SCCA2 contributing to the inhibition mechanism against Der p 1, different from the interaction of SCCA1 with papain. The analyses of substitution of each amino acid suggested that Leu-354 at SCCA2 would be critical for resistance against Der p 1 because SCCA2 mut4, in which Leu-354 was replaced with Ser, was susceptible to cleavage by Der p 1, diminishing the inhibitory activity against Der p 1 (Table II and data not shown). It is assumed that Leu-354 would block the nucleophilic reaction of Cys-34 at Der p 1 toward the P1 residue (Glul-353), based on the homology modeling of the interaction between SCCA2 and Der p 1. In addition to the comparison between SCCA1 and SCCA2, by substituting each amino acid in the RSL of SCCA2, we unexpectedly succeeded in generating a very potent inhibitor (SCCA2 tm) when compared with native SCCA2 (Fig. 6). The analyses of amino acid replacement suggested that removal of ionic strength in Glul-353 would stabilize the interaction of the RSL and the cleft of Der p 1, leaving the SCCA molecule more resistant to the cleavage by Der p 1 (Table II and Fig. 5). Analysis of the comparison between SCCA2 and SCCA2 tm also supported the notion that enhancement of the resistance against cleavage by Der p 1 would lead to enhancement of the inhibitory activity of the SCCA2 molecule.

Although the precise role of the catalytic activity of Der p 1 in the pathogenesis of allergic diseases remains unclear, it is hoped that an inhibitor against its catalytic activity has the potential to be developed into a therapeutic reagent for allergic diseases arising from mite allergens. Actually, it has been reported that a peptide-based inhibitor, PTL11028, showed inhibitory effects for the catalytic activities of group I mite allergens and improved airway hyperreactivity, inflammation, and systemic sensitization induced by Der p 1 in rats (40, 41). Structural analyses of the interaction between SCCA/Der p 1 would give us a hint as to how to develop a novel low molecular weight compound to block the catalytic activity of group I mite allergens.

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