The interaction of streptokinase (SK) with human plasminogen (HPlg) was investigated using truncated SK peptides prepared by gene cloning techniques. SK(16–414) and SK(16–378) could activate HPlg as efficiently as the authentic SK. SK(60–414), which had been preincubated with SK(1–59), could also activate HPlg. SK(91–414), SK(127–414), and SK(158–414), at a concentration of one-tenth of HPlg, all failed to activate HPlg. However, the truncated SK peptides in complexes with equimolar HPlg could form amidolytically active virgin enzymes that slowly converted to human plasmin (HPlm) after a lag period of 15 min. SK(16–316) could not activate HPlg. No virgin enzyme was detected when SK(16–316) was incubated with equimolar HPlg, but the HPlg in the complex was modified to HPlm after reaction for 20 min. SK(220–414) and SK(16–251) had no ability to transform HPlg to virgin enzyme or to HPlm in equimolar complex with HPlg, although they could bind to HPlg. The functions of five regions in the SK molecule (a, Ile1–Lys59; b, Ser60–Asn90; c, Val158–Arg219; d, Tyr252–Ala316; e, Ser317–Ala378) in interaction with HPlg are deduced. Region a is important in stabilizing the conformation of the SK molecule, and region b is essential for HPlg activation. Region c is required for induction of the conformational changes of HPlg to virgin enzyme. Regions c and d are required for the conversion of HPlg to HPlm in the HPlgSK equimolar complex. Coordination of regions c, d, and e is essential for a virgin enzyme formation, and coordination of regions b, c, d, and e is required for an effective SK-type HPlg activator.

STUDIED WITH TRUNCATED STREPTOKINASE PEPTIDES*

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The abbreviations used are: SK, streptokinase; Plg, plasminogen; Plm, plasmin; HPlg, human plasminogen; HPlm, human plasmin; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; HPLC, high performance liquid chromatography.

Infarction.

Studies of the activation of HPlg by SK suggest that more than one intermediate step is involved in the entire process. In the first step, a 1:1 stoichiometric complex of HPlg and SK is formed, and the conformation of the catalytic domain of HPlg is altered to expose its enzyme-active center (6, 7, 9). The HPlg moiety in the complex has been named virgin enzyme since it has similar catalytic activity to human plasmin (HPlm), but the activating peptide bond of Arg360–Val561 is not cleaved (12). In the second reaction step, the HPlgSK complex is converted to HPlmSK, and then in the final reaction step, the HPlmSK catalyzes the hydrolysis of the specific activating peptide bond of Arg360–Val561 on substrate HPlg, resulting in the formation of HPlm (5, 8, 13–15).

The exact interaction sites of SK with HPlg and their functions, however, have not been determined. Proteolytic SK fragments obtained in the reactions of SK with human, rabbit, and dog Plg(m)s have been used to study the functions of the SK molecule (15–18). A 36 and a 25.7-kDa fragment obtained in the reaction with HPlm and dog Plg, respectively, can activate HPlg (15–17). A 17-kDa SK fragment consisting of Val143 (Glu148) to Arg289 (Lys293) obtained in the reaction with Sepharose-immobilized HPlg (HPlm) is the smallest SK fragment that can bind to HPlm (18). A SK fragment, SKo, Ser60–Lys333 (or Ser60–Lys332 according to the numbering used in this paper), is essential for minimal SK activator activity (19). Obviously, the COOH-terminal peptide of SKp, Ala334–Lys387 (or Ala334–Lys386), is required for strong binding with HPlm. The NH2-terminal 59-amino acid peptide is important in maintaining the proper conformation of SK for full activator activity (19). These studies imply that domain-like structures may exist in the SK molecule that exert various functions in HPlg activation. This study was undertaken to elucidate the functions of various domains of SK and to determine more precisely the interaction sites between SK and HPlg using a series of truncated SK peptides lacking NH2 and/or COOH-terminal amino acid residues.

EXPERIMENTAL PROCEDURES

Materials—Enzymes used in DNA manipulation were purchased from Boehringer Mannheim, Life Technologies, Inc., or Framo and were used according to the Cold Spring Harbor Manual or the recommendations of the suppliers. The two oligonucleotides used in SK gene amplification by polymerase chain reaction (PCR) were custom synthesized by Pan Asia Hospital Supply Co. (Taiwan, Republic of China). The oligonucleotide for sense primer was 5'-GGAGGGATCCATGAAAAAT-3' (nucleotide position 809–836 by the numbering convention for the Patl fragment containing SK) and for antisense primer was 5'-AAGAGGATCTTTTGGCTAGGTT-3' (nucleotide position 2151–2124) (1). For cloning convenience, BamHI recognition sequences (underlined) were created by replacing some of the nucleotides. Blue-Sepharose CL 6B, Lys-Sepharose, and DEAE-cellulose were from Pharmacia Biotech Inc. NH2–Val–Leu–Lys–p-nitroanilide (S-2251) was obtained from Sigma. SK antibody was prepared in our laboratory from
mice. All other chemicals were of the highest grade commercially available.

Proteins and Enzymes—HPlg was prepared from pooled human plasma by a modification of the Deutsch and Mertz method (20). Forms 1 and 2 of native HPlg were separated by chromatography on Lys-Sepharose column (21). Form 2 of HPlg was used throughout this study. HPlg was purified by activating HPlg with Sepharose-bound uridine diphosphate glucose (UDPG) as described previously (22). Native SK (Behringwerke AG, Marburg, Germany) was further purified by passing it through a Blue-Sepharose CL 6B column to remove serum albumin (15).

Construction of SK and Truncated SK Genes—The SK gene was amplified by PCR by the standard procedure from Streptococcus equisimilis 7.4 M (H46A) (24) using the 5′- and 3′-end primers described by Deutsch and Mertz (20), respectively. The 1.3-kb SK gene was subcloned into the BamHI site of the multiple cloning region of pGEM-Z vector (Promega) and propagated in Escherichia coli M109. Unidirectional deletion of the SK gene was carried out with exonuclease III using the Erase-a-Base system (Promega) to construct truncated SK genes from the NH₂ or COOH terminus. Nucleotide sequences of the SK and truncated genes were determined by dyeoxy sequencing method (23, 24).

Expression and Purification of Recombinant Truncated SK Peptides—Truncated SK genes were subcloned in-frame into the BamHI site of the overproducing plasmid pET-3 (Novagen), in which the cloned genes were inducibly expressed under the control of TT promoter in E. coli strain BL21(DE3)pLyS. Bacterial cells were grown to mid-log phase, and the target gene expression was induced by shifting the incubation temperature to 33 °C and adding 1 mg isopropy-1-thio-β-D-galactopyranoside. 3 h later, cells were harvested, washed, and disintegrated by French press, then the proteins were concentrated by ammonium sulfate precipitation. The precipitated proteins were disintegrated by French press, then the proteins were concentrated by ammonium sulfate precipitation. The precipitated proteins were dialyzed and loaded onto a DEAE cellulose column. After elution with salt gradient, the active fractions were detected by HPlg activation with S-2251. Because the HPlg activator activities of some short peptides of SK were too low to detect, the fractions containing SK peptides were also analyzed by SDS-PAGE and Western blotting. Quantitation of the activities of truncated SK peptides in each purification step was conducted in flat bottomed 96-well plates by activating HPlg with S-2251. The HPlg activator activities of some short peptides of SK were too low to detect, the fractions containing SK peptides were also analyzed by SDS-PAGE and Western blotting. Quantitation of the activities of truncated SK peptides in each purification step was conducted in flat bottomed 96-well plates by activating HPlg to hydrolyze S-2251. A series diluted native SK (93,750 IU/mg) solution was used as standard.

Protein Concentration—Protein concentrations of SK peptides were determined with Folin reagent (Merck) by the Lowry method (25).

Amidolytic Activity—The amidolytic activity was measured by adding aliquots of the HPlg-SK-peptides (final concentration, 0.2 μM) in an assay cuvette containing 0.5 mM S-2251 in 0.05 M Tris buffer, pH 7.4, and 0.1 mM NaCl. The absorbance at 405 nm was monitored.

Continuous Assays of Amidolytic Activity for HPlg-SK Peptide—Virgin Enzyme Complexes—Incubations of equimolar HPlg and truncated SK peptides (final concentration, 2 μM) at 25 °C in 10 mM HEPES/NaOH, pH 7.4, for 30 min determined by the above method to achieve maximal active-site formation. The amidolytic activity was measured by adding aliquots of the HPlg-SK-peptides (final concentration, 0.2 μM) in an assay cuvette containing 0.5 mM S-2251 in 0.05 M Tris buffer, pH 7.4, and 0.1 mM NaCl. The absorbance at 405 nm was monitored.

Amidase Parameters of the HPlg-SK Peptide—Virgin Enzyme Complexes—HPlg (final concentration, 2 μM) was incubated with equimolar SK peptides in 10 mM HEPES/NaOH, pH 7.4, at 25 °C for 4–7 min to achieve the maximal active-site formation. The amidolytic activity was measured by adding aliquots of the HPlg-SK-peptides (final concentration, 0.2 μM) in an assay cuvette containing 0.5 mM S-2251 in 0.05 M Tris buffer, pH 7.4, and 0.1 mM NaCl. The changes in absorbance at 405 nm were monitored. The initial rate and substrate concentration data were analyzed on a Lineweaver-Burk plot.

Binding of Truncated SK Peptides to HPlg—SK or truncated SK peptide was labeled with 125I by the method described previously (31, 32). The binding analysis was carried out by coating a radiomunno-assay strip plate (Costar) with 0.2 μl of HPlg (2 mg/ml) in 0.05 M carbonate/bicarbonate buffer, pH 9.6 for 36 h at 4 °C, and blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS). After washing, 125I labeled SK or truncated SK peptide at increasing concentrations was added to the wells and incubated at 4 °C for 1 h. The unbound peptide was thoroughly washed out with PBS containing 0.05% Tween 20, and the radioactivity was determined with a LKB γ-counter. Nonspecific binding of the tested peptide was measured in the presence of a 30-fold excess of each unlabeled peptide and was subtracted from the total binding.

RESULTS

The SK gene of 1.3 kilobases was amplified by PCR with standard procedures from S. equisimilis H46A and was constructed into pGEM-3Z and pET3 plasmid. Unidirectional deletion of SK gene from either end was carried out with exonuclease III. Nine truncated SKs were prepared and designated SK (16–414), SK (60–414), SK (91–414), SK (127–414), SK (158–414), SK (220–414), SK (16–378), SK (16–316), and SK (16–251). The numbers in the parenthesis indicate positions of the initial and terminal amino acids of the corresponding truncated SK peptide according to the published amino acid sequence deduced from the nucleotide sequence of SK gene (1, 38). In sequencing the PCR-amplified full-length SK DNA, one primer mutation at Lys⁸⁸ (5′-AAG) and one mutation at Arg⁵⁰ (5′-AAG) to Pro⁵⁰ (5′-CTT) were observed, thereby maintaining with the SK gene sequences (1). The slightly modified SK DNA was used for preparation of truncated SK peptides without correction of these mutations. Apparently the alteration had no effect on the HPlg activator activity of recombinant SK since purified SK (16–414) had a specific activity of 118,755 IU/mg (Table I), which is comparable with purified commercially available native SK. The expressed recombinant SK and its truncated peptides were also sequenced and found to be identical to the published sequence (1, 2) except that an additional fusion peptide of 14–18 amino acid residues derived from the pET3 plasmid was attached at their NH₂ termini. The SK (16–414) DNA was used to prepare COOH-truncated SK, since SK (16–414) protein had the same HPlg activator activity as native SK, and the SK peptides without NH₂-terminal 15 amino acids were more easily overexpressed in the E. coli cells than the full-length SK. In general the NH₂- and COOH-truncated SK peptides overexpressed in the E. coli system consisted of more than 70% of the total amount of proteins in the crude extracts of the E. coli cells. Homogeneous SK peptides were obtained after ammonium sulfate precipitation and DEAE column chromatography as shown in SDS-PAGE (Fig. 1). All of the SK peptides could be detected by a polyclonal antiserum raised against the native SK. The recovery and specific activities of...
SK peptides during each purification step are summarized in Table I. The specific HPlg activator activities of purified SK(16–414) and SK(16–378) were higher than 100,000 IU/mg and were comparable with that of the purified commercial native SK. SK(60–414), SK(91–414), SK(127–414), SK(158–414), and SK(16–316) had low specific HPlg activator activities (Table I). The HPlg activator activities of the two short peptides, SK(16–251) and SK(220–414), were too low to detect.

SK(16–414) and SK(16–378) at a catalytic concentration (0.02 μM) could effectively catalyze the conversion of HPlg (2 μM) to HPlm, and an increasing rate of substrate hydrolysis was observed as more HPlm was produced (Fig. 2 A). The conversion of HPlg to HPlm was completed in 10 min as confirmed by SDS-PAGE. The bands of HPlg (94 kDa) disappeared, and both the heavy (65 kDa) and light (26 kDa) chains were detected (Fig. 2 B). The second-order rate constants of HPlg activation, k_{Plg}/K_{Plg}, of native SK, SK(16–414), and SK(16–378) were similar (Table II). SK(60–414) had low HPlg activator activity (Table I). However, its activator activity could be dose-dependently enhanced by incubating with a catalytic amount of native SK with immobilized HPlm and purified by HPLC (Fig. 2 A). After 10 min of incubation, about half of HPlg was activated by SK(1–59) modified SK(60–414)*, while complete activation occurred after 30 min of incubation (Fig. 2 B). This result suggests that SK(1–59) may induce a conformational change in SK(60–414) to form an efficient activator. SK(91–414), SK(127–414), or SK(158–414) alone at a catalytic amount could not activate HPlg nor in the presence of SK(1–59).

SK(91–414) could form a catalytically active virgin enzyme

### Table I

| Truncated SK     | Step                  | Protein | Total activity | Specific activity | Yield | Purification |
|------------------|-----------------------|---------|----------------|-------------------|-------|--------------|
| SK(16–414)       | starting              | 954.0   | 1147800        | 1203              | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 157.0   | 950985         | 6057              | 82.9  | 5.0          |
|                  | DEAE                  | 5.0     | 593775         | 118755            | 51.7  | 98.7         |
| SK(60–414)       | starting              | 590.2   | 22280          | 38                | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 51.9    | 4488           | 87                | 20.1  | 2.3          |
|                  | DEAE                  | 3.7     | 3790           | 1024              | 17.0  | 26.9         |
| SK(91–414)       | starting              | 777.5   | 139500         | 179               | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 123.6   | 96600          | 782               | 69.2  | 4.4          |
|                  | DEAE                  | 14.0    | 75000          | 5357              | 53.8  | 29.9         |
| SK(127–414)      | starting              | 902.2   | 237300         | 263               | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 124.4   | 162200         | 1304              | 68.4  | 5.0          |
|                  | DEAE                  | 13.6    | 74636         | 5488               | 31.5  | 20.9         |
| SK(158–414)      | starting              | 616.0   | 5440           | 9                  | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 152.4   | 4376           | 29                | 80.4  | 3.2          |
|                  | DEAE                  | 11.7    | 3790           | 323               | 69.5  | 35.9         |
| SK(16–378)       | starting              | 1034.0  | 2516000        | 2433              | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 197.8   | 1006400        | 5088              | 40.0  | 2.1          |
|                  | DEAE                  | 7.6     | 920000         | 121053            | 36.6  | 49.8         |
| SK(16–316)       | starting              | 620.2   | 36880          | 60                | 100.0 | 1.0          |
|                  | (NH₄)₂SO₄             | 52.3    | 22034          | 421               | 59.7  | 7.0          |
|                  | DEAE                  | 2.6     | 2197           | 845               | 6.0   | 14.1         |

**Fig. 1.** SDS-PAGE of purified truncated SK peptides. Lane M, molecular mass marker; lane a, purified native SK; lane b, SK(16–414); lane c, SK(60–414); lane d, SK(91–414); lane e, SK(127–414); lane f, SK(158–414); lane g, SK(220–414); lane h, SK(16–378); lane i, SK(16–316); lane j, SK(16–251).

**Fig. 2.** Activation of HPlg by SK(16–414), SK(16–378), and SK(60–414)*. A, rate of activation; B, SDS-PAGE analysis. HPlg (2 μM) was activated by incubation with a catalytic amount of activator, 0.02 μM SK(16–414) (●), 0.02 μM SK(16–378) (○), or 0.2 μM SK(60–414)* (□), at 37 °C in 0.05 M Tris, 0.1 M NaCl, pH 7.4, containing 0.5 mM S-2251. SK(60–414)* was prepared by premixing SK(60–414) (0.2 μM) and SK(1–59) 0.01 μM (△) or SK(1–59) 0.005 μM (□) at 25 °C for 1 min. An incubation with SK(1–59) 0.01 μM was used as control (○). The change in absorbance at 405 nm was monitored. Parallel samples of HPlg activation for 10 min and 30 min at 37 °C were also taken for SDS-PAGE analysis (B). Lane M, molecular mass marker; lane a, HPlg control; lane b, SK(16–414), 10 min; lane c, SK(16–378), 10 min; lane d, SK(16–378), 30 min; lane e, SK(16–378), 30 min; lane f, SK(60–414)*, 10 min; lane g, SK(60–414)*, 30 min; lane h, HPlm control.
with HPlg in a one-to-one equimolar complex. The equimolar HPlg and SK(91–414) had a maximal amidolytic activity after reaction for 7 min (Fig. 3A). However, no cleavage of the activating peptide bond, Arg^560-Val^563, of HPlg was observed at up to 13 min determined at intervals as shown by SDS-PAGE analysis (Fig. 3B), suggesting that a virgin enzyme, which consisted of intact HPlg and SK(91–414) equimolar complex, was induced. However, after reaction for more than 15 min, some HPlg was hydrolyzed, heavy and light chains of HPlm were detected, and SK(91–414) was degraded (Fig. 3B, lanes e and f). The cleavage of the activating peptide bond was also confirmed by NH$_2$-terminal amino acid determination of the HPlm light chain. Virgin enzymes could also be induced in stoichiometric complexes of HPlg and other truncated SK peptides, although different durations of incubation were required to reach maximal amidolytic activities. For example, to achieve the maximal amidolytic activities, it took 4 min for HPlg and SK(16–414) or SK(16–378), but 7 min for HPlg and SK(91–414), SK(127–414), or SK(158–414). The rates for hydrolysis of S-2251 by the virgin enzyme complexes of HPlg and truncated SK peptides are shown in Fig. 4A. The HPlgSK(16–414) and HPlgSK(16–378) had similar second-order reaction constant ($k_{app}/K_m$) as HPlm in hydrolysis of substrate S-2251 (Table III). The virgin enzymes of HPlgSK(91–414), HPlgSK(127–414), and HPlgSK(158–414) had lower reaction constants (Table III). Initially, no HPlm conversion was observed in each HPlgSK-peptide virgin enzyme complex, since only HPlg and the corresponding SK peptide were detected by SDS-PAGE (Fig. 4B). However, after incubation for 15 min, HPlm formation was also observed in the equimolar complexes of HPlgSK(127–414) and HPlgSK(158–414) as that of HPlgSK(91–414), suggesting that the activation peptide bond in these complexes was also cleaved.

The COOH-terminally truncated, SK(16–316) at a concentration one-tenth of HPlg, had no HPlg activator activity. It also failed to induce a virgin enzyme formation in an equimolar stoichiometric complex with HPlg under the same conditions described previously. However, enzyme activity of HPlm con-

![FIG. 3. Discontinuous assay for measuring the rates of activation site generation in HPlg and SK(91–414) complex. A, rate of amidolytic activity; B, SDS-PAGE analysis. HPlg (final concentration, 2 $\mu$M) was incubated with equimolar of SK(91–414) at 25 °C in 10 mM HEPES/NaOH, pH 7.4. Aliquots (final concentration, 0.2 $\mu$M) were removed at intervals for assay of amidolytic activity using S-2251 at a final concentration of 0.5 mM. Amidolytic activity was expressed as $\mu$M S-2251 hydrolyzed per min using an extinction coefficient (1 M, 1 cm, 405 nm) of 9559 as shown in A, parallel samples were also taken for SDS-PAGE as shown in B. Lane M, molecular mass marker; lane a, 1 min; lane b, 5 min; lane c, 11 min; lane d, 13 min; lane e, 15 min; lane f, 30 min.

![FIG. 4. Virgin enzyme formation of HPlg with various SK peptides in stoichiometric complexes. A, continuous assay for determination of virgin enzyme activity; B, SDS-PAGE analysis. HPlg (final concentration, 2 $\mu$M) was preincubated with equimolar of SK(16–414) (●) or SK(16–378) (○) for 4 min, or with SK(91–414) (□), SK(127–414) ( ), or SK(158–414) ( ) for 7 min in 10 mM HEPES/NaOH, pH 7.4, at 25 °C to form a virgin enzyme complex. The complex (final concentration, 0.2 $\mu$M) was then added into 150 $\mu$L of 0.05 M Tris-HCl/0.1 M NaCl, pH 7.4, containing 0.5 mM S-2251 at 37 °C. The increment of absorbance at 405 nm of the mixture was recorded. The amidolytic activity of human plasmin (0.2 $\mu$M) ( ) was also shown as control. Parallel samples of virgin enzyme complex were also taken for SDS-PAGE as shown in B. Lane M, molecular mass marker; lane a, HPlgSK(16–414), 30 s; lane b, HPlgSK(16–414), 4 min; lane c, HPlgSK(16–378), 30 s; lane d, HPlgSK(16–378), 4 min; lane e, HPlgSK(91–414), 30 s; lane f, HPlgSK(91–414), 7 min; lane g, HPlgSK(127–414), 30 s; lane h, HPlgSK(127–414), 7 min; lane i, HPlgSK(158–414), 30 s; lane j, HPlgSK(158–414), 7 min.]}
**DISCUSSION**

The secondary structures of truncated SK peptides used in this study were determined by circular dichroism (CD) spectroscopy and were all found to be similar to that of the corresponding regions of the native SK moiety reported (34) except for SK(60–414). This result suggests that the recombinant truncated SK peptides were suitable for the study of structure-function relationship of SK. SK(60–414) had a majority of disordered secondary structure according to the CD spectroscopy. It also had a lower HPlg activator activity compared with the SK-p, Ser60-Lys387 (or Ser60-Lys386 according to the numbering used in this study) obtained by limited digestion of SK. The fusion peptide of 14 amino acid residues in the NH2-terminal of SK(60–414) might interrupt the proper conformation of SK(60–414). However, after incubation with SK(1–59), SK(60–414) was shifted to SK(60–414)*, with elevation of HPlg activator activity. This might be due to a conformational change in SK(60–414) induced by SK(1–59). Therefore, SK(1–59) was important in maintaining the proper conformation of the core region of SK (19).

Since SK(16–414) and SK(16–378) were as active as native SK in HPlg activation, the peptides Ile1-Ala382 (or Ile1-Ala383) and Ser379-Lys384 were of little functional importance for SK. The study in which two SK gene products, cSKL, Ile1-Ala384 (or Ile1-Ala383) according to the numbering used in this study), and cSKS, Ile1-Leu383 (or Ile1-Leu382 according to the numbering used in this study), were cloned suggested that Ala384 (or Ala383) was essential for activator activity of SK (35), since Ile1-Ala384 (or Ile1-Ala383) had full activator activity, while Ile1-Leu383 (or Ile1-Leu382) retained only 25% activity. However, SK(16–378) prepared in this study was as active as native SK in HPlg activation and also had a properly folded secondary structure. The low activator activity of cSKS might be due to reasons other than Ala384 (or Ala383) directly involved in activation of HPlg.

In this report, we defined five important regions in SK molecule as a HPlg activator. These regions were as follows: a, Ile1-Lys59; b, Ser60-Asn90; c, Val158-Arg219; d, Tyr252-Ala316; e, Ser317-Ala378 as shown in Scheme I.

Region a functioned to stabilize the conformation of SK in maintaining its full activator activity. SK(60–414), which contained regions b, c, d, and e, was a competent HPlg activator, although the activation rate was slower than those of SK(16–414) and SK(16–378) (Fig. 2A). SK(91–414), at a molar ratio of one-tenth of HPlg, could not activate free HPlg. However, an equimolar complex of HPlg and SK(91–414) was amido-labilely active. The complex of HPlg and SK(127–414) or SK(158–414) had properties similar to SK(91–414). Therefore, the SK peptide, which contained regions c, d, and e, had the ability to form a so-called virgin enzyme complex with HPlg, and region b was essential for HPlg activation. The conversion of HPlg to HPlm in these one-to-one complexes of SK peptides and HPlg was detected after a lag period of 15 min (Fig. 3B). The reason of this slow HPlg conversion to HPlm in the complexes remains unclear. It is possible that the conformation of HPlg is transformed in the complex and the activating peptide bond is more vulnerable for hydrolysis by the less effective activators. In regard to the formation of virgin enzyme complex with HPlg, SK(220–414), which contained regions d and e, lost this ability. Therefore, the region c, Val158-Arg219, was thought to possess one of the essential interaction cores for virgin enzyme formation. The COOH-terminal truncated SK(16–378) had all the essential regions to form a virgin enzyme complex with HPlg and to catalyze HPlg activation. The SK(16–316), which consisted of regions a, b, c, and d but not e, could not form virgin enzyme and could not catalyze the activation of HPlg into HPlm as a typical SK-type HPlg activator. However, the HPlg moiety was slowly converted to HPlm in equimolar HPlgSK(16–316) complex but not in HPlgSK(16–251), in

**TABLE III**

| Enzyme species | Amidase parameters | Values are the mean ± S.E.M. of three experiments. |
|----------------|--------------------|--------------------------------------------------|
|                | $K_m \text{ (\mu M)}$ | $k_{cat} \text{ (s}^{-1})$ | $k_{cat}/K_m$ (s$^{-1}$·\mu M)$^{-1}$ |
| HPlm           | 355 ± 6            | 27.9 ± 0.3          | 0.078            |
| HPlg-SK(16–414)| 880 ± 36           | 58.7 ± 1.1          | 0.067            |
| HPlg-SK(16–378)| 878 ± 22           | 57.5 ± 1.1          | 0.065            |
| HPlg-SK(91–414)| 691 ± 9            | 27.1 ± 0.9          | 0.039            |
| HPlg-SK(127–414)| 617 ± 5           | 28.8 ± 0.2          | 0.047            |
| HPlg-SK(158–414)| 617 ± 5          | 3.6 ± 0.02          | 0.0091           |

**Fig. 5.** Discontinuous assay for measuring the amidolytic activity of HPlg and SK(16–316) complex. A, rate of amidolytic activity; B, SDS-PAGE analysis. HPlg (final concentration, 2 \mu M) was incubated with equimolar of SK(16–316) at 25°C in 10 mM HEPES-NaOH, pH 7.4. Aliquots (final concentration, 0.2 \mu M) were removed at intervals for assay of amidolytic activity using S-2251 at a final concentration of 0.5 mM. Parallel samples were also taken for SDS-PAGE as shown in B. Lane M, molecular-mass marker; lane a, 1 min; lane b, 20 min; lane c, 30 min; lane d, 45 min; lane e, 60 min; lane f, HPlm control.
Fig. 6. Binding of $^{[125]I}$-SK, $^{[125]I}$-SK(220–414), and $^{[125]I}$-SK(16–251) to HPIg. HPIg (2 mg/ml) was coated on a radioimmunoassay strip plate. $^{[125]I}$-Labeled native SK (●), $^{[125]I}$-labeled SK(220–414) (○), and $^{[125]I}$-labeled SK(16–251) (□) at various concentrations from 0.03 to 1 μM were added to the wells and incubated at 4 °C for 1 h. After washing out extensively, the radioactivity was determined. Each point represents the mean ± S.D. of three independent determinations and the nonspecific binding has been subtracted.

which SK(16–251) contained only regions a, b, and c. Therefore, region d apparently was involved in the induction of the conformational changes in HPIg, so that it could be activated in the complex.

In conclusion, by studying with the truncated SK peptides, five regions of defined functions in SK molecules were deduced. The functional studies of the truncated SK peptides provided the evidence that more than one region on SK could interact with HPIg. By comparing the functions of the truncated SK, we were able to define the function of each region of SK. These findings were consistent with the results of NMR and CD spectroscopy studies of SK in which a flexible structure of SK was observed of at least three or four domains was proposed (36, 37). The elucidation of the function of each of these specific regions of SK molecule is very important for understanding the molecular mechanism of its interaction with HPIg. Further studies on the functions of specific point mutation of SK in different regions might provide more critical information needed for the delineation of the intriguing interaction between SK and HPIg.

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REFERENCES
1. Malke, H., Roe, B., and Ferretti, J. J. (1985) Gene (Amst.) 34, 357–362
2. Jackson, K. W., and Tang, J. (1982) Biochemistry 21, 6620–6625
3. Tillet, W. S., and Garner, R. L. (1933) J. Exp. Med. 58, 489–502
4. Ling, C. M., Summaria, L., and Robbins, K. C. (1963) J. Biol. Chem. 240, 4213–4218
5. Wulf, R. J., and Mertz, E. T. (1969) Can. J. Biochem. 47, 927–931
6. McIntock, D. K., and Bell, P. H. (1971) Biochem. Biophys. Res. Commun. 43, 694–702
7. Reddy, K. N. N., and Markus, G. (1972) J. Biol. Chem. 247, 1683–1691
8. Schick, L. A., and Castellino, F. J. (1973) Biochemistry 12, 4315–4321
9. Reddy, K. N. N., and Markus, G. (1974) J. Biol. Chem. 249, 4851–4857
10. Schick, L. A., and Castellino, F. J. (1974) Biochem. Biophys. Res. Commun. 57, 47–54
11. Bai, S. P., and Castellino, F. J. (1977) J. Biol. Chem. 252, 492–498
12. Summaria, L., Wohls, R. C., Boreisha, I. G., and Robbins, K. C. (1982) Biochemistry 21, 2056–2059
13. Gonzalez-Gronow, M., Siefring, G. E., Jr., and Castellino, F. J. (1977) J. Biol. Chem. 252, 1090–1094
14. Shi, G. Y., Chang, B. I., Wu, D. H., and Wu, H. L. (1993) Biochem. Biophys. Res. Commun. 195, 192–200
15. Siefring, G. E., Jr., and Castellino, F. J. (1976) J. Biol. Chem. 251, 3913–3920
16. Brockway, W. J., and Castellino, F. J. (1974) Biochemistry 13, 2063–2070
17. Reddy, K. N. N., and Kline, D. L. (1976) Thromb. Res. 9, 407–411
18. Rodriguez, R., Fuentes, F., Munoz, E., Orta, D., Alburquerque, S., Perez, S., Besada, V., and Herrera, L. (1994) Fibrinolysis 8, 276–285
19. Shi, G. Y., Chang, B. I., Chen, S. M., Wu, D. H., and Wu, H. L. (1994) Biochem. J. 304, 235–241
20. Deutsch, D. G., and Mertz, E. T. (1970) Science 170, 1095–1096
21. Brockway, W. J., and Castellino, F. J. (1972) Arch. Biochem. Biophys. 151, 194–199
22. Wu, H. L., Shi, G. Y., and Bender, M. L. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8292–8295
23. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
24. Chen, E. Y., and Seeburg, P. H. (1985) DNA (N. Y.) 4, 165–170
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
26. Laemmli, U. K. (1970) Nature 227, 680–685
27. Burnette, W. N. (1981) Anal. Biochem. 112, 195–203
28. Wohls, R. C., Summaria, L., and Robbins, K. C. (1980) J. Biol. Chem. 255, 2052–2053
29. Shi, G. Y., Chang, B. I., Wu, D. H., Ha, Y. M., and Wu, H. L. (1990) Thromb. Res. 58, 317–329
30. Chibber, B. A. K., Radek, J. T., Morris, J. P., and Castellino, F. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1237–1241
31. Wu, H. L., Wu, Y. S., Fang, R. Y., Hau, J., Wu, D. H., Chang, B. I., Lin, T. M., and Shi, G. Y. (1992) Biochem. Biophys. Res. Commun. 188, 703–711
32. Wu, H. L., Chang, B. I., Wu, D. H., Chang, C. L., Gong, C. C., Lou, K. L., and Shi, G. Y. (1990) J. Biol. Chem. 265, 19658–19664
33. Misselwitz, R., Kraft, R., Kostak, S., Fabian, H., Welfle, K., Pfeil, W., and Welfle, H. (1992) Int. J. Biol. Macromol. 14, 107–116
34. Radek, J. T., and Castellino, F. J. (1989) J. Biol. Chem. 264, 9915–9922
35. Jackson, K. W., Malke, H., Gerlach, D., Ferretti, J. J., and Jordan, T. R. (1986) Biochemistry 25, 108–114
36. Teuten, A. J., Broadhurst, R. W., Smith, R. A. G., and Dobson, C. M. (1993) Biochem. J. 290, 313–319
37. Damaschun, G., Damaschun, H., Gast, K., Gerlach, D., Misselwitz, R., Welfle, H., and Zirwer, D. (1992) Eur. Biochem. J. 20, 355–361.
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