Plasminogen Activation by Streptokinase via a Unique Mechanism*

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The mechanism of human plasminogen (HPlg) activation by streptokinase (SK)-type activator was investigated with recombinant truncated SK peptides. An enzyme-substrate intermediate of HPlg-SK-HPlg ternary complex was demonstrated by a sandwich-binding experiment. Formation of the ternary complex was saturable, HPlg-specific, and inhibited by 6-aminocaproic acid. Three interaction sites between SK and HPlg were demonstrated. SK-(220–414) bound to HPlg with two binding sites: one to the micro-HPlg region, the catalytic domain of HPlg, and one to the kringle 1–5 region, with $K_d$ values of $1.8 \times 10^{-7}$ and $2.44 \times 10^{-8} \text{M}$, respectively. SK-(16–251) bound to a single site on the kringle 1–5 region of HPlg with a $K_d$ of $4.09 \times 10^{-7} \text{M}$. SK-(220–414) and SK-(16–251) competed for binding on the same or nearby location on the human kringle 1–5 domain. Combination of SK-(220–414) and SK-(16–251), but not either peptide alone, could effectively activate HPlg. In addition, SK-(16–251) dose-dependently enhanced the activation of HPlg by SK-(16–414), while the HPlg activation by SK-(16–414) was inhibited by SK-(220–414). We conclude that the HPlg that binds to the COOH-terminal domains of SK functions as an enzyme to catalyze the conversion of substrate HPlg that binds to the NH$_2$-terminal domain of SK to human plasmin.

Streptokinase (SK), a potent plasminogen (Plg) activator, is a single-chain secretory protein produced by strains of $\beta$-hemolytic Streptococcus (1–3). The SK and human plasminogen (HPlg) complex can activate Plg to plasmin (Plm) from different mammalian species (3–10). Plm thus produced in the blood can activate Plg. However, Plg alone does not have enzymatic activity. However, HPlg in interacting with SK becomes a virgin enzyme, which has amidolytic activity, although the activating peptide Arg$_{560}$–Val$_{561}$ remains intact (5, 6, 11). Shortly after complex formation, HPlg-SK is converted to HPlm-SK (12), both of which can act as a Plg activator to catalyze the hydrolysis of the activating peptide bond of other Plgs (7, 13, 14). Plgs from many different mammalian species have been tested for their sensitivity to activation by SK, but only those from human, monkey, and cat are found to be sensitive (15), while those from cow, bovine, sheep, pig, mouse, and rat are not (3). The regions of amino acid sequences of Plgs that may account for the species specificity were determined with HPlg mutants (16).

The detailed three-dimensional structure of SK or Plg-SK complex based on x-ray crystallography has not been solved. According to amino acid sequence alignment, two internal homologous domains, SK-(1–173) and SK-(254–415) were suggested (2). NMR and CD spectroscopy studies suggest that SK consists of at least three to four independent domains linked by mobile segments of the protein chain (17, 18). Therefore, truncated SK proteins obviously would contain some intact structure of these domains. In the previous report, we demonstrated that most of the truncated SK peptides obtained by gene cloning techniques had secondary structures similar to those of the corresponding regions on native SK moieties, and they could be used to study the functions of the SK domains independently (19). Based on the study, five functional regions (a, Ile$_1$–Lys$_59$; b, Ser$_{60}$–Asn$_{110}$; c, Val$_{158}$–Arg$_{219}$; d, Tyr$_{252}$–Ala$_{316}$; e, Ser$_{217}$–Ala$_{275}$) in the SK molecule for interaction with HPlg were deduced. From the results, we concluded that coordination of SK region Val$_{158}$–Ala$_{275}$ is essential for a virgin enzyme formation and that coordination of SK region Ser$_{60}$–Ala$_{275}$ is required for an effective SK-type Plg activator. Additionally, the smaller SK fragments, SK-(16–251) and SK-(220–414), can bind to HPlg significantly, although they have neither virgin enzyme activity in a stoichiometric complex with HPlg nor Plg activator activity. Multiple binding sites of HPlg and SK molecules have been proposed (20–23). Nevertheless, how the separate HPlg-binding sites in the SK sequence interact with HPlg during HPlg activation remains a fundamental question to be resolved. In the present study, experiments were performed to elucidate the functions of NH$_2$- and COOH-terminal SK binding regions. A more detailed mechanism of HPlg activation by SK was proposed.

**EXPERIMENTAL PROCEDURES**

Materials—Enzymes used in DNA manipulation were purchased from Boehringer Mannheim, Bethesda Research, or Promega Laboratories and were used according to the Cold Spring Harbor Manual or the recommendations by the suppliers. The full-length SK gene was obtained from Streptococcus equisimilis H46A (ATCC 12449) by the amplification with polymerase chain reaction as previously reported (19). Lys-Sepharose, CNBr-activated Sepharose 4B, and DEAE-cellulose were from Pharmacia Biotech Inc. Soybean trypsin inhibitor and NH$_2$-Val-Leu-Lys-$p$-nitroanilide (S-2251) were obtained from Sigma. Bis-(sulfosuccinimidyl) suberate was purchased from Pierce; 6-aminocaproic acid (EACA) was from Merck. All chemicals were of the highest purity available.
grade commercially available. HPlg and SK antiserum were prepared in our laboratory from mice.

Preparation of HPlg, BPlg, Micro-HPlg, and Human Kringle 1–5—HPlg and BPlg were prepared from pooled human and bovine plasma as described (19, 24). Forms 1 and 2 of native Plgs were separated by chromatography on a Lys-Sepharose and soybean-trypsin inhibitor-Sepharose 4B as described in previous reports (27–29).

Expression and Purification of Recombinant SK Transcanted Peptides—SK peptides used in this study were obtained by gene cloning techniques as previously reported (19). Briefly, the full-length SK gene was unidirectionally deleted by exonuclease III from the NH2 or COOH terminus. Then the fragments coding for the truncated peptides were subcloned in frame into the overproducing plasmid pET-3a or pET-3b or pET-3c (Novagen) at the specific binding. The binding affinities of these 125I-SK peptides to HPlg addition of a 30-fold molar excess of each unlabeled peptide, and the DEAE-cellulose column. were harvested, washed, and disintegrated. Then the target proteins containing 0.05% Tween 20, the amount of 125I-SK peptide was determined by an LKB described.125I-SK peptides at various concentrations were added to the say strip plate were coated with HPlg and blocked with BSA as above and Determination of the Binding Affinity—conducting the same procedures as in the sandwich binding assay, we place of unlabeled Plgs for a coating plate in one assay and 125I-SK in the surface of each well and the amount of bound SK as a bridge were estimated by two separate groups of experiments. We used125I-Plgs in our laboratory from mice.

Preparation of HPlg, BPlg, Micro-HPlg, and Human Kringle 1–5—HPlg and SK antiserum were prepared grade commercially available. HPlg and SK antiserum were prepared in our laboratory from mice.

Plasminogen Activation by Streptokinase 3111

RESULTS

Sandwich binding assays were performed to study the formation of the HPlg-SKPlg complex. Wells of a radiolabeled assay stripe plate were coated with HPlg or BPlg at a concentration of 10 μg/ml in 0.05 mM carbonate/bicarbonate buffer, pH 9.6, for 36 h at 4 °C and blocked with 1% BSA in phosphate-buffered saline (PBS) for 1 h. Native SK (1 μM) was added to the wells as a bridge and incubated for 30 min at 4 °C. After washing with the same buffer, the radioactivity and the amount of 125I-Plg was determined by using BSA instead of SK protein. This was subtracted from the total binding to obtain the amount of specific binding. The binding of 125I-HPlg and SK 1:1 complex, preformed in complex solution, that of the HPlg bound to the HPlg complex in a dose-dependent and saturable manner and shows that 4.69 ± 0.30 pmol of 125I-HPlg was bound in the saturated condition. No binding of 125I-HPlg was observed when BSA was used in place of SK. The binding of 125I-HPlg and SK 1:1 complex, in solution, to the HPlg precoated plate was similar to the binding of 125I-HPlg to the SK-coated plate (Fig. 1). In separate experiments, the 125I-labeled proteins were used to determine the molarity of Plgs coated on the plate surface and the SK that bound to the Plgs on the surface. The amount of HPlg and BPlg coating on the surface of each well and the amount of bound SK as a bridge were estimated by two separate groups of experiments. We used in place of unlabeled Plgs for a coating plate in one assay and 125I-SK in the surface of each well and the amount of bound SK as a bridge and incubated for 30 min at 4 °C. After washing with the same buffer, the radioactivity and the amount of 125I-Plg was determined by using BSA instead of SK protein. This was subtracted from the total binding to obtain the amount of specific binding. The binding of 125I-HPlg and SK 1:1 complex, preformed in complex solution, to the HPlg precoated plate was similar to the binding of 125I-HPlg to the HPlg-SKPlg complex (Fig. 1). The level of bound 125I-HPlg decreased in the presence of 10 μg/ml EACA (Fig. 1). Only limited amount of ternary complex formed if BPlg was tested instead of HPlg (Fig. 1). In separate experiments, the 125I-labeled proteins were used to determine the molarity of Plgs coated on the plate surface and the SK that bound to the Plgs on the surface. The amount of HPlg and BPlg coating on the surface of each well was 4.97 ± 0.07 and 3.91 ± 0.06 pmol (n = 6), respectively. The amount of SK that bound to the immobilized HPlg and BPlg was 5.01 ± 0.07 and 3.08 ± 0.08 pmol (n = 5), respectively. Therefore, it appeared that the binding of SK to either of the Plgs precoated on the well surface is very close to 1:1 stoichiometry. I also confirmed that a nearly 1:1 of HPlg-SK-HPlg ternary complex existed in the sandwich assay when referred to the amount of HPlg that was bound on the HPlg-SKPlg coated plate in the saturated condition (Fig. 1). However, in case of BPlg, only the BPlgSK complex, not the BPlgSK-BPlg ternary complex, was detected.

Recombinant truncated SK peptides of COOH-terminal or NH2-terminal domains, SK-(220–414) and SK-(16–251), were

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used to identify the interaction mode in the HPlg-SK-HPlg ternary complex. The concentration-dependent binding of $^{125}\text{I}\text{-SK-(220–414)}$ or $^{125}\text{I}\text{-SK-(16–251)}$ to HPlg was determined. Typical saturable binding curves are shown in Fig. 2. Based on the Scatchard transformation of the data, SK-(220–414) bound to two classes of sites of HPlg with $K_d$ of $1.50 \pm 0.13 \times 10^{-6} \text{M}$ and $2.44 \pm 0.16 \times 10^{-6} \text{M}$ (mean $\pm$ S.D., $n=3$), while SK-(16–251) bound to a single class of sites with a $K_d$ of $4.09 \pm 0.35 \times 10^{-7} \text{M}$ (mean $\pm$ S.D., $n=3$). Similar experiments were also performed using kringle 1–5 of HPlg and BPlg instead of HPlg.

The binding of SK-(220–414) to human kringle 1–5 had only one class of sites with a $K_d$ of $3.26 \pm 0.13 \times 10^{-6} \text{M}$, and SK-(16–251) bound to human kringle 1–5 with a $K_d$ of $4.62 \pm 0.25 \times 10^{-7} \text{M}$. SK-(220–414) bound to two classes of sites of BPlg with $K_d$ of $0.52 \pm 0.07 \times 10^{-6} \text{M}$ and $2.25 \pm 0.16 \times 10^{-6} \text{M}$, while SK-(16–251) bound to a single class of sites with a $K_d$ of $1.43 \pm 0.23 \times 10^{-6} \text{M}$.

Competition between human kringle 1–5 or micro-HPlg with intact HPlg for SK binding sites was evaluated by a competitive binding assay. The binding of $^{125}\text{I}\text{-SK-(16–251)}$ with HPlg was effectively competed for by human kringle 1–5, not by micro-HPlg (active site domain of HPlg) (Fig. 3A). Thus, SK-(16–251) obviously could only interact with human kringle 1–5. On the other hand, both human kringle 1–5 and micro-HPlg could compete with HPlg for the binding of $^{125}\text{I}\text{-SK-(220–414)}$ (Fig. 3B). At a high concentration of competitor peptide, 40–60% of the binding was inhibited (Fig. 3B). These data confirmed that SK-(220–414) could bind to HPlg through interaction with kringle domain and catalytic domain independently. The result of the cross-link experiment also confirmed that native SK can react with kringle domain of HPlg. Two high molecular weight protein bands were observed in the SDS-PAGE of cross-linked products of human kringle 1–5 and native SK (Fig. 4, lane 3). These bands contained both SK and HPlg proteins, since both bands showed positive reaction with anti-HPlg and anti-SK polyclonal antibodies (Fig. 4, lanes 4 and 5). The protein bands of 100 and 155 kDa represented a 1:1 SK-human kringle 1–5 complex and a 1:1:1 kringle 1–5:SK:kringle 1–5 ternary complex, respectively. The binding of NH$_2$- or COOH-terminal domains of SK to the kringle domain of HPlg would result in the formation of 1:1 SK and kringle domain complex, while the binding of the COOH-terminal domains of SK to one kringle domain of HPlg and the binding of the NH$_2$-terminal domain of the same SK to a second kringle domain would lead to the formation of a ternary complex.

Competition between different SK moieties for binding to HPlg was also studied. The binding of SK-(16–251) to HPlg was completely inhibited by SK-(220–414) (Fig. 5A), and the binding of SK-(220–414) to HPlg was partially inhibited by SK-(16–251) as well as SK-(16–191) (Fig. 5B). Therefore, the bind-
binding to HPlg with excess unlabeled HPlg fragments. Wells of
( ) at final concentrations ranging from 0 to 3 ( ), or BSA ( ) in Fig. 1. Unlabeled human kringle 1–5 ( ) was added to the HPlg-precoated wells for 5 min at 4 °C. The binding procedures were performed as in Fig. 2, and the amount of bound labeled SK peptides was determined. The percentage of labeled SK peptide binding was determined by the fractional binding of the labeled SK peptides to HPlg in the presence of a given competitor in comparison with that occurring in the absence of any unlabeled competitor. Each point represents the mean ± S. D. of three independent determinations.

Enzymatic assays were performed to determine the functions of the two significant HPIg-binding SK peptides. Neither SK-(220–414) nor SK-(16–251) alone at a catalytic concentration could activate HPlg (Fig. 6A). However, when these two SK domains were added to HPlg simultaneously, HPlg was activated (Fig. 6A). In the parallel SDS-PAGE analysis, the conversion of HPlg to HPlm by the combination of SK-(220–414) and SK-(16–251) was completed in 30 min, as the band of HPlg (94-kDa) disappeared and both the heavy (66-kDa) and light (26-kDa) chains were detected (Fig. 6B, lane 4). To determine if SK-(220–414) and SK-(16–251) have effects on the activation of HPlg by full-length SK, HPlg was incubated with a catalytic amount of SK-(16–414) alone or in the presence of various concentrations of SK fragments. SK-(16–251) in a nanomolar concentration range could dose-dependently amplify the activation of HPlg by SK-(16–414). Increasing the concentrations of SK-(16–251) to micromolar range caused more rapid enhancement, and the extent of amplification reached a plateau (Fig. 7A). In contrast, nanomolar concentrations of SK-(220–414) had no effect on the HPlg activation, and increasing amounts of SK-(220–414) to micromolar concentrations progressively inhibited the activation of HPlg by SK-(16–414) (Fig. 7B).

**DISCUSSION**

We have previously demonstrated that several regions in the SK molecule were essential for its functions as a plasminogen activator (19). In this study, we have used peptide fragments of SK and HPlg to further elucidate the interaction between these two molecules. It has been clearly demonstrated and widely accepted that formation of a 1:1 complex of SK and HPlg, as the catalytically active unit, is the first step in Plg activation (5, 6, 12). In the process of free HPlg activation, an enzyme-substrate intermediate, in the form of HPlg-SK-HPlg, might be present temporarily during activation. In our study, the possible existence of a HPlg-SK-HPlg ternary complex was demonstrated by a sandwich-binding experiment (Fig. 1). We also demonstrated that preformed HPlg-SK complex could bind to HPlg in an approximately 1:1 ratio. In contrast, in the report of Summaria et al. (20), a HPlm B-chain-SK-HPlg ternary complex, but not a HPlg-SK-HPlg ternary complex, was proposed based on the results of agarose double diffusion analysis. Two binding sites for the SK and HPlg interaction have been proposed, and when SK is bound to intact HPlg, the two interaction sites of SK are occupied by one single HPlg molecule. In the HPlm B-chain complex, only one of the two sites on SK is occupied, leaving the other one free for interacting with a second HPlg molecule. However, no explanation for the precipitin zones around the wells containing the HPlg and SK mixture was given (Fig. 1, A (well 1) and B (well 4) of Ref. 20). One possible explanation is that HPlg and SK might form insoluble polymers in wells of agarose, which can not migrate into the agarose gel. The precipitin reaction of SK and HPlg, in a manner analogous to an antigen-antibody reaction, might be the result of formation of insoluble polymers in which each molecule can bind to more than one reacting molecule to form an insoluble network (20). Similar high molecular weight polymers were also observed in sedimentation velocity analysis and native gel electrophoresis.
of HPlgSK complexes (34, 35). Results of the sandwich binding assays give direct evidence to support the hypothesis that one SK molecule can interact with two HPlg molecules simultaneously.

Although two interaction sites on SK for HPlg were suggested in the previous report (20), the specific locations have not been identified. A straight line was obtained in the Scatchard plot of the binding of the NH2-terminal domain of SK to HPlg, indicating only one binding site was involved. On the other hand, a curved line was obtained in the similar plot of the binding of COOH-terminal domains of SK to HPlg, indicating in this case that two binding sites were involved (Fig. 2). The binding of the NH2-terminal domain of SK with HPlg was competed for by human kringle 1–5, but not by micro-HPlg, while the binding of COOH-terminal domains of SK with HPlg could be competed for by both kringle 1–5 and micro-HPlg (Fig. 3). Therefore, we conclude that SK had three binding sites for HPlg. One binding site located at the NH2-terminal domain of SK is specific for the interaction with the kringle domain of HPlg. There are two other binding sites on the COOH-terminal domains of SK; one specifically interacts with the catalytic domain, and the other interacts with the kringle domain of HPlg. Since the binding of the COOH- and NH2-terminal domains of SK is mutually exclusive for binding to the HPlg kringle domain (Fig. 5), these two binding sites of SK cannot interact with the same HPlg molecule simultaneously. On the basis of the binding constants we determined, we conclude that the order of binding strength was as follows: catalytic domain SK-(220–414) > kringle domain SK-(16–251). Taken together, the binding of COOH-terminal domains of SK to the catalytic and kringle domains of the first HPlg molecule and the binding of the NH2-terminal domain of the same SK to the kringle domain of a second HPlg molecule would result in the formation of the HPlgSK-HPlg ternary complex as demonstrated in the sandwich binding assay (Fig. 1). The result of the cross-linking experiment (Fig. 4) also supports the existence of the ternary complex.
complex. The hypothetical binding mode could also explain the presence of high molecular weight complexes of these two molecules (34, 35).

Formation of the ternary complex is inhibited by 10 mM EACA (Fig. 1), since it might interfere with the interaction of SK and lysine-binding sites on the kringle domain of HPlg. A similar explanation could also be applied to the observations that high molecular weight complexes of SK and HPlg shifted to a 1:1 complex in 100 mM EACA (34, 36). Activation of HPlg by SK was substantially inhibited by EACA as described in a previous report (37) and our own observation.2 The inhibitory effects of EACA could be partially due to its interference on the formation of the HPlg-SK-HPlg complex. When SK is used as a thrombolytic agent, with HPlg in excess and no EACA, the condition would be in favor of the formation of the HPlg-SK-HPlg ternary enzyme-substrate complex. The ternary complex might represent the intermediate of the two-stage activation process proposed by Reddy and Markus (6). It was clearly demonstrated that HPlg-SK as well as the HPlm-SK complex can activate Plgs with cleavage site-resistant HPlm (38). The HPlg-SK complex is remarkable in that SK induces a typical serine protease active site in the HPlg molecule. Finding the interaction sites between SK and HPlg should be valuable in revealing the mechanism and the species specificity of the active complex formation. Two peptide segments, Arg579–Met584 and Arg625–Lys627 (by the numbering convention for the full-length HPlg) were believed to be involved in a reaction with SK, by using the human-bovine hybrid micro-HPlg (39). In this report, we demonstrated that COOH-terminal, not NH2-terminal, domains of SK had high affinity for micro-HPlg. Therefore, we assume that the COOH-terminal domains play a major role in interaction with micro-HPlg and are essential for induction of the active site in HPlg. The inertness of SK toward BPlg was also attributed to the specific interaction sites. The weak binding between BPlg and COOH-terminal domains of SK is consistent with the incapacity of SK to form activator with BPlg. However, the binding of BPlg and COOH-terminal domains was observed, although weaker than HPlg. There should be still some unidentified protein segments in HPlg that are involved in the interaction between Plgs and SK.

According to the reaction model proposed by Markus et al. (40), the role of the SK moiety in HPlg-SK-HPlg ternary complex may have two components: (a) SK may open the active center on one HPlg, thereby inducing it to serve as an activator; (b) SK may modulate the geometry near the Arg560–Val561 bond of the substrate HPlg, and the activating peptide bond was brought toward the active center of the activator HPlg to form the transient Michaelis complex. The COOH-terminal domains of SK Val158–Ala378 were previously demonstrated to be responsible for the formation of enzymatic center in complexing with HPlg (19). The present study showed that HPlg could be effectively activated by combination of the two functionless SK peptides, SK-(220–414) and SK-(16–251). We assumed that the NH2-terminal domain SK-(16–251) might function as a substrate modulator, SK-(220–414) to form the activator with HPlg. In addition, SK-(16–251) might also function as a substrate modulator, since it could amplify the activation of HPlg by SK-(16–414) (Fig. 7A). The most plausible explanation for this observation is that HPlg, by interacting with the NH2-terminal domain of SK with its kringle 1–5 domain, becomes a better substrate for activation by the HPlg-SK activator complex. The hypothesis is also in consistent with the following observations: (a) a series of NH2-terminal truncated SK peptides still retains the comparable activities of virgin enzyme complex with HPlg but has lower rates of HPlg activation (19); (b) micro-HPlg, which lacks kringle domain, is activated by SK less effectively than Hg-HPlg (29). However, further studies are required to reveal the specific binding sites on the NH2-domain of SK and kringle of HPlg as well as their reaction mechanism. It is possible that binding of SK-(16–251) to the kringle domain of HPlg induces the conversion of Hg-HPlg to the Lys-HPlg conformation, which might be attributed to the stimulating effect of the NH2-terminal domain of SK, since SK-(16–251) cannot enhance activation of Lys-HPlg by SK.2 However, it is also possible that the NH2-terminal domain of SK could induce the conformational changes near the activating peptide bond.
Plasminogen Activation by Streptokinase

(Arg\textsuperscript{560}–Val\textsuperscript{561}) of HPlg through a different mechanism. On the other hand, the HPlg activation by SK(-16–414) is inhibited by SK(220–414). It could be due to the fact that excess SK(220–414) would compete with SK(-16–414) for binding to kringle 1–5 as well as to the catalytic domain of HPlg and inhibit the formation of the HPlg activator (Fig. 7B). This observation is in agreement with the previous finding that SK(-244–352) dose-dependently inhibited the generation of an active site by full-length SK and HPlg (41).

The activation scheme of HPlg by SK is proposed, based on the findings on multiple functions of SK as shown in Scheme 1.

The COOH-terminal domains of SK can bind to the kringle and the catalytic domains of HPlg. The binding of the catalytic domain of HPlg to the COOH-terminal domains of SK is most important in inducing a conformational change in the active center of HPlg (Scheme 1, part a) and, therefore, forming an activator of HPlg, which can specifically hydrolyze the activating peptide bond Arg\textsuperscript{560}–Val\textsuperscript{561} of the substrate HPlg. The NH\textsubscript{2}-terminal domain of SK, by interaction with the kringle ing peptide bond Arg\textsuperscript{560}–Val\textsuperscript{561} of the substrate HPlg. The activator of HPlg, which can specifically hydrolyze the activator of HPlg (Fig. 7B) and make it more effectively converted to HPlm (part c). The ternary complex in Scheme 1, HPlgSK-HPlm, which was demonstrated in the sandwich binding assays, might also represent the transient Michaelis complex proposed by Markus et al. (40). The multiple functions of SK domains in reacting with HPlg render SK a very efficient HPlg activator. In the case of BPlg, the binding of its catalytic domain to the COOH-terminal domains of SK is significantly weaker than HPlg, and no ternary complex of BPlg and SK formed. This might be one of the reasons why BPlg cannot be activated directly by SK (3).

The reaction of SK and HPlg is a unique example of protein-protein interaction that is very important in the regulation of various biological functions and deserves further delineation. SK, as a simple protein cofactor in the activation of HPlg, may provide a unique opportunity to study the mechanism of reaction between the protein cofactors and enzymes. The detailed mechanism, when elucidated, may be exploited to design a new generation of better SK as a thrombolytic agent.

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