A Comparison of Bovine Serum Albumins, Modified with a Variety of Carboxyl Group Agents, as Stimulators of Tissue-Type Plasminogen Activator-Catalyzed Activation of Plasminogen¹⁾

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Bovine serum albumins (BSA), modified with a variety of carboxyl group agents, stimulated the tissue-type plasminogen activator (t-PA)-catalyzed activation of human plasminogen. Modification with taurine (tau) and putrescine (put) provided the best stimulants. The tauBSA and putBSA were effective at a concentration of 5 $\mu\text{g/ml}$ and enhanced the Lys-plasminogen activation by two-chain t-PA in a dose-dependent manner to a maximum of 44- to 46-fold at 200 $\mu\text{g/ml}$. The K_m values for the activation of Glu-plasminogen by t-PA in the presence of tauBSA and putBSA (100 $\mu\text{g/ml}$) were 1.7 and 1.8 μM , while the k_{cat} values were 0.059 and 0.062 s^{-1} , respectively. T-PA was bound to both tauBSA and putBSA, which were immobilized on agarose beads, with K_D values of 163 and 138 nM , respectively. The two modified BSAs were good substrates for plasmin and were hydrolyzed by the enzyme to small peptides. All of these modified BSA-related actions were inhibited by lysine analogs (e.g. tranexamic acid) which were adjusted to the concentrations required for the inhibition of the plasminogen (Kringle 1 domain) binding to fibrin. On the other hand, acetylation or succinylation of the amino groups of BSA was not effective, while alkylation of the thiol groups of this protein resulted in a moderate stimulation of the plasmin generation. The present results show that t-PA and plasminogen form complexes with certain charge-modified BSAs via their lysine-binding sites. The different stimulation potency of modified BSAs may provide a model for *in vivo* counterparts of fibrin.

Keywords plasminogen; tissue-type plasminogen activator; binding; charge-modified albumin

In the fluid phase, tissue-type plasminogen activator (t-PA; EC 3.4.21.31)-catalyzed activation of plasminogen is kinetically unfavorable with a K_m greater than the plasma plasminogen concentration.²⁾ However, immobilization of plasminogen and t-PA on fibrin via their Kringle domains^{2,3)} efficiently improves their kinetic parameters and facilitates a localized generation of plasmin. Hence, the specific binding of t-PA and plasminogen to fibrin is an essential feature for the regulation of fibrinolysis.

Recently, t-PA has prompted renewed interest in relation to its additional role in the extracellular matrix, since the extracellular components such as fibronectin⁴⁾ and thrombospondin⁵⁾ can provide a surface on which t-PA efficiently activates plasminogen. Radcliffe and Heinze⁶⁾ previously suggested that other *in vivo* analogs of fibrin such as aged protein, scar tissue and tumors, as well as complement complexes on cell surfaces, may also serve as stimulators of t-PA. However, it is not well-known what features of the denatured proteins act as the determinants to stimulate the t-PA-related plasminogen-plasmin system. Furthermore, studies were not performed to determine whether plasminogen and/or t-PA bind directly to the denatured proteins. Thus, information about the chemical and physical nature of the stimulants may enhance our understanding of the precise physiological role of t-PA in non-fibrin-containing environments.

As a part of studies on the regulation of the fibrinolytic system, we have synthesized BSA derivatives, modified with a variety of amino, carboxyl and sulfhydryl group agents, and characterized their abilities to stimulate t-PA-catalyzed activation of human plasminogen. In this paper, we demonstrate that specific charge modifications of the carboxyl groups of BSA enable t-PA to activate plasminogen efficiently via the mechanism of cyclic ternary complex formation.

Methods

Proteins and Materials Porcine one-chain t-PA was purified as de-

scribed previously.⁷⁾ The two-chain form of t-PA was prepared by the treatment of one-chain t-PA with immobilized plasmin.⁸⁾ Glu-plasminogen (20 CU/mg protein) was obtained from Kabi, Stockholm, Sweden. Lys-plasminogen was prepared by the method of Lucas *et al.*⁹⁾ BSA, lysine and its analogs were purchased from Sigma, St. Louis, MO, U.S.A. HD-Ile-Pro-Arg-*p*-nitroanilide (S-2288), HD-Val-Leu-Lys-*p*-nitroanilide (S-2251) and human fibrinogen were from Kabi. Other reagents were of analytical grade available from commercial suppliers.

Chemical Modification of BSA The carboxyl groups of BSA were modified by the method of Hoare and Koshland¹⁰⁾ with a slight alteration. Ethylenediamine, 1,4-diaminobutane (putrescine), 1,6-diaminohexane (hexamethylenediamine), ethylamine, *n*-butylamine and 2-aminoethane-1-sulfonic acid (taurine) were used as nucleophilic agents. To a solution at pH 4.75 and 25°C containing BSA (15 mg/ml), 1.5 M nucleophile and 7.5 M urea was added 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide dissolved in 7.5 M urea. The pH of the reaction mixture was maintained at 4.75 by the addition of 0.5 M HCl as required. After 1 h, 10 volumes of 1 M acetate buffer, pH 4.75, were added to quench the reaction. The mixture was allowed to stand for a few minutes at 25°C and concentrated by ultrafiltration on a PM-10 membrane (Amicon). The concentrate was applied to a Sephadex G-25 column (1.5 × 20 cm) equilibrated with 1 M HCl. The modified BSA was completely separated from the reagents by this gel filtration. For reduction and S-alkylation, 60 mg of BSA was dissolved in 8 ml of 50 mM Tris-HCl buffer, pH 7.6, containing 5 M guanidine hydrochloride, and to this, 190 mg of dithiothreitol (final concentration, 150 mM) was added. After reduction for 1 h at 37°C under a nitrogen atmosphere, the free SH groups were alkylated by the addition of 1 ml of either 2 M iodoacetate or 2 M iodoacetamide for 1 h at 37°C in the dark. The mixture was dialyzed against 50 mM Tris-HCl buffer, pH 7.6, at 4°C. Acetylation and succinylation of the amino groups of BSA were carried out as described by Tanaka *et al.*¹¹⁾

Chemical Modification of Immobilized BSA BSA was immobilized on CNBr-activated Sepharose 4B (Pharmacia Fine Chem. Co.) according to the manufacturer's instructions. About 65 mg of BSA were added to 10 ml of the gel swollen with a coupling buffer (0.5 M NaCl/0.1 M NaHCO₃, pH 8.0) and the gel suspension was mixed by magnetic stirring overnight at 4°C. The gel was first washed with the coupling buffer and then with 20 mM Tris-HCl buffer, pH 7.5, containing 0.15 M NaCl (500 ml of each per ml of gel). The amount of BSA bound to the gel was calculated from the difference between the amount of original protein and that found in the supernatant after the coupling reaction. The modification of carboxyl groups of the immobilized BSA (6.1 mg BSA/ml of gel) with carbodiimide at pH 4.75 in the presence of taurine or putrescine was performed as described above. The reagents were removed by washing the gels with Tris-buffered saline.

Protein Determination Protein concentration was determined by the method of Lowry *et al.*,¹²⁾ using BSA as the standard.

Assay of Plasminogen Activation The plasminogen activation by t-PA was assayed by measuring the formation of plasmin with S-2251. The assay mixture contained the following components in a total volume of 250 μ l: 1 mM S-2251, 1.5 μ M Lys-plasminogen, 1.5–2.0 nM two-chain t-PA, 0.001% (v/v) Tween 80, 0.1 M Tris-HCl buffer (pH 7.5)/0.15 M NaCl and, if indicated, a chemically modified BSA as a stimulator. After a specified time of incubation at 37 °C, the reaction was stopped by addition of 500 μ l of 2% (w/v) citric acid solution. The absorbance at 405 nm was measured with a UV-730 Microflow spectrophotometer (Shimadzu Co., Japan). Kinetics of plasminogen activation by t-PA were determined by the method of Hoylaerts *et al.*²⁾ with a slight modification. The assay was performed with 5 IU of two-chain t-PA, 0–100 μ M Glu-plasminogen and 0.6 mM S-2251 and, if indicated, a stimulator (fibrin monomer or modified BSA) in a total volume of 250 μ l of 50 mM Tris-HCl buffer (pH 7.5)/0.15 M NaCl.

Assay of Amidolytic Activity of Plasmin or t-PA The amidolytic activity of plasmin was measured using S-2251 as a substrate. Fifty microliters of plasmin (0.3 CU/ml) was mixed with 50 μ l of 4.9 mM S-2251 and 100 μ l of 0.1 M Tris-HCl buffer (pH 7.5)/0.15 M NaCl, containing a given concentration of BSA derivative. After incubation for 20 min at 37 °C, the reaction was stopped by addition of 500 μ l of 2% citric acid solution and the A_{405} was measured. The amidolytic activity of t-PA was measured with S-2288. Fifty microliters of t-PA (2,500 IU/ml) was mixed with 50 μ l of 4.5 mM S-2288 and 100 μ l of 0.1 M Tris-HCl buffer (pH 8.0)/0.15 M NaCl/0.001% Tween 80 containing a given concentration of BSA derivative, and then the procedure described above was followed.

Degradation of Carboxyl Group-Modified BSA by Plasmin The degradation of the modified BSA by plasmin was analyzed by the use of the following two assay methods. One was a qualitative analysis by SDS-polyacrylamide gel electrophoresis. The analytical method was essentially the same as that described by Laemmli.¹³⁾ Acrylamide concentration was 3.3% in the stacking gel and 10% in the separating gel. The modified BSA (330–350 μ g) was dissolved in 1 ml of 0.2 M Tris-HCl buffer (pH 7.5)/0.15 M NaCl/1 mM EDTA/0.005% Tween 80 and incubated with 0.05 CU of plasmin at 37 °C. At specified times, 50 μ l aliquots were withdrawn and added to 50 μ l of Laemmli's sample buffer containing 20 μ M DFP. The solutions were loaded on the slab gels and subjected to electrophoresis. The gels were stained with Coomassie Brilliant Blue R-250. For quantitative analysis, the protein determination method of Lowry *et al.*¹²⁾ was used. At specified times, 50 μ l aliquots were withdrawn from the reaction mixture described above and mixed with 150 μ l of 15% (w/v) trichloroacetic acid solution. After standing at room temperature for 15 min, the solutions were centrifuged at 10000 $\times g$ for 10 min at 4 °C and 100 μ l of the supernatant was subjected to the determination of protein concentration.

Binding of t-PA to Immobilized Carboxyl Group-Modified BSA In a typical binding assay, 40 μ l of Sepharose beads, which contained 90 nmol of ligand per ml of gel, was incubated with various amounts of t-PA (0–120 μ g) in 0.1 M Tris-HCl buffer (pH 8.0)/0.15 M NaCl/0.01% Tween 80 (total volume, 600 μ l). After incubation at 37 °C for 30 min, the reaction was terminated by centrifugation at 2000 $\times g$ for 10 min (4 °C) and 50 μ l of the supernatant was subjected to the assay of amidolytic activity of t-PA. The assay method was the same as that described above, except that the reaction was carried out for 60 min, and the enzyme activity obtained is based on unbound t-PA. Various amounts of t-PA were incubated with S-2288 at 37 °C for 60 min in the absence of immobilized BSA derivative. When the A_{405} versus the amount of t-PA used was plotted, a straight line was obtained (data not shown). Therefore, the amount of unbound t-PA was determined from the calibration curve.

Results

Activation of Plasminogen by t-PA in the Presence of Various Chemically Modified Derivatives of BSA The effects of various carboxyl group-modified BSAs on the rate of Lys-plasminogen activation by two-chain t-PA are shown in Fig. 1. Among the BSA derivatives tested, tauBSA and putBSA were the most effective stimulators, which linearly increased the generation of plasmin for at least 20 min (30- to 33-fold). Ethylaminated BSA (eaBSA), which is a positively charged product like putBSA, also

Fig. 1. Effects of Various Carboxyl Group-Modified Derivatives of BSA on the Rate of Plasminogen Activation by t-PA

The assay system contained 1.5 μ M Lys-plasminogen, 1.8 nM two-chain t-PA, 1 mM S-2251 and 100 μ g/ml native or chemically modified BSA in a total volume of 250 μ l. The assay of plasmin generated was carried out as described in Methods. The symbols represent: ($\times$), native BSA; ($\blacktriangle$), ethylated BSA (eBSA); ($\triangle$), *n*-butylated BSA (bBSA); ($\square$), ethylaminated BSA (eaBSA); ($\circ$), *n*-butylaminated BSA (putBSA); ($\blacksquare$), hexylaminated BSA (hexBSA); ($\bullet$), sulfoethylated BSA (tauBSA).

Fig. 2. Concentration Dependence of Stimulatory Effects of Carboxyl Group-Modified BSA Derivatives and Poly-D-lysine on the Activation of Plasminogen by t-PA

The assay mixture, which contained 1.8 μ M Lys-plasminogen, 1.6 nM two-chain t-PA and a solution (20 μ l) of various concentrations of the BSA derivatives or poly-D-lysine in a total volume of 200 μ l of 0.1 M Tris-HCl buffer (pH 8.0)/0.15 M NaCl/0.001% Tween 80, was incubated at 37 °C for 20 min. The amount of plasmin generated was measured by addition of 50 μ l of 4.9 mM S-2251 and subsequent incubation at 37 °C for 10 min. The stimulation factor (vertical axis) is the ratio of the amount of plasmin generated in the presence and that in the absence of the modified BSA. The symbols are the same as those shown in Fig. 1, except that ($\triangle$) represents poly-D-lysine.

stimulated the plasminogen activation (13-fold). The other carboxyl group-modified BSAs had only slight stimulative effects. On the other hand, acetylation or succinylation of the amino groups of BSA was not effective. Carboxymethylation or carboxamidomethylation of the thiol groups of BSA resulted in a moderate stimulation of the plasmin generation (10-fold, data not shown). Furthermore, the stimulation of t-PA-catalyzed plasminogen activation by these BSA derivatives was not affected by the addition of native BSA.

To clarify the cause of the stimulative effect described

Fig. 3. Lineweaver-Burk Plots of t-PA-Catalyzed Plasminogen Activation in the Absence (A, —▲—) or Presence of tauBSA (B, —○—), putBSA (B, —○—) and Fibrin (C, —□—)

TABLE I. Kinetic Parameters of Plasminogen Activation by t-PA in the Presence of tauBSA and putBSA

Effector	K_m (μM Plg)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{mM}^{-1} \text{s}^{-1}$)
None	78	0.045	0.58
putBSA ^{a)}	1.8	0.062	34.4
tauBSA ^{a)}	1.7	0.059	34.7
Fibrin ^{b)}	0.85	0.095	111.8

The experiments were carried out as described in Methods. a) 200 $\mu\text{g}/\text{ml}$; b) 1 mg/ml .

above, we tested the direct effect of the modified BSA on the amidolytic activity of plasmin or t-PA. At 100 $\mu\text{g}/\text{ml}$, the BSA derivatives had no effect on the activity of t-PA, while putBSA or tauBSA slightly stimulated the amidolytic activity of plasmin (2- to 3-fold).

Figure 2 shows the concentration dependence of stimulative effects of carboxyl group-modified BSAs on t-PA-catalyzed plasminogen activation. Poly-D-lysine (M.W. = 78000), which was used as a control stimulator, increased the rate of plasmin formation and showed the maximum effect (22-fold stimulation) at a concentration of 100 $\mu\text{g}/\text{ml}$. Both putBSA and tauBSA stimulated the plasmin formation at 5 $\mu\text{g}/\text{ml}$ or more. The effects of these derivatives increased with an increase in their concentrations and reached a maximum of 44- to 46-fold at 200 $\mu\text{g}/\text{ml}$. The stimulation by these BSA derivatives was optimal between pH 7.5 and 8.5.

The kinetics of Glu-plasminogen activation by two-chain

Fig. 4. Effects of Lysine Analogs on the Stimulatory Effect of putBSA or tauBSA on t-PA-Catalyzed Plasminogen Activation

Experimental conditions were similar to those in the legend to Fig. 1, except that the assay mixture contained various amounts of lysine analogs and the reaction was carried out for 30 min. The percent inhibition was calculated by comparison with the enzyme activity obtained from the control (not contained lysine analogs). A: putBSA (200 $\mu\text{g}/\text{ml}$) + tranexamic acid (t-AMCHA, □), 6-aminoheptanoic acid (6-AHA, ●) or L-lysine (Lys, ○); B: tauBSA (200 $\mu\text{g}/\text{ml}$) + t-AMCHA, 6-AHA or Lys.

TABLE II. Effects of ω -Aminocarboxylic Acids on the Stimulation of t-PA-Catalyzed Plasminogen Activation by putBSA or tauBSA

ω -Aminocarboxylic acid	Concentration required for 50% inhibition ^{a)}	
	Stimulation by putBSA ^{b)} (μM)	Stimulation by tauBSA ^{b)} (μM)
Tranexamic acid	7.3 $\pm$ 0.4	5.2 $\pm$ 0.6
6-Aminoheptanoic acid	38 $\pm$ 23	45 $\pm$ 18
Lysine	450 $\pm$ 210	530 $\pm$ 160

The experimental conditions were similar to those in the legend to Fig. 1. a) Values represent the means $\pm$ S.D. of three experiments; b) 200 $\mu\text{g}/\text{ml}$.

t-PA in the presence of putBSA, tauBSA or fibrin monomer are shown in Fig. 3 and Table I. The predominant effect of tauBSA or putBSA was a 45-fold decrease in the K_m . This increase in affinity of t-PA for plasminogen resulted in a nearly 60-fold increase in the catalytic efficiency (k_{cat}/K_m). In comparison, the K_m of t-PA in the presence of fibrin decreased approximately 90-fold, and its catalytic efficiency increased 190-fold.

Effects of Lysine Analogs on the Stimulation of t-PA-Catalyzed Plasminogen Activation by Carboxyl Group-Modified BSA We studied the effects of lysine, 6-aminoheptanoic acid and tranexamic acid on the stimulation of t-PA-catalyzed plasminogen activation by tauBSA or putBSA. Figure 4 indicates that the BSA derivative-induced stimulation was inhibited by the lysine analogs in a dose-dependent manner. In all cases, 85% or greater inhibition was observed at high concentrations of the analogs. The concentrations of each analog required for 50% inhibition of the stimulation by tauBSA were similar to those in the case of putBSA (Table II).

Fig. 5. Binding of t-PA to Immobilized tauBSA or putBSA as a Function of One-Chain t-PA Concentration

A; various amounts of t-PA (0–120 μg) were incubated at 37°C for 30 min with 40 μl of immobilized tauBSA (●) or putBSA (○). B; Scatchard plot of the data in A.

Binding of t-PA to Immobilized Carboxyl Group-Modified BSA As shown in Fig. 5A, the binding of t-PA to immobilized putBSA and tauBSA derivatives was dose-dependent. On the other hand, t-PA did not bind immobilized native BSA to a significant extent. The Scatchard plot of the binding of t-PA to the two immobilized derivatives of BSA is shown in Fig. 5B. Apparent K_D values for t-PA binding to the immobilized putBSA and tauBSA were approximately 138 and 163 nM, respectively. Furthermore, a complete inhibition (94–96%) of the binding of t-PA to the two immobilized ligands was produced by the addition of 6-aminohexanoic acid (10 mM or more) (data not shown).

Degradation of Carboxyl Group-Modified BSA by Plasmin As shown in Fig. 6, both putBSA and tauBSA were good substrates for plasmin. Plasmin extensively cleaved the substrates in a time-dependent manner to form an array of peptides. The electrophoretic patterns suggest that these BSA derivatives are heterogeneous and the degradation products may be caused by unlimited proteolysis by plasmin. In contrast, BSA, which was treated with carbodiimide at pH 4.75 for 1 h in the absence of nucleophile (*e.g.* taurine) and carbodiimide was not cleaved by plasmin. In addition, the degradation of putBSA or tauBSA was completely inhibited by the addition of 20 μM tranexamic acid (data not shown).

Discussion

The present study demonstrates that specific modifications of BSA with a variety of carboxyl agents provide stimulants for t-PA-catalyzed plasminogen activation. The degree of the stimulation was in the order of tauBSA > putBSA > eaBSA > hexBSA > bBSA > eBSA (Figs. 1 and 2). For the class of positively charged derivatives of

Fig. 6. Degradation of Carboxyl Group-Modified Derivatives of BSA by Plasmin

Plasmin (0.05 CU) was incubated at 37°C with 330 μg of putBSA or 350 μg of tauBSA. At specified times, 50 μl aliquots were withdrawn and the degree of degradation of the modified BSA was determined as described in Methods. A, substrate = putBSA; B, substrate = tauBSA; + Pln, in the presence of plasmin; - Pln, in the absence of plasmin.

BSA (eaBSA, putBSA and hexBSA), the $-\text{CH}_2-$ length of the introduced diamines seems to be important for determining the stimulatory potency. In fact, the greatest stimulation was obtained by introducing putrescine onto BSA to an extent of 15–20 equivalents with respect to lysine residues (analytical data not shown). Many investigators have suggested that lysine residues in fibrin and its degradation products play a key role in the stimulation of t-PA-related plasmin generation. Together with the report of Radcliffe and Heinze,⁶⁾ our data suggest that also in the case of stimulants other than fibrin, the exposure of lysyl ϵ -amino groups may be important in the stimulation. On the other hand, a replacement of the carboxyl group with a neutral group gave a weak stimulator (bBSA or eBSA). This implies that an increase in the hydrophobicity (or a change in the aggregation state) of BSA may partly contribute to the stimulation. Simple gel filtration experi-

ments (Toyopearl HW-55S column) on these six carboxyl group-modified BSAs showed that although all of the BSA derivatives were in a considerably aggregated state, bBSA and eBSA were the most aggregative proteins (data not shown). Furthermore, the stimulatory effects of these derivatives were considerably decreased by the addition of 0.25 M or more NaCl. Therefore, different solubility or state of association of the carboxyl group-modified BSAs may also cause the difference in their stimulatory potencies.

We found that the introduction of several sulfate groups onto the carboxyl groups of BSA provide the best stimulant (tauBSA). It perplexed us that the stimulatory potency of tauBSA was very higher than that of the ethylenediamine-introduced BSA (eaBSA) and was indistinguishable from that of putBSA, since these are oppositely charged products. Whereas, the kinetic analyses indicated that both tauBSA and putBSA increased the catalytic efficiency (k_{cat}/K_m) of t-PA by a factor of 60-fold (Table I). The binding constants of t-PA for the two immobilized ligands were concluded to be almost the same (Fig. 5). These bindings were completely inhibited by the addition of 10 mM or more 6-aminohexanoic acid (data not shown). Also, the stimulation of plasminogen activation by tauBSA or putBSA was inhibited in a dose-dependent manner by ω -aminocarboxylic acids (Fig. 4). The concentrations of these agents required for 50% inhibition of the stimulation by tauBSA or putBSA were similar to the dissociation constants for the interactions of these analogs with the high affinity lysine-binding site (Kringle 1) of plasminogen, which is required for the interaction with fibrin.¹⁴ These findings suggest that t-PA and plasminogen can equally recognize these carboxyl group-modified BSAs as a fibrin-like protein and probably form a ternary complex through their lysine-binding sites.

Recently, Voskuilen *et al.*¹⁵ have synthesized peptide analogs to parts of A α 148–197 of human fibrinogen and revealed that lysine residue A α 157 is essential for the accelerating action of fibrin on the t-PA-catalyzed plasminogen activation. However, they have suggested that amino acid residues other than the lysine residue A α 157 are also involved in the rate-enhancing effect, since the concentration of fibrinopeptide fragment required to obtain half-maximal rate enhancement increases with a decrease in the fragment size. In the case of lysine analogs, their plasminogen activation rate-enhancing properties have been mainly ascribed to carboxyl-terminal lysines.^{16–18} Furthermore, it has recently been demonstrated that a sulfated polysaccharide, heparin, interacts with both plasminogen and t-PA, thereby increasing the plasminogen activation.^{19,20} The binding site of plasminogen or t-PA to heparin may be closely related to the fibrin-binding site, since the heparin stimulation was diminished by the presence of fibrin degradation products.²⁰ From these facts, the finding that the oppositely charged products, tauBSA and putBSA, had indistinguishable effects on the plasminogen activation may be explained as follows. As described above, the two modified BSAs were in a considerably aggregated state under low ionic and/or non-detergent-containing conditions. In such an aggregation state, they may take a suitable configuration for interaction with both t-PA and plasminogen molecules. Namely, sulfoethyl (taurine) or lysyl (putrescine) residues in the aggregated BSA may be arranged in a position where they can easily bind to the Kringle

domains of t-PA and plasminogen. However, the functional groups of tauBSA and putBSA are oppositely charged ones. Consequently, we assume that each functional group binds to different position(s) in the Kringle domain. As suggested by Voskuilen *et al.*,¹⁵ fibrin probably exposes both lysine residue A α 157 and another unknown residue on its surface for accelerating t-PA-catalyzed plasminogen activation. The aggregation of these BSA derivatives may also cause the exposure of another functional group in a position where the denatured proteins can mimic the fibrin functions. If this hypothesis is true, the other crucial residues of tauBSA and putBSA seem to be similar or identical to each other. Namely, a certain neutral group may be the functional residue, since it is not affected by the modification of BSA. In connection with this hypothesis, we have recently observed that a newly synthesized polyanion having certain neutral groups stimulates t-PA-catalyzed plasminogen activation and the neutral groups play a key role in the stimulation (unpublished data). On the basis of the present study, we suggested that the sulfate groups as well as the lysyl ϵ -amino groups of denatured protein (BSA) may have a crucial role in the stimulation of t-PA-catalyzed plasminogen activation. When taurine was introduced into BSA, this amino acid may in part couple with the γ -carboxyl groups of glutamyl residues. It is of interest to note that acidic peptides containing the γ -glutamyl-aurine bond are enriched in the synaptic vesicles of calf brain,²¹ since, on the vesicle membrane, plasminogen activators may play a role in the processing of precursors for peptide hormones and/or neurotransmitters.²² Sulfatide groups are present in various matrix or cell constituents as sulfated polysaccharides and sulfatides. In the previous paper, we indicated that the reconstitution of t-PA onto sulfatide vesicles lowered the K_m value of t-PA for plasminogen activation 20-fold.²³ However, the mechanism of the regulatory effect of these sulfate groups on t-PA activity has yet to be sufficiently clarified. Therefore, tauBSA may offer a useful model system for the study of the regulatory mechanism of t-PA-related plasminogen activation in sulfate-containing environments.

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References and Notes

- 1) Abbreviations used: BSA, bovine serum albumin; eBSA, COOH-group-ethylated BSA; bBSA, COOH-group-*n*-butylated BSA; eaBSA, COOH-group-ethylaminated BSA; putBSA, COOH-group-*n*-butylaminated BSA; hexBSA, COOH-group-hexylaminated BSA; tauBSA, COOH-group-sulfoethylated BSA; t-PA, tissue-type plasminogen activator; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; DFP, diisopropyl fluorophosphate.
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