Binding of the COOH-terminal Lysine Residue of Streptokinase to Plasminogen(ogen) Kringle Enhances Formation of the Streptokinase-Plasminogen(ogen) Catalytic Complexes*

Received for publication, July 3, 2006.
Published, JBC Papers in Press, July 20, 2006, DOI 10.1074/jbc.C600171200
Peter Panizzi, Paul D. Boxrud, Ingrid M. Verhamme, and Paul E. Bock
From the Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2561

Streptokinase (SK) activates human fibrinolysis by inducing non-proteolytic activation of the serine protease zymogen, plasminogen (Pg), in the SK-Pg* catalytic complex. SK-Pg* proteolytically activates Pg to plasmin (Pm). SK-induced Pg activation is enhanced by lysine-binding site (LBS) interactions with kringles on Pg and Pm, as evidenced by inhibition of the reactions by the lysine analogue, 6-aminohexanoic acid. Equilibrium binding analysis and [Lys]Pg activation kinetics with wild-type SK, carboxypeptidase B-treated SK, and a COOH-terminal Lys414 deletion mutant (SKΔK414) demonstrated a critical role for Lys414 in the enhancement of [Lys]Pg and [Lys]Pm binding and conformational [Lys]Pg activation. The LBS-independent affinity of SK for [Glu]Pg was unaffected by deletion of Lys414. By contrast, removal of SK Lys414 caused 19- and 14-fold decreases in SK affinity for [Lys]Pg and [Lys]Pm binding in the catalytic mode, respectively. In kinetic studies of the coupled conformational and proteolytic activation of [Lys]Pg, SKΔK414 exhibited a corresponding 17-fold affinity decrease for formation of the SKΔK414-[Lys]Pg* complex. SKΔK414 binding to [Lys]Pg and [Lys]Pm and conformational [Lys]Pg activation were LBS-independent, whereas [Lys]Pm substrate binding and proteolytic [Lys]Pm generation remained LBS-dependent. We conclude that binding of SK Lys414 to [Lys]Pg and [Lys]Pm kringle enhances SK-[Lys]Pg* and SK-[Lys]Pm catalytic complex formation. This interaction is distinct structurally and functionally from LBS-dependent Pg substrate recognition by these complexes.

Streptokinase (SK)2 activates the human fibrinolytic system by activating the zymogen, plasminogen (Pg) to form the fibrin-degrading protease, plasmin (Pm) (1). The mechanism of SK-activated Pm formation is unique in that it is initiated by formation of an SK-Pg* complex in which the zymogen catalytic site is activated non-proteolytically (2–5). SK-Pg* binds free Pg and converts it into Pm by intermolecular proteolytic cleavage (6). Pm binds tightly to SK in the catalytic mode (7, 8) and SK-Pm propagates proteolytic Pg activation (6, 9, 10) through expression of a Pg substrate binding exosite (7). The mechanism is regulated by intrinsic differences in affinity of SK for [Glu]Pg, [Lys]Pg, and [Lys]Pm (5–8, 11). [Glu]Pg consists of an NH2-terminal 77-residue peptide, five kringle domains, and a serine proteinase catalytic domain (12). [Glu]Pm is maintained in a compact conformation through intramolecular interaction of the NH2-terminal peptide with kringle 4 and 5 (13–15). Pm cleavage of the NH2-terminal peptide of [Glu]Pg generates the more reactive [Lys]Pg, which assumes an extended conformation with expression of enhanced lysine-binding site (LBS) interactions (12, 14–16).

Formation of SK-[Lys]Pg* and SK-[Lys]Pm catalytic complexes and subsequent [Lys]Pm substrate recognition are enhanced by interactions of SK with LBS of Pg and Pm kringle domains (5–8, 11, 17–20). Recent studies of the Pg activation mechanism demonstrate that LBS interactions enhance SK-[Lys]Pg* catalytic complex formation and Pg substrate binding but are not absolutely required for these interactions (5, 6). SK binding to the compact conformation of [Glu]Pg in the catalytic mode is LBS-independent (5, 6, 8, 11).

Several studies have sought to define SK lysine residues that mediate its interactions with Pg and Pm kringles. The crystal structure of SK bound to the isolated Pm catalytic domain (micro-Pm) shows that SK consists of three homologous, independently folded β-grasp domains connected by flexible linking segments (21). SK forms a “crater” around the Pm catalytic site which provides a surface for Pg substrate binding (21). Studies of SK domain truncation and deletion mutants, isolated domains, and point mutants have led to diverse interpretations, indicating that each of the SK domains may participate in kringle interactions (18–20, 22, 23). Some studies support a role for the flexible 250-loops of the SK β-domain in LBS-dependent Pg substrate binding (19, 20). By contrast, no studies have demonstrated the structural basis for the LBS dependence of catalytic complex formation.

Kringles of Pg and Pm bind zwitterionic COOH-terminal lysine residues and lysine analogues specifically, notably 6-aminohexanoic acid (6-AHA) (13, 24). This is the basis for Pg binding by several proteins, including fibrin (25, 26), antiplasmin (27), histidine-rich glycoprotein (28), and tetranectin (29). In fibrinolysis, LBS interactions with fibrin mediated by COOH-terminal lysine residues localize and accelerate Pg activation.

* This work was supported by National Institutes of Health/NHLBI Grant HL056181 (to P. E. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby indicated “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1. To whom correspondence should be addressed: Dept. of Pathology, Vanderbilt University School of Medicine, C3321A Medical Center North, Nashville, TN 37232-2561. Tel.: 615-343-9863; Fax: 615-322-1855; E-mail: paul.boxrud@vanderbilt.edu.

2. The abbreviations used are: SK, streptokinase; wtSK, recombinant wild-type SK; SKΔK414, streptokinase mutant lacking the COOH-terminal lysine; CbP, carboxypeptidase B; 6-AHA, 6-aminohexanoic acid; pNA, p-nitroaniline; Pg, plasminogen; [Glu]Pg, compact form of Pg; [Lys]Pg, [Glu]Pg lacking the 77-residue NH2-terminal peptide; [Lys]Pm, Pm, plasmin; fluorescein-labeled analogues of Pg or Pm prepared with Nε-[(acetylthio)acetyl]-L-[Phe]-Phe-Arg-CH2Cl and 5-(iodoacetamido)fluorescein are represented by [5F]FFR-Pg or –Pm; LBS, lysine-binding site; TEV, tobacco etch virus.
and fibrin degradation by Pm (25, 26, 30) and protect fibrin-bound Pm from inactivation by antiplasmin (31). Surprisingly, the fact that the COOH-terminal residue of SK is Lys$^{414}$ (32) has been overlooked in previous studies. Here, we show that Lys$^{414}$ is responsible for the LBS-dependent enhancement in affinity of SK-[Lys]$^*$Pg and SK-[Lys]Pm catalytic complex formation. This interaction is shown to be structurally and functionally distinct from the LBS-dependent binding of [Lys]$^*$Pg as a substrate of the catalytic complexes.

**EXPERIMENTAL PROCEDURES**

**Wild-type SK and an SK Mutant Lacking the COOH-terminal Lys**—Wild-type SK (wtSK) was prepared by methods described previously (33, 34) or was expressed as a fusion protein with a tobacco etch virus (TEV) proteinase cleavage site (underlined) encoded before the wtSK protein, Met-His$_6$-Ser-Ala-Gly-Gly-Ser-Pro-Trp-Asn-Glu-Asn-Leu-Try-Phe-Gln-SK$_{414}$ (His$_6$-wtSK). From a pET30a(+) vector backbone, a single nucleotide substitution mutated the P1$^3$ residue of a thrombin cleavage site (from Arg to Trp), which eliminated this unnecessary site and generated a Ncol restriction site. Flanking Ncol and Xhol restriction sites (underlined) were incorporated into the 5’-and 3’-PCR primers, respectively, with the sense primer, 5’TACACTCCGGGGTGTTAGGGTTATCAGG-3, the same for wtSK and SK$\Delta K414$ constructs, and the antisense primer, 5’-ATAATGGTGCTC-GAGATTTTTGCTTATGGTTATACGG-3’, only differed by the Lys$^{414}$ codon (bold). A construct that encoded a His$_6$-tagged TEV proteinase was kindly provided by Dr. Laura Mizoue of the Vanderbilt University Center for Structural Biology and used to remove the His$_6$-tag from the NH$_2$ terminus of SK$_{414}$K.

His$_6$-wtSK was expressed from Rosetta(DE3) pLysS cells induced with 20 g/liter lactose for 12–16 h at 37 °C. Cells were harvested by centrifugation, resuspended in 50 mM Hepes, 125 mM NaCl, 1 mg/ml polyethylene glycol 8000, pH 7.4 (Buffer A) with 1 mM EDTA and 0.2% sodium azide, lysed by three cycles of sonication (~45 s cycles) on ice, and centrifuged to clarify lysates. The pellet was resuspended in Buffer A containing 3 M NaSCN. The solubilized wtSK was dialyzed into 50 mM Hepes, 400 mM NaCl, 50 mM imidazole, pH 7.4 (Buffer B) and purified by Ni$^{2+}$-iminodiacetic acid-Sepharose chromatography with a 50–500 mM imidazole gradient in Buffer B. TEV proteinase was added to the eluted protein in a 1 to 5 molar ratio of enzyme to substrate. The reaction mixture was first dialyzed overnight into 50 mM Hepes, 300 mM NaCl, 1 mM dithiothreitol, 5% glycerol, pH 7.8 at 4 °C, and subsequently dialyzed back into Buffer B. Uncleaved fusion protein, cleaved His$_6$-tag, and the TEV proteinase bound to Ni$^{2+}$-iminodiacetic acid-Sepharose, and wtSK was obtained from the column flow-through. wtSK was dialyzed against Buffer A without polyethylene glycol, quick-frozen, and stored at ~80 °C. SK$\Delta K414$K was prepared following an identical procedure. The correct NH$_2$-terminal sequence for wtSK and SK$\Delta K414$K was confirmed.

**Native and Carboxypeptidase B (CpB)-treated SK**—Native SK purchased from Diapharma and purified as described previously (7, 8, 11) was treated with porcine pancreatic CpB (Sigma Type-I, diisopropylfluorophosphate-treated, 4.7 mg/ml in 0.1 M NaCl) in 50 mM Hepes, 125 mM NaCl, pH 7.4. SK (2.5 mg/ml) was incubated with CpB (35 μg/ml) for 30 min at 25 °C, and the reaction was stopped by addition of 10 mM EDTA. Titrations of [5F]FFR- [Lys]$^*$Pg were performed in 50 mM Hepes, 125 mM NaCl, 1 mM EDTA, 10 μM Val-Phe-Arg-CH$_2$Cl, 1 mg/ml bovine serum albumin, ±100 mM 6-AHA, pH 7.4.

**Fluorescence Equilibrium Binding**—[Glu]$^*$Pg, [Lys]$^*$Pg, and the active site-labeled fluorescein analogues were prepared as described (5, 6, 11). Fluorescence titrations were performed in Buffer A containing 1 mM EDTA and 1 μM Phe-Phe-Arg-CH$_2$Cl ± 10 mM 6-AHA as described previously (5, 6, 33). Fluorescence changes ($F_{obs} - F_o$)/$F_o = \Delta F/F_o$ as a function of total SK concentration, were fit by the quadratic binding equation to determine the maximum fluorescence change ($\Delta F_{max}/F_o$) and dissociation constant ($K_D$), with the stoichiometric factor (n) fixed at 1. Competitive binding titrations of native [Lys]$^*$Pg were performed by addition of wtSK or SK$\Delta K414$ to mixtures of [5F]FFR-[Lys]$^*$Pg as a function of native [Lys]$^*$Pg concentration, as described previously (5). Results were analyzed by fitting of the cubic binding equation to determine the $K_D$ of wtSK and SK$\Delta K414$ for native [Lys]$^*$Pg.

**Plasminogen Activation Kinetics**—Coupled conformational and proteolytic activation of [Lys]$^*$Pg by wtSK and SK$\Delta K414$ was quantitated as described previously (5, 6). Fitting of parabolic progress curves of d-Val-Leu-Lys-pNA (VLK-pNA) hydrolysis at 200 μM, in the presence of 15 nM [Lys]$^*$Pg and increasing wtSK and SK$\Delta K414$ concentrations gave the initial rates ($v_i$) of VLK-pNA hydrolysis, reflecting conformational activation of the SK-Pg* complex, and the rates of activity increase ($v_j$), reflecting Pm generation. The SK dependences of $v_i$ and $v_j$ were analyzed using the simplified equations described previously (5, 6).

$$K_A + Pm \rightleftharpoons K_{pm}$$

$$SK + Pm \rightleftharpoons SK-Pm + Pm$$

$$K_A + Pm \rightleftharpoons K_{pm}$$

$$SK + Pm \rightleftharpoons SK-Pm + Pm$$

**SCHEME 1**

The data were also analyzed by fitting of the family of progress curves as a function of SK concentration with a more complete mechanism including both SK-Pg*- and SK-Pm-catalyzed Pm activation pathways under bimolecular reaction conditions (Scheme 1). For this analysis, the $K_m$ values for chromogenic substrate hydrolysis by Pm, SK-Pg*, and SK-Pm were fixed at the previously determined values, whereas the corresponding $k_{cat}$ values were allowed to vary within the experimental error of their determination to optimize the fit. $K_{cat}$ for SK-Pm binding was fixed at 12 pm (7). The fitted parameters were $K_A$ for SK-Pg* and $K_{pm}$ for SK-Pm activation pathways under bimolecular reaction conditions.
ACCELERATED PUBLICATION: Interactions of the COOH-terminal Lysine of Streptokinase

**RESULTS AND DISCUSSION**

Binding of Native SK and CpB-treated SK to [Lys]Pg—Native SK was treated with CpB to remove the COOH-terminal lysine residue, under conditions where there was no detectable degradation of SK observable by SDS-gel electrophoresis (not shown). Titrations of [5F]FFR-[Lys]Pg with native SK and CpB-SK were performed in the absence and presence of saturating 6-AHA to evaluate the effect of CpB treatment on the LBS dependence of SK affinity (Fig. 1A). In the absence and presence of 6-AHA, native SK bound fluorescein-labeled [Lys]Pg with $K_D$ 20 ± 5 nM and 380 ± 40 nM, respectively, and with $\Delta F_{\text{max}}/F_o$ of −15 ± 1% and −19 ± 1%. By contrast, CpB-SK bound labeled [Lys]Pg with $K_D$ 130 ± 20 nM and $\Delta F_{\text{max}}/F_o$ of −17 ± 1% in the absence of 6-AHA, and with $K_D$ 300 ± 50 nM and $\Delta F_{\text{max}}/F_o$ of −19 ± 1% in the presence of 6-AHA. The affinities for native and CpB-treated SK in the presence of 6-AHA were indistinguishable. CpB treatment of SK decreased the effect of 6-AHA on [Lys]Pg affinity from 19-fold to 2.3-fold. This demonstrated that CpB treatment of SK resulted in a selective loss of LBS-dependent affinity for labeled [Lys]Pg.

Binding of wtSK and SKΔK414 to [Lys]Pg—Titrations of [5F]FFR-[Lys]Pg with wtSK and SKΔK414 were performed in the absence and presence of 10 mM 6-AHA (Fig. 1C). In the absence and presence of 6-AHA, wtSK bound labeled [Lys]Pg with $K_D$ 930 ± 120 nM and 471 ± 140 nM, respectively, and with $\Delta F_{\text{max}}/F_o$ of −37 ± 1% and −19 ± 1%. SKΔK414 bound labeled [Lys]Pg with $K_D$ 634 ± 160 nM and 560 ± 150 nM in the absence and presence of 6-AHA, respectively, and with $\Delta F_{\text{max}}/F_o$ of −30 ± 2% and −19 ± 1%. Although the amplitudes of the fluorescence changes were decreased by 6-AHA, the affinities of wtSK and SKΔK414 for labeled [Lys]Pg in the presence of 6-AHA were indistinguishable and LBS-independent. This was consistent with the LBS independence of SK binding to [Lys]Pg in the compact conformation (5, 8, 11) and an independence of the affinity on deletion of Lys414.

Binding of wtSK and SKΔK414 to [Glu]Pg—Titrations of [5F]FFR-[Glu]Pg with wtSK and SKΔK414 were performed in the absence and presence of 10 mM 6-AHA (Fig. 1C). In the absence and presence of 6-AHA, wtSK bound labeled [Glu]Pg with $K_D$ 390 ± 120 nM and 471 ± 140 nM, respectively, and with $\Delta F_{\text{max}}/F_o$ of −37 ± 1% and −19 ± 1%. SKΔK414 bound labeled [Glu]Pg with $K_D$ 634 ± 160 nM and 560 ± 150 nM in the absence and presence of 6-AHA, respectively, and with $\Delta F_{\text{max}}/F_o$ of −30 ± 2% and −19 ± 1%. Although the amplitudes of the fluorescence changes were decreased by 6-AHA, the affinities of wtSK and SKΔK414 for labeled [Glu]Pg in the presence of 6-AHA were indistinguishable and LBS-independent. This was consistent with the LBS independence of SK binding to [Glu]Pg in the compact conformation (5, 8, 11) and an independence of the affinity on deletion of Lys414.

Binding of wtSK and SKΔK414 to [Lys]Pg—To determine whether the COOH-terminal lysine of SK also interacted with [Lys]Pg kringles, titrations of [5F]FFR-Pm with wtSK and SKΔK414 were performed in the absence and presence of 10 mM 6-AHA (Fig. 1D). wtSK bound labeled [Lys]Pg with $K_D$ 19 ± 7 nM, consistent with the previously reported affinity (7) and $\Delta F_{\text{max}}/F_o$ of −54 ± 2% in the absence of 6-AHA, compared with a 14-fold higher $K_D$ of 260 ± 70 nM and $\Delta F_{\text{max}}/F_o$ of −49 ± 2% in the presence of 6-AHA. SKΔK414 bound labeled [Lys]Pg with impaired affinity represented by $K_D$ 190 ± 40 PM and 350 ± 80 PM in the absence and presence of 6-AHA, respectively, and with $\Delta F_{\text{max}}/F_o$ of −50 ± 1% and −47 ± 2%. The affinities of wtSK for [5F]FFR-Pm in the presence of 6-AHA and that of SKΔK414 in the absence and presence of 6-AHA were indistinguishable within the experimental error. The results indicated that similar to [Lys]Pg, the affinity for complex formation between wtSK and [Lys]Pg was enhanced by interaction of SK Lys414 with kringles on [Lys]Pg.

Binding of SKΔK414 and wtSK to Native Pg—The affinities of active site-labeled [Glu]Pg and [Lys]Pg for SK are consistently ~5-fold lower than those of the native proteins, as determined in previous studies, but maintain the same magnitude of the effects of 6-AHA on binding (5, 11). To characterize binding of wtSK and SKΔK414 to native [Lys]Pg, [5F]FFR-[Lys]Pg was used as a probe of the native and labeled [Lys]Pg competitive binding equilibria in the absence of proteinase inhibitors. To resolve the rapid binding equilibrium from slower proteolytic cleavage, individual measurements were made as a function of time after addition of wtSK or SKΔK414 to mixtures of [5F]FFR-[Lys]Pg and various concentrations of native [Lys]Pg at fixed wtSK and SKΔK414 concentrations as described previ-
Simultaneous fits of the direct titration and the competitive titration data in Fig. 2 by the cubic binding equation gave a $K_D$ of 8.8 ± 4.3 nM for wtSK and SKΔK414. A, the fractional change in fluorescence ($ΔF/F_0$) of 15 nM [5F]-FFR-[Lys]Pg plotted against the total concentration of native [Lys]Pg ([Pg]₀) for 150 nM (○) and 1 μM (■) wtSK. B, similar titrations are shown in the presence of 10 mM 6-AHA for 100 nM (▲) and 1 μM (▲) wtSK. C, titrations at 1 μM SKΔK414 in the absence (■) and presence (▲) of 10 mM 6-AHA. The lines represent the fit by the cubic competitive binding equation with the parameters given in the “Results and Discussion” and $n$ fixed at 1. Fluorescence titrations were performed and analyzed as described under “Experimental Procedures.”

**Plasminogen Activation Kinetics**—Determination of the affinities of wtSK and SKΔK414 for native [Lys]Pg was necessary to interpret the kinetics of native [Lys]Pg activation by the SK mutant. The kinetics of [Lys]Pg activation were examined by analysis of reaction progress curves in the presence of VLK-pNA, monitored by hydrolysis of the chromogenic substrate. As previously detailed (5, 6), the parabolic progress curves were resolved into an initial rate of substrate hydrolysis ($v_1$) representing the activity of the conformationally activated SK-Pg* complex and the rate of acceleration ($v_2$) representing the subsequent proteolytic generation of Pm. The dependences of $v_1$ and $v_2$ on SK concentration were analyzed as described under “Experimental Procedures.” A second method was also used for the analysis in which families of progress curves collected as a function of SK concentration were fit by numerical integration of the rate equations for the complete mechanism, including both SK-Pg* and SK-Pm-catalyzed sequential reactions (Scheme 1 and see “Experimental Procedures”).

Analysis of the hyperbolic $v_1$ dependence on SK concentration in the absence of 6-AHA gave apparent $K_A$ of 2 ± 1 nM and 68 ± 27 nM for wtSK and SKΔK414, respectively (Fig. 3A). In the presence of 6-AHA, the formation of the wtSK-[Lys]Pg* complex was weakened to 43 ± 12 nM (22-fold), whereas the affinity of SKΔK414 was indistinguishable at 87 ± 28 nM, indicating that formation of the SKΔK414-[Lys]Pg* catalytic complex was LBS-independent. Analysis of the bimodal $v_2$ dependence on wtSK concentration in the absence of 6-AHA gave a
similar affinity of 1.5 ± 0.1 nM for SK-[Lys]Pgs formation. The $v_2$ dependence of SKΔK414 was shifted to higher SK concentration, due to coupling between the formation of SKΔK414-[Lys]Pgs and Pm generation, corresponding to $K_A$ 63 ± 54 nM for SKΔK414-[Lys]Pgs formation. Values of $K_A$ determined kinetically in the absence and presence of 6-AHA were in good agreement with the dissociation constants determined above by competitive binding. The bimolecular rate constants ($k_{pg}$), representing Pg substrate binding and Pm formation, were indistinguishable for wtSK and SKΔK414, as indicated by the same $v_2$ maxima. In the presence of 6-AHA, $v_2$ was decreased 20-fold for wtSK and an indistinguishable ~25-fold for SKΔK414. Analysis of reaction progress curves by numerical integration of the rate equations gave $K_A$ 3.2 ± 0.1 nM and 88 ± 7 nM for wtSK and SKΔK414, respectively, in the absence of 6-AHA, and indistinguishable $k_{pg}$ of 0.0006 ± 0.0001 nM$^{-1}$ s$^{-1}$, whereas in the presence of 6-AHA, $K_A$ for wtSK and SKΔK414 increased to 39 ± 1 nM (12-fold) and 131 ± 8 nM (1.5-fold), respectively.

In conclusion, results of quantitative equilibrium binding and [Lys]Pg activation kinetic studies with wtSK, Cpβ-treated SK, and a COOH-terminal SKΔK414 deletion mutant demonstrated a critical role for the COOH-terminal lysine of SK in the enhancement of [Lys]Pg and [Lys]Pm binding and conformational [Lys]Pg activation. Removal of SK Lys414 resulted in complete loss of the LBS dependence of SK binding to [Lys]Pg and [Lys]Pm in the catalytic mode and a corresponding loss of LBS-dependent affinity for formation of the conformationally activated SK-[Lys]Pgs complex measured kinetically.

SKΔK414 binding to [Lys]Pg and [Lys]Pm and conformational [Lys]Pg activation were LBS-independent, as shown by the absence of a significant effect of 6-AHA, whereas [Lys]Pg substrate binding remained LBS-dependent to a comparable extent as that of wtSK. We conclude that binding of SK Lys414 to [Lys]Pg and [Lys]Pm kringles enhances SK-Pgs* and SK-Pm catalytic complex formation. The Pg substrate interaction is concluded to be mediated by a distinct SK structure responsible for Pg substrate recognition by the SK-Pgs* and SK-Pm complexes. Further studies will be required to clarify the SK structure responsible for the LBS dependence of Pg substrate recognition.

Acknowledgments—We thank Malabika Laha and Ronald R. Bean for excellent technical assistance.

REFERENCES
1. Collen, D., and Lijnen, H. R. (1991) Blood 78, 3114–3124
2. McClintock, D. K., and Bell, P. H. (1971) Biochem. Biophys. Res. Commun. 43, 694–702
3. Reddy, K. N., and Markus, G. (1972) J. Biol. Chem. 247, 1683–1691
4. Schick, L. A., and Castellino, F. J. (1974) Biochem. Biophys. Res. Commun. 57, 47–54
5. Boxrud, P. D., Verhamme, I. M., and Bock, P. E. (2004) J. Biol. Chem. 279, 36633–36641
6. Boxrud, P. D., and Bock, P. E. (2004) J. Biol. Chem. 279, 36642–36649
7. Boxrud, P. D., Fay, W. P., and Bock, P. E. (2000) J. Biol. Chem. 275, 14579–14589
8. Boxrud, P. D., and Bock, P. E. (2000) Biochemistry 39, 13974–13981
9. Gonzalez-Gronow, M., Siefried, G. E., Jr., and Castellino, F. J. (1978) J. Biol. Chem. 253, 1090–1094
10. Wohl, R. C., Summaria, L., and Robbins, W. J. (1980) J. Biol. Chem. 255, 2005–2013
11. Bock, P. E., Day, D. E., Verhamme, I. M., Bernardo, M. M., Olson, S. T., and Shore, J. D. (1996) J. Biol. Chem. 271, 1072–1080
12. Ponting, C. P., Marshall, J. M., and Cederholm-Williams, S. A. (1992) Blood Coagul. Fibrinolysis 3, 605–614
13. Ponting, C. P., Holland, S. K., Cederholm-Williams, S. A., Marshall, J. M., Brown, A. J., Spraggon, G., and Blake, C. C. (1992) Biochim. Biophys. Acta 1159, 155–161
14. Marshall, J. M., Brown, A. J., and Ponting, C. P. (1994) Biochemistry 33, 3599–3606
15. McCance, S. G., and Castellino, F. J. (1995) Biochemistry 34, 9581–9586
16. Violand, B. N., Byrne, R., and Castellino, F. J. (1978) J. Biol. Chem. 253, 5395–5401
17. Lin, L. F., Houg, A., and Reed, G. L. (2000) Biochemistry 39, 4740–4745
18. Conejero-Lara, F., Parrado, J., and Ponting, C. P. (1998) Protein Sci. 7, 2190–2199
19. Dhar, J., Pande, A. H., Sundram, V., Nanda, J. S., Mande, S. C., and Sahni, G. (2002) J. Biol. Chem. 277, 13257–13267
20. Chaudhary, A., Vasudha, S., Rajagopal, K., Komath, S. S., Garg, N., Yadav, M., Mande, S. C., and Sahni, G. (1999) Protein Sci. 8, 2791–2805
21. Wang, X., Lin, X., Loy, J. A., Tang, J., and Zhang, X. C. (1998) Science 281, 1662–1665
22. Loy, J. A., Lin, X., Schenone, M., Castellino, F. J., Zhang, X. C., and Tang, J. (2001) Biochemistry 40, 14686–14695
23. Sazonova, I. Y., Robinson, B. R., Glaydsheva, I. P., Castellino, F. J., and Reed, G. L. (2004) J. Biol. Chem. 279, 24994–25001
24. Castellino, F. J., and McCance, S. G. (1997) Ciba Found. Symp. 212, 46–60
25. Lucas, M. A., Fretto, L. J., and McKee, P. A. (1983) J. Biol. Chem. 258, 4249–4256
26. Bok, R. A., and Mangel, W. F. (1985) Biochemistry 24, 3279–3286
27. Hortin, G. L., Gibson, B. L., and Fok, K. F. (1988) Biochem. Biophys. Res. Commun. 155, 591–596
28. Lijnen, H. R., Hoylaers, M., and Collen, D. (1980) J. Biol. Chem. 255, 10214–10222
29. Clemmensen, I., Petersen, L. C., and Kluft, C. (1986) Eur. J. Biochem. 156, 327–333
30. Wiman, B., and Collen, D. (1978) Nature 272, 549–550
31. Wiman, B., and Collen, D. (1978) Eur. J. Biochem. 84, 573–578
32. Jackson, K. W., and Tang, J. (1982) Biochemistry 21, 6620–6625
33. Bean, R. R., Verhamme, I. M., and Bock, P. E. (2005) J. Biol. Chem. 280, 7504–7510
34. Boxrud, P. D., Verhamme, I. M., Fay, W. P., and Bock, P. E. (2001) J. Biol. Chem. 276, 26084–26089
35. Kuzmic, P. (1996) Anal. Biochem. 237, 260–273
36. Schechter, L., and Berger, A. (1967) Biochim. Biophys. Res. Commun. 27, 157–162