Kinetics and Mechanism of Platelet-Surface Plasminogen Activation by Tissue-Type Plasminogen Activator†

Helene Ouimet, Jane E. Freedman, and Joseph Loscalzo*

Cardiovascular Divisions, Brigham and Women's Hospital and Brockton/West Roxbury VA Medical Center, Harvard Medical School, Boston, Massachusetts 02115

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ABSTRACT: Plasminogen and tissue-type plasminogen activator bind to the platelet surface, and as a result, the catalytic efficiency of plasminogen activation is significantly enhanced. The plasmin that is generated on or near the platelet is known to affect a number of platelet surface events. For this reason, we examined the effect of plasmin on platelet-surface plasminogen activation and its determinants. Specifically, we measured the effects of plasmin treatment of platelets (1 caseinolytic unit/mL for 1 h at 37 °C) on plasminogen, tissue-type plasminogen activator, and plasmin binding to the unactivated and ADP-activated platelet surface; and on the kinetics of plasminogen activation on the platelet surface. Following plasmin treatment, the number of plasminogen binding sites on unactivated platelets increased by 78% (from 46 000 ± 4000 to 88 000 ± 9000 sites/platelet), while the number of tissue-type plasminogen activator sites did not change, and the number of diisopropyl fluorophosphate (DFP)-inactivated plasmin (DFP-plasmin) binding sites decreased by 31% (from 92 000 ± 11 000 to 65 000 ± 7000 sites/platelet); the dissociation constants (K_ds) for each of these binding processes did not change significantly following treatment. On ADP-activated platelets, plasmin treatment increased the number of plasminogen binding sites by 41% (from 188 000 ± 17 000 to 265 000 ± 25 000 sites/platelet), decreased the number of plasmin binding sites by 28% (from 219 000 ± 41 000 to 157 000 ± 24 000 sites/platelet), and did not affect the number of tissue-type plasminogen activator sites; again, the K_ds for each of these binding processes did not change significantly following treatment. Competitive binding studies revealed that plasminogen and DFP-plasmin partially competed for platelet surface binding, suggesting that both common and independent binding sites are involved in the binding mechanism(s). Furthermore, plasminogen was 14-fold more effective as a competitive inhibitor of DFP-plasmin binding to activated than unactivated platelets (IC_{50} = 0.5 μM vs 7.0 μM, respectively). Plasmin treatment led to an approximate 2-fold increase in the catalytic efficiency of tissue-type plasminogen activator activity, and ADP-activation led to an approximate 9-fold increase in the catalytic efficiency, while ADP-activation following plasmin treatment led to an approximate 5-fold increase in catalytic efficiency. A kinetic model is represented that fits the experimental observations well and supports a mechanism whereby plasmin treatment enhances the catalytic efficiency of tissue-type plasminogen activator on the platelet surface as a consequence of enhanced substrate binding and reduced product binding, leading to increased turnover. These observations suggest that plasminogen activation by tissue-type plasminogen activator may be considered an autocatalytic process on the platelet surface and support the view that a unique reciprocating mechanism regulates the interaction between platelets and the plasminogen activator system.

Cell-surface plasminogen activation is increasingly recognized as an important determinant of a number of biologic processes, including embryogenesis (Plow & Miles, 1991), metastasis (Burtin & Fondoneche, 1988; Miles & Plow, 1988), and the maintenance of blood fluidity (Blasi, 1988; Miles & Plow, 1988). The assembly of a plasminogen activator and its substrate on the cell surface has a number of biochemical consequences that, in general, promote the formation of active plasmin. These include enhanced catalytic efficiency of plasminogen activation (Miles & Plow, 1985; Hajjar et al., 1986; Stephens et al., 1989), protection of cell-associated plasmin from α_2-antiplasmin (Plow et al., 1986), and local elaboration of plasmin activity in a relatively protected environment (Miles & Plow, 1985; Plow et al., 1986).

The plasminogen activator system can assemble on a variety of peripheral blood cells, including platelets (Miles et al., 1988; Adelman et al., 1989). Plasminogen and tissue-type plasminogen activator (t-PA) both bind directly to platelets (Miles & Plow, 1985; Vaughan et al., 1989), and as a result, the catalytic efficiency of plasminogen activation by t-PA is enhanced (Gao et al., 1990). Since plasmin generated on or near the platelet surface can variably affect a variety of platelet-surface events, we chose to examine the effects of plasmin exposure on platelet-surface plasminogen activation on unactivated and ADP-activated platelets.

MATERIALS AND METHODS

Materials. Sephadex G-25, lysine–Sepharose 2B and 4B, and precast 8–25% polyacrylamide gradient gels were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Tissue-type plasminogen activator (t-PA) was provided by Genentech, Inc., S. San Francisco, CA, and urokinase was purchased

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* To whom correspondence should be addressed. Current address: Whitaker Cardiovascular Institute, Center for Advanced Biomedical Research, Boston University School of Medicine, 700 Albany St., Boston, MA 02118.

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from Abbott Laboratories, Evanston, IL. Plasmin and H-D-valyl-L-leucyl-L-lysine-*p*-nitroanilide (S-2251) were obtained from Kabi Vitrum, Stockholm, Sweden. $Na^{125}I$ was purchased from New England Nuclear, Boston, MA, and Iodobeads were obtained from Pierce Chemical Co., Rockford, IL. Tranexamic acid, bovine serum albumin (BSA), aprotinin, diisopropyl fluorophosphate (DFP), prostaglandin (PGE_1), L-arginine, L-lysine, and Tween 80 were purchased from Sigma Chemical Co., St. Louis, MO. Silicone oil was obtained from William F. Nye, Inc., New Bedford, MA. Monoclonal antibody S12 directed against P-selectin was a kind gift from Dr. Rodger McEver, University of Oklahoma, Oklahoma City, OK. All other chemicals were reagent grade or better.

Protein Preparation. Glu-plasminogen was purified from human plasma by affinity chromatography on L-lysine–Sepharose 4B (Deutsch & Mertz, 1970). The purified Glu-plasminogen (99.2% Glu form by amino-terminal analysis) was then dialyzed against either 10 mM sodium phosphate, pH 7.4, and 0.15 M NaCl (PBS) or 50 mM sodium phosphate, pH 7.4, and 50 mM L-lysine (PLB) and concentrated using Centricones (Amicon Corp., Danvers, MA). Protein concentrations were determined by the method of Lowry and colleagues (1951) or of Bradford (1976). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) using the Phast System to ensure the purity of the plasminogen. Gels were run with low- and high-molecular-weight standards (Bio-Rad, Richmond, CA) and stained with Coomassie Brilliant Blue.

Plasmin was prepared by incubating plasminogen dialyzed before use against PLB with urokinase (500 units/mL) for 20 min at 37 °C. Plasmin activity was determined using the chromogenic substrate, S-2251, and found to be 2 CU/nmol.

Diisopropyl fluorophosphate-inactivated plasmin (DFP-plasmin) was prepared by incubating plasmin with 5 mM diisopropyl fluorophosphate in PBS for 24 h at 4 °C. Following dialysis to remove excess diisopropyl fluorophosphate, the final preparation was tested for residual plasmin activity, which never exceeded 0.1% of the original activity.

Collection and Preparation of Platelets. Venous blood was obtained from healthy volunteer donors, none of whom had taken aspirin or any drug likely to affect platelet function for at least 10 days. Blood was collected in 13 mM citrate, 16 mM NaH_2PO_4, and 142 mM dextrose (CPD) containing 11 μM PGE_1. Whole blood was centrifuged at 120*g* for 10 min at 22 °C to prepare platelet-rich plasma (PRP).

Plasmin Treatment of Platelets. PRP was incubated with plasmin at a final activity of 1.0 CU/mL for 1 h at 37 °C. Following this incubation period, aprotinin was added to a final concentration of 350 KIU/mL to end the reaction.

Gel Filtration of Platelets. Platelets were separated from plasma using a Sepharose 2B column equilibrated with 5.8 mM sodium HEPES, pH 7.35, 140 mM NaCl, 6.11 mM KCl, 2.53 mM $MgSO_4$, 2.4 mM Na_2SO_4, 59 μM bovine serum albumin, and 5.63 mM dextrose (HBS). The column was developed at a flow rate of 2 mL/min at room temperature (Timmons & Hawiger, 1978). Platelet counts were adjusted to 1.5×10^8/mL with HBS using a Coulter Counter Model ZM (Coulter Electronics, Hialeah, FL).

Radioiodination. Plasminogen, DFP-plasmin, and t-PA were radioiodinated using Iodobeads (Pierce Chemical Co., Rockford, IL). After preincubating two Iodobeads with 1.0 mCi of $Na^{125}I$ for 10 min at 25 °C, 1.0 mL of 0.8 mg/mL protein was added and the reaction allowed to proceed for 15 min. The reaction solution was then removed from the Iodobeads and passed over a Sephadex G-25 column that had been first developed with 20 mM Tris, pH 7.8, and 0.15 M NaCl (TBS) with 1% bovine serum albumin, followed by TBS. Fifteen 0.15-mL fractions were then collected and assayed for total and protein-bound radioactivity. Typically, protein fractions contained at least 94% precipitable radioactivity (using trichloroacetic acid precipitation) and had specific activities of 40–85, 55–96, and 45–92 cpm/ng for plasminogen, DFP-plasmin, and t-PA, respectively.

Binding Assays. Gel-filtered untreated or gel-filtered plasmin-treated platelets (270 μL at 1.5×10^8 platelets/mL) in HBS were incubated with 22–308 μg of ^{125}I-plasminogen, yielding final concentrations of 0.25–3.5 μM, respectively. HBS or tranexamic acid (10 mM) was added to bring the final volume to 450 μL. The platelet suspension was incubated for 60 min, after which bound ligand was separated from free ligand by layering 200-μL aliquots over 250 μL of silicone oil (1 part #556 AC:2 parts #550 AE) in duplicate. After centrifugation for 5 min at 8000*g* in a microfuge, the Eppendorf tubes were inverted and drained of fluid and the tips amputated for γ counting. Specific binding was defined as the difference between total and nonspecific binding, the latter measured in the presence of 10 mM tranexamic acid or a 30-fold molar excess of unlabeled ligand. A similar experimental protocol was used to evaluate the binding of ^{125}I-DFP-plasmin and ^{125}I-t-PA (Vaughan et al., 1989) to gel-filtered platelets. Competitive binding studies were performed using a fixed concentration of radioiodinated ligand (twice the K_d) and increasing concentrations of unlabeled competing ligand.

Binding of the monoclonal antibody against P-selectin, S12, to control and plasmin-treated platelets was evaluated using fluorescence-activated cell sorting, with the kind help of Dr. Alan Michelson and Mr. Marc Bernard (University of Massachusetts, Worcester, MA). Following inhibition of plasmin with aprotinin, a 50-μL aliquot of platelet suspension was mixed with an equal volume of 2% formaldehyde in HBS. After 30 min, 900 μL of HBS was added and the platelet suspensions were subjected to flow cytometry of surface-bound S12, according to previously published reports (Michelson & Bernard, 1990).

Kinetic Assays. Gel-filtered untreated or plasmin-treated platelets at 1.5×10^8/mL were incubated with 0.75–2.5 μM plasminogen in PBS for 30 min at 37 °C. Certain experiments were performed without platelets as platelet-free controls. S-2251 was added to a final concentration of 0.8 mM, and 100 μL of this reaction mixture was added to microtiter wells. One hundred nanomolar t-PA was added to start the reaction, and the optical density was read every 30 s for 7 min using a Dynatech MR 5000 card reader (Dynatech, Chantilly, VA). Comparative blanks included all reaction mixture components except t-PA. In experiments in which the kinetics of S-2251 hydrolysis by plasmin were measured directly, gel-filtered untreated or plasmin-treated platelets at 1.5×10^8/mL were incubated with 0.01–5.0 mM S-2251, and 100 μL of this reaction mixture was added to microtiter wells. Fifty nanomolar plasmin was added to start the reaction, and the optical density was read every 30 s for 7 min using a Dynatech MR 5000 card reader. Comparative blanks included all reaction components except plasmin. The initial reaction velocity was derived from a plot of change in absorbance at 405 nm vs t^2 for assays of plasminogen activation by t-PA, as previously described (Ranby, 1982; Gao et al., 1990).

Kinetic Modeling. A kinetic model was developed utilizing the binding and kinetic parameters derived from the experimental observations made with untreated and plasmin-treated

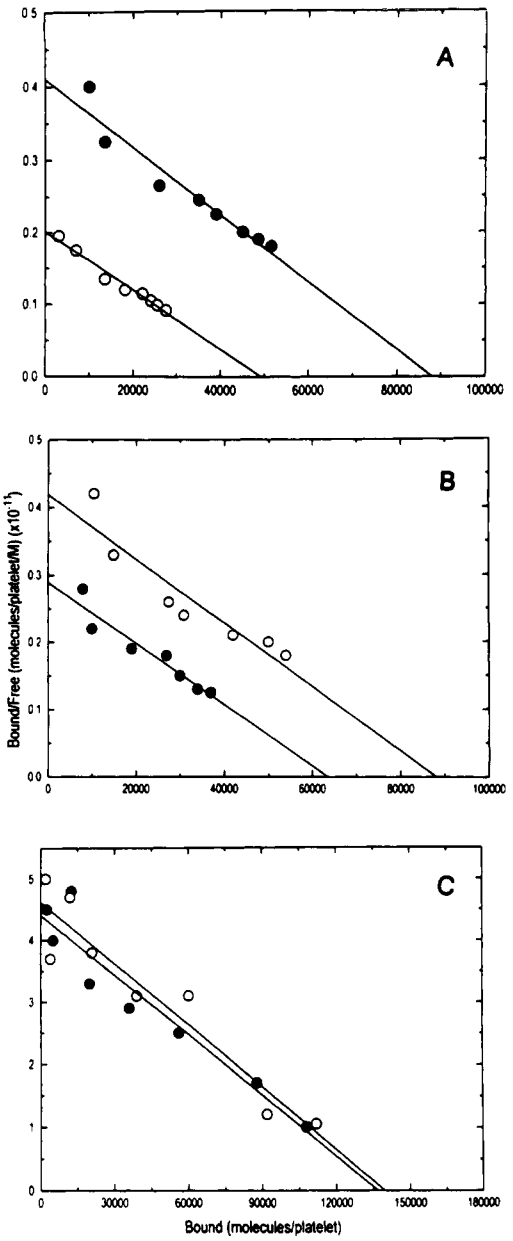

FIGURE 1: Scatchard analysis of the binding of components of the plasminogen activator system to unactivated platelets. Binding analyses were performed as described in Materials and Methods for plasminogen (panel A), DFP-plasmin (panel B), and t-PA (panel C) binding to unactivated platelets before (open circles) and after (closed circles) plasmin treatment.

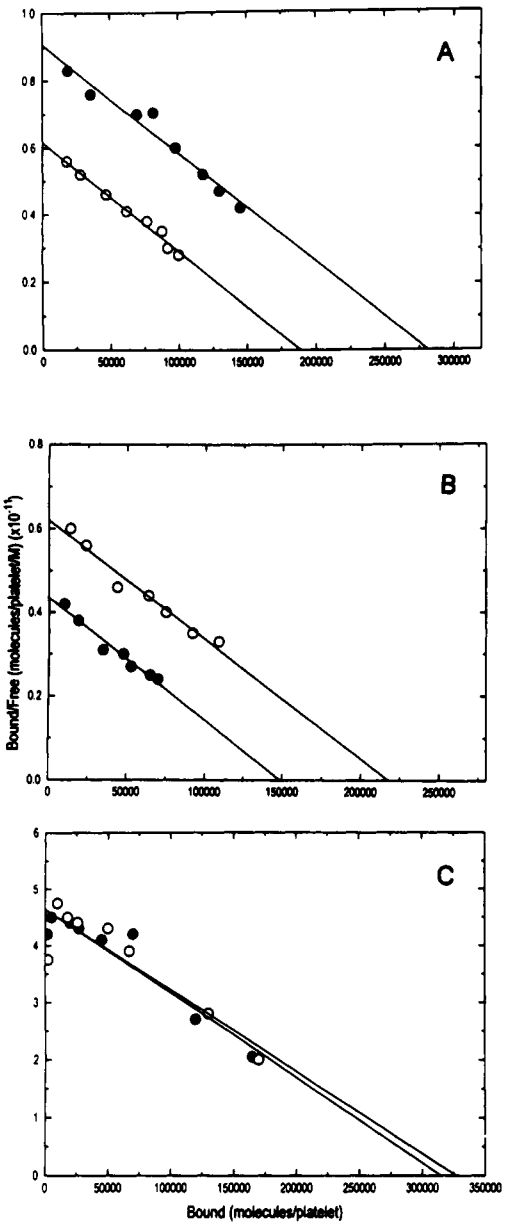

FIGURE 2: Scatchard analysis of the binding of components of the plasminogen activator system to ADP-activated platelets. Binding analyses were performed as described in Materials and Methods for plasminogen (panel A), DFP-plasmin (panel B), and t-PA (panel C) binding to ADP-activated platelets before (open circles) and after (closed circles) plasmin treatment.

platelets. These data were modeled using an IBM personal computer programmed in BASIC.

Statistical Analysis. Data were subjected to analysis of variance methods followed by a Newman–Keuls comparison. p values less than 0.05 were considered significant.

RESULTS

Effect of Plasmin Treatment on Plasminogen, DFP-Plasmin, and t-PA Binding to Unactivated Platelets. Human platelets in platelet-rich plasma were incubated with 1 CU/mL plasmin for 1 h at 37 °C or with buffer, after which they were gel-filtered as described in Materials and Methods. The gel-filtered platelets were then incubated with radioiodinated plasminogen, DFP-plasmin, or t-PA and binding isotherms derived. As shown in the Scatchard analyses of Figure 1, we found that untreated platelets bound 46 000 ± 4000 molecules of plasminogen at saturation and that this number of sites increased by 78% following plasmin treatment to 88 000 ± 9000 ($p < 0.00001$) without affecting the K_d (=2.2 μM) (Figure 1A). By contrast, platelets bound 92 000 ± 11 000 DFP-plasmin molecules/cell at saturation, and this number of sites decreased by 31% to 65 000 ± 7000 ($p < 0.05$), again without affecting the K_d (=2.3 μM) (Figure 1B). Plasmin treatment had no effect on t-PA binding to platelets, as shown by the equivalent number of binding sites before and after treatment (137 000 ± 11 000 vs 144 000 ± 8000, respectively; p = NS), and by equivalent K_ds (=0.31 μM) (Figure 1C).

In all cases, the Scatchard analyses were consistent with a single class of binding sites. In addition, in each case the extent of nonspecific binding accounted for not more than 30% of total binding.

Effect of Plasmin Treatment on Plasminogen, DFP-Plasmin, and t-PA Binding to ADP-Activated Platelets. Gel-filtered untreated and plasmin-treated platelets were activated with 5 μM ADP, after which the binding of radioiodinated

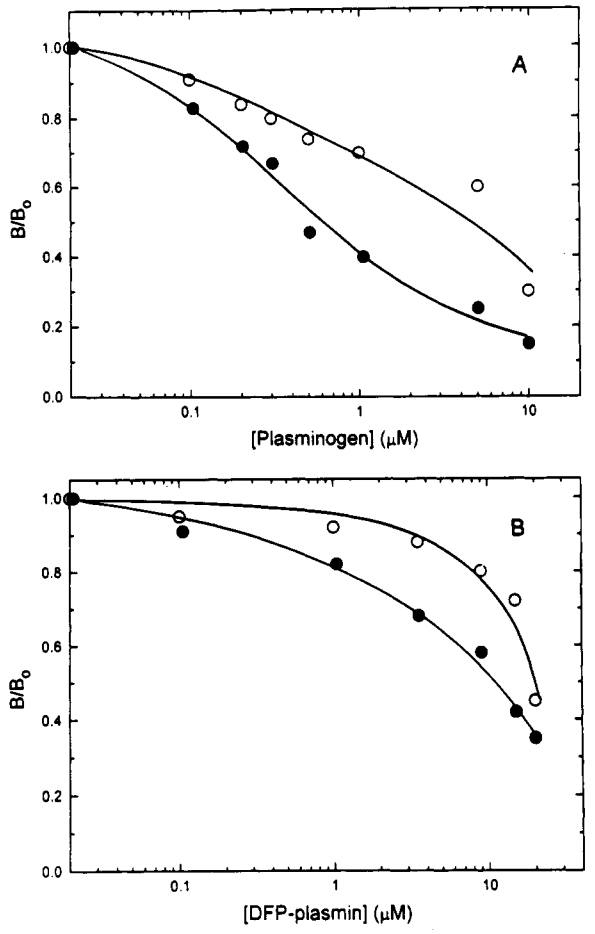

FIGURE 3: Competitive binding of plasminogen and DFP-plasmin to platelets. Increasing concentrations of plasminogen were added in the presence of a fixed concentration of radioiodinated DFP-plasmin (4.6 μM) to unactivated platelets before (open circles) and after (closed circles) plasmin treatment (panel A). Increasing concentrations of DFP-plasmin were added in the presence of a fixed concentration of radioiodinated plasminogen (4.4 μM) before (open circles) and after (closed circles) plasmin treatment (panel B).

plasminogen, DFP-plasmin, and t-PA was assessed. As shown in the Scatchard analyses of Figure 2A, we found that ADP-activated platelets not treated with plasmin bound significantly more plasminogen than unactivated platelets, 188 000 ± 17 000 molecules/cell, and that plasmin treatment increased the number of sites by 41% to 265 000 ± 25 000/cell without affecting the K_d (=2.8 μM). ADP-activated platelets not treated with plasmin also bound considerably more DFP-plasmin than unactivated platelets, 219 000 ± 41 000 molecules/cell; however, plasmin treatment decreased the number of sites by 28% to 157 000 ± 24 000/cell, again without affecting the K_d (=3.5 μM) (Figure 2B). ADP-activated platelets not treated with plasmin bound more t-PA than unactivated platelets (301 000 ± 36 000 molecules/cell), as well, and plasmin treatment had no significant effect on the maximal number of binding sites nor on the K_d (0.72 μM) (Figure 2C).

Competitive Binding of Plasminogen and DFP-Plasmin on Unactivated Platelets. Plasminogen competed effectively with DFP-plasmin for platelet binding, having an IC_{50} of 6.5 μM for untreated platelets. Following plasmin treatment, the IC_{50} decreased to 0.5 μM (Figure 3A). By contrast, DFP-plasmin competed significantly less well with plasminogen for platelet binding, having IC_{50}s of 19.0 μM and 13.5 μM, respectively, for untreated and plasmin-treated platelets (Figure 3B).

Table 1: Effect of Plasmin Treatment of Platelets on the Kinetics of Plasminogen Activation by t-PA[a]

	K_m (μM)	k_{cat} (s)	k_{cat}/K_m (μM^{-1} s^{-1})
unactivated platelets			
untreated	2.60	0.0090	0.0035
plasmin-treated	5.71	0.0350	0.0061
ADP-activated platelets			
untreated	0.45	0.014	0.031
plasmin-treated	1.43	0.023	0.016
no platelets	10.40	0.0072	0.00069

[a] Each value represents the mean of two experiments each performed in duplicate.

Table 2: Kinetic Constants for S-2251 Hydrolysis by Plasmin[a]

	K_m (mM)	k_{cat} (s)	k_{cat}/K_m (mM^{-1} s^{-1})
unactivated platelets			
untreated	0.16	4.5	28.1
plasmin-treated	0.15	4.4	29.3
ADP-activated platelets			
untreated	0.15	4.6	30.7
plasmin-treated	0.15	4.6	30.7
no platelets	0.44	4.3	9.7

[a] Each value represents the mean of two experiments each performed in duplicate.

Effect of Plasmin Treatment on Platelet-Surface Plasminogen Activation. The functional effects of these differences in platelet surface binding were examined by measuring the kinetics of plasminogen activation on the surface of untreated and plasmin-treated platelets which were either unactivated or ADP-activated. As shown in Table 1, the K_m of t-PA increased 2.2-fold, the k_{cat} increased 3.9-fold, and the catalytic efficiency increased 1.7-fold following plasmin treatment of unactivated platelets. With ADP activation, the K_m decreased by 83%, the k_{cat} increased 1.6-fold, and the catalytic efficacy increased 8.9-fold compared with unactivated, untreated platelets. Plasmin treatment prior to ADP activation decreased the K_m by 45%, increased the k_{cat} 2.6-fold, and increased the catalytic efficiency 4.6-fold compared with unactivated, untreated platelets.

The measured kinetic constants for plasmin activity on the surface of unactivated, untreated platelets; unactivated, plasmin-treated platelets; ADP-activated, untreated platelets; or ADP-activated, plasmin-treated platelets; and without platelets are shown in Table 2. Platelets enhanced the catalytic efficiency of plasmin itself approximately 3-fold and did so without significant modulation following plasmin treatment.

Kinetic Modeling of Platelet-Surface Plasminogen Activation: Effect of Plasmin Treatment with or without ADP Activation. A kinetic model was developed utilizing the kinetic and equilibrium binding constants determined in the experiments described above. In this model, the equations of state describing the system are as follows:

$$P + PR \rightleftharpoons PPR \quad (1)$$

$$N + NR \rightleftharpoons NNR \quad (2)$$

$$T + TR \rightleftharpoons TTR \quad (3)$$

$$P + PR' \rightleftharpoons PPR' \quad (4)$$

$$N + NR' \rightleftharpoons NNR' \quad (5)$$

$$T + TR' \rightleftharpoons TTR' \quad (6)$$

$$P + T \rightleftharpoons PT \rightarrow N + T \quad (7)$$

$$PPR + TTR \rightleftharpoons PPT \rightarrow NNR + T \quad (8)$$

$$PPR' + TTR' \rightleftharpoons PPT' \rightarrow NNR' + T \quad (9)$$

$$N + S \rightleftharpoons NS \rightarrow N + pNA \quad (10)$$

$$NNR + S \rightleftharpoons NNRS \rightarrow NNR + pNA \quad (11)$$

$$NNR' + S \rightleftharpoons NNRS' \rightarrow NNR' + pNA \quad (12)$$

where P = plasminogen, N = plasmin, T = t-PA, PR = plasminogen receptor, NR = plasmin receptor, TR = t-PA receptor, S = S-2251, and pNA = *p*-nitroanilide, and a prime (′) indicates a change in the state of the receptor(s) induced by plasmin treatment of platelets. Experimentally measured values (as described above), simplifying assumptions, and substitutions used were defined as follows. Plasmin treatment of platelets does not modify receptor affinity (as supported by the data of Figures 1 and 2):

$$K_P = K_P' \quad (13)$$

$$K_T = K_T' \quad (14)$$

$$K_N = K_N' \quad (15)$$

There are clearly detectable differences in platelet receptor number (availability) for plasminogen and plasmin, and these change with plasmin treatment of platelets:

$$PR' = 1.9(PR) = 3.8(NR) \quad (16)$$

$$TR' = TR \quad (17)$$

$$NR = 2.0(PR) \quad (18)$$

$$NR' = 1.4(PR) \quad (19)$$

The affinities of platelet receptor(s) for plasminogen and plasmin are identical, both before and after plasmin treatment (as supported by the data of Figures 1 and 2):

$$K_N = K_P = K_{N'} = K_{P'} = 2.2\ \mu M \quad (20)$$

The affinity of platelet receptors for t-PA does not change following plasmin treatment of platelets:

$$K_T = K_{T'} = 0.3\ \mu M \quad (21)$$

The Michaelis constant of plasmin bound to its platelet receptor for substrate (S-2251) is 36% that of free plasmin and is unaffected by plasmin treatment of platelets:

$$K_{SN} = K_{SN'} = 0.36K_{mS} = 160\ \mu M \quad (22)$$

$$K_{mS} = 440\ \mu M \quad (23)$$

The catalytic constant of free plasmin is 4.5-fold lower than that for platelet-bound plasmin, and the latter is unaffected by plasmin treatment:

$$k_S = 4.3\ s^{-1} \quad (24)$$

$$k_{SN} = k_{SN'} = 4.5k_S = 19.2\ s^{-1} \quad (25)$$

The Michaelis constant for free t-PA is approximately 10^3 greater than that for t-PA bound to its platelet receptor, and the latter is increased approximately 2-fold following plasmin treatment:

$$K_{mf} = 10.4\ \mu M \quad (26)$$

$$K_{mP} = 9.4\ nM \quad (27)$$

$$K_{mP}' = 18.8\ nM \quad (28)$$

The catalytic constant of free t-PA is 80% that of t-PA bound to its platelet receptor; plasmin treatment of the platelet increases the latter approximately 4-fold:

$$k_f = 0.8k_P = 0.0072\ s^{-1} \quad (29)$$

$$k_P = 0.009\ s^{-1} \quad (30)$$

$$k_P' = 3.9k_P = 0.035\ s^{-1} \quad (31)$$

Using these measured values, simplifying assumptions, and identities, the following equations were derived for reiterative calculations:

$$P_T = P + PPR + N + NNR \quad (32)$$

$$N_T = N + NNR \quad (33)$$

$$P_T' = P' + PPR' \quad (34)$$

$$N_T' = N' + NNR' \quad (35)$$

From these equations, using appropriate substitutions, receptor-bound plasminogen, plasmin, and t-PA concentrations were calculated from the following quadratic equations:

$$PPR = \{(P_T + PR + K_P) - [(P_T + PR + K_P)^2 - 4(P_T)(PR)]^{1/2}\}/2 \quad (36)$$

$$TTR = \{(T_T + TR + K_T) - [(T_T + TR + K_T)^2 - 4(T_T)(TR)]^{1/2}\}/2 \quad (37)$$

$$NNR = \{[N_T + 2(PR) + K_P] - [[N_T + 2(PR) + K_P]^2 - 8(N_T)(PR)]^{1/2}\}/2$$
$$= \{[(P_T - P - PPR) + 2(PR) + K_P] - [[(P_T - P - PPR) + 2(PR) + K_P]^2 - 8(P_T - P - PPR)PR]^{1/2}\}/2 \quad (38)$$

Michaelis–Menten rate equations were calculated using these values as follows:

$$v_T t = (v_P + v_{PR})t \quad (39)$$

$$v_P t = \{[[0.8k_P(T_T - TTR)](P_T - PPR)]/[4K_{MP} + (P_T - PPR)]\}t \quad (40)$$

$$v_{PR} t = \{[k_P(PPR)(TTR)]/(K_{MP} + PPR)\}t \quad (41)$$

$$v_{TS} t = (v_S + v_{NS})t \quad (42)$$

$$v_S t = [(k_S)(NS)/(K_{MS} + S)]t \quad (43)$$

$$v_{SR} t = \{[4.5k_S(NNR)(S)]/(0.36K_{MS} + S)\}t \quad (44)$$

Solving for N and NNR, then substituting in eqs 43 and 44, we obtain

$$v_S t = [(k_S v_P t S)/(K_{MS} + S)]t = [(k_S v_P S)(K_{MS} + S)]t^2 \quad (45)$$

$$v_{SR} t = [(4.5 k_S v_{PR} t S)/(0.36 K_{MS} + S)]t = [(4.5 k_S v_{PR} S)/(0.36 K_{MS} + S)]t^2 \quad (46)$$

Absorbance at any t is calculated by substituting eqs 44 and 45 into eq 39 and multiplying by the molar extinction coefficient of p-nitroanilide at 405 nm, 8800 M^{-1} cm^{-1}. The results of this kinetic model compare well with experimental data, as shown in Figure 4.

DISCUSSION

In this study, we have assessed the equilibrium and kinetic constants that govern the interactions of platelets with the components of the plasminogen activator system. We specifically characterized the interactions among plasminogen, plasmin, t-PA, and platelets and did so following platelet treatment with plasmin for both unactivated and ADP-activated platelets. We confirmed earlier work showing that t-PA and plasminogen bind directly and saturably to unactivated platelets and showed for the first time that (enzymatically inactivated) plasmin, like plasminogen, binds directly and saturably to resting and ADP-activated platelets. Although platelets manifest similar affinities for both plasminogen and plasmin, the binding capacities for these ligands differ considerably and, more importantly, are differentially affected by plasmin treatment and following activation with ADP. Taken together with the partial competition between plasminogen and plasmin for platelet surface binding, these data suggest that a unique class of receptor(s) distinct from that (those) for plasminogen is at least partially responsible for plasmin binding to the platelet surface. An alternative explanation for these results is that a single class of receptors is available for plasminogen and plasmin binding, and these common receptors have differing capacities for plasmin and its zymogen owing to steric constraints, receptor multimerization, or both.

Platelet activation and plasmin treatment of the platelet modulate the kinetics of plasminogen activation by t-PA significantly. The catalytic efficiency for plasminogen activation by t-PA increases 8.9-fold following activation by ADP. Plasmin treatment of unactivated platelets also increased the catalytic efficiency of plasminogen activation by t-PA, but by only approximately 2-fold. Plasmin treatment of platelets attenuated the degree to which ADP activation increased this catalytic efficiency, leading to a 4.6-fold increase compared with unactivated, untreated platelets. These measured constants were readily applicable to kinetic modeling of the process of plasminogen activation on the platelet surface utilizing a few simplifying assumptions, and such analysis showed a very good correlation with the observed kinetics.

These observations suggest that plasmin may play a role in accelerating its own generation from plasminogen by enhancing plasminogen binding and decreasing plasmin binding to both the unactivated and ADP-activated platelet. This change in binding facilitates increased turnover of substrate for t-PA on the platelet surface. In addition, as a consequence of plasmin treatment, the catalytic efficiency of plasmin itself is increased approximately 3-fold, primarily as a consequence of a decrease in K_m for plasmin substrate. These observations are consistent with those reported by Gonzalez-Gronow and colleagues (1991) for U-937 cells in which system plasmin treatment led to an enhancement of plasminogen binding and an increase in the catalytic efficiency of surface-bound plasmin approximately 12-fold compared with that of free plasmin.

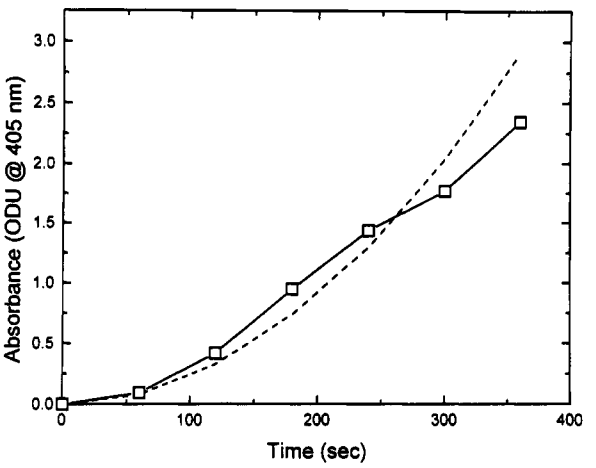

FIGURE 4: Kinetic modeling of plasminogen activation in the presence of platelets. Modeled values for the change in absorbance vs time (dashed line) were compared with the observed values (solid line) for unactivated platelets.

The specific identity of cell-surface plasminogen receptor(s) remains somewhat controversial. Initial reports of plasminogen binding to platelets (Miles et al., 1986) suggested a role for glycoprotein IIb/IIIa. Subsequent data supported the potential role for platelet surface-bound fibrinogen (perhaps arising from platelet α granules) (Adelman et al., 1989). This latter mechanism may be particularly important in the context of ADP activation of platelets. Furthermore, plasmin degradation of surface-bound fibrin(ogen) would increase the number of binding sites for plasminogen (and plasmin). While this mechanism cannot be excluded with the results presented here, other possible binding mechanism(s) need also be considered in light of more recent results. α-Enolase, a 54 000 molecular weight protein with a carboxy-terminal lysine, has recently been identified as a potential plasminogen receptor on U-937 cells (Miles et al., 1991). Gangliosides have also been recognized as participating in defining the plasminogen receptor on both U-937 cells and platelets (Miles et al., 1989; Ezratty et al., 1993). In addition, recent evidence supports the view that a common receptor exists for both plasminogen and plasmin in a carcinoma cell line (Durliat et al., 1991). The role that these determinants of plasminogen (and plasmin) binding to the platelet surface have following modulation of that surface by plasmin, ADP activation, or both has yet to be determined.

In summary, these data demonstrate that plasmin treatment and ADP activation increase surface-stimulated plasminogen activation by t-PA, primarily by enhancing plasminogen binding and decreasing plasmin binding to the platelet surface. These observations suggest that plasminogen activation may be autocatalytic on the platelet surface and support the view that a unique reciprocating mechanism governs the interaction between platelets and the plasminogen activator system.

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