RESEARCH COMMUNICATION

The peptide LSARLAF causes platelet secretion and aggregation by directly activating the integrin $\alpha_{IIb}\beta_3$

Jerry M. DERRICK*, Donald B. TAYLOR†, Robert G. LOUDON* and T. Kent GARTNER*[1]

*Microbiology and Molecular Cell Sciences, University of Memphis, Campus Box 526041, Memphis, TN 38152, U.S.A., and †Department of Biological Sciences, Benedictine University, Lisle, IL 60532, U.S.A.

A novel peptide (designed to bind to $\alpha_{IIb}\beta_3$) caused platelet aggregation and aggregation-independent secretion of the contents of α-granules in an $\alpha_{IIb}\beta_3$-dependent fashion. The agonist peptide induced secretion in the presence of prostaglandin E_1. In cell-free assays, $\alpha_{IIb}\beta_3$ bound specifically to immobilized agonist peptide and the peptide enhanced the binding of fibrinogen to immobilized $\alpha_{IIb}\beta_3$. The agonist peptide apparently activates $\alpha_{IIb}\beta_3$ by directly inducing a conformational change in the receptor. This change activates the platelets and causes secretion in a manner independent of fibrinogen binding.

INTRODUCTION

Platelet aggregation, under conditions of low shear stress, is dependent on the binding of fibrinogen to its receptor (the integrin $\alpha_{IIb}\beta_3$) on the surface of the platelets [1,2]. The glycoproteins α_{IIb} and β_3 exist as inactive heterodimeric calcium-dependent complexes on non-activated, resting [prostaglandin E_1 (PGE_1) present] platelets [1]. Attachment of fibrinogen to activated $\alpha_{IIb}\beta_3$ on activated platelets mediates aggregation by cross-linking adjacent platelets [1]. The α subunit, α_{IIb}, of the platelet fibrinogen receptor, $\alpha_{IIb}\beta_3$, is thought to be unique to platelets and megakaryocytes. The results of peptide cross-linking studies, the characterization of peptides derived from α_{IIb} and the characterization of chimeric receptors implicate residues 294–314 of α_{IIb} as being at least part of a presumptive fibrinogen γ-chain binding site [3–7]. The sequence ESRADRK (ESR), corresponding to residues 315–321 of α_{IIb}, is adjacent to the presumed fibrinogen binding site on α_{IIb} [8]. Because of its proximity to the presumptive fibrinogen γ-chain binding site, the binding of a synthetic probe to the ESR sequence of α_{IIb} might disrupt some fibrinogen–platelet-interaction-dependent function(s). Consequently, a peptide was designed to bind to residues 315–321 of α_{IIb} [9–11]. Surprisingly the peptide designed for that purpose, LSARLAF (LSA), activated $\alpha_{IIb}\beta_3$.

MATERIALS AND METHODS

Peptide synthesis and design

Peptides were synthesized and purified in our laboratory and the amino-acid compositions and sequences were confirmed as described previously [12]. The molecular-recognition hypothesis was used to design a peptide to bind the target residues 315–321, ESR, on α_{IIb} of the integrin $\alpha_{IIb}\beta_3$ [9–11]. The α_{IIb} mRNA nucleotide sequence for ESR is shown below. The complement of this nucleotide sequence is labelled cRNA. According to the molecular-recognition hypothesis, the complementary peptide predicted by the cRNA should bind to the region of the protein coded for by the corresponding mRNA. Translation of this cRNA in the $3' \rightarrow 5'$ and $5' \rightarrow 3'$ directions generates the amino-acid sequences LSA and FSVCPAL respectively:

α_{IIb} :	Glu	Ser	Arg	Ala	Asp	Arg	Lys	(ESRADRK)
DNA (3'-5') :	CTC	TCG	GCC	CGT	CTG	GCT	TTT	(TEMPLATE)
mRNA (5'-3') :	GAG	AGC	CGG	GCA	GAC	CGA	AAA	
cRNA (3'-5') :	CUC	UCG	GCC	CGU	CUG	GCU	UUU	
PEPTIDE (3'-5') :	^{H}Leu	Ser	Ala	Arg	Leu	Ala	Phe ^{OH}	(LSARLAF)
PEPTIDE (5'-3') :	^{HO}Leu	Ala	Pro	Cys	Val	Ser	Phe ^{H*}	(FSVCPAL)

Note that for the $5' \rightarrow 3'$ translation the amino terminus of the peptide is on the right and is designated by superscript ^{H}. The carboxyl terminus is designated by the superscript ^{HO} or ^{OH}.

Only LSA was synthesized and characterized because it had the better hydropathic profile (Figure 1) of the two peptides (the plot for FSVCPAL is not shown).

Antibodies

Mouse IgG (technical grade) was purchased from Sigma. Antibodies to ESR (anti-ESR, against residues 315–321 of α_{IIb}) were obtained from salt precipitate rabbit serum as described previously [13]. Control IgG was obtained from the same rabbits, but before immunization with ESR, and was designated preimmune IgG (PI) [14]. Monoclonal antibodies (mAbs) were gifts (see Acknowledgments section).

Aggregation

Aggregation studies using platelets in plasma (Figures 2a and 2b) and gel-filtered platelets (Figure 2c) were performed as described [12]. Platelets in plasma and platelets to be gel-filtered were prepared from blood drawn into White's anticoagulant [2.94% (w/v) citrate/2.45% (w/v) glucose, pH 6.5]. Dissociation of receptor subunits was accomplished by treating platelets in plasma with the calcium chelator EDTA (5 mM) at 37 °C under alkaline (pH 7.8) conditions for 20 min. Acidic conditions (pH 6.6) were used for the non-dissociation control. The platelets were removed from EDTA-containing plasma by low-speed centrifugation (650 ***g***, 10 min) and were suspended in normal, platelet-poor plasma before testing for their ability to aggregate in response to LSA. Gel-filtered platelets were freed of fibrinogen in Ca^{2+}-free Tyrodes buffer containing 0.5 mM Mg^{2+} [12]. Resting

Abbreviations used: PGE_1, prostaglandin E_1; ESR, ESRADRK, residues 315–321 of α_{IIb}; LSA, LSARLAF, synthetic peptide designed to bind to ESR; FRA, FRALASL, peptide with the same amino-acid composition but different sequence to LSA; anti-ESR, antibodies raised to ESR; TRAP, thrombin-receptor-activating peptide; mAb, monoclonal antibody; PF-4, platelet factor 4; PI, preimmune IgG.

[1] To whom correspondence should be addressed.

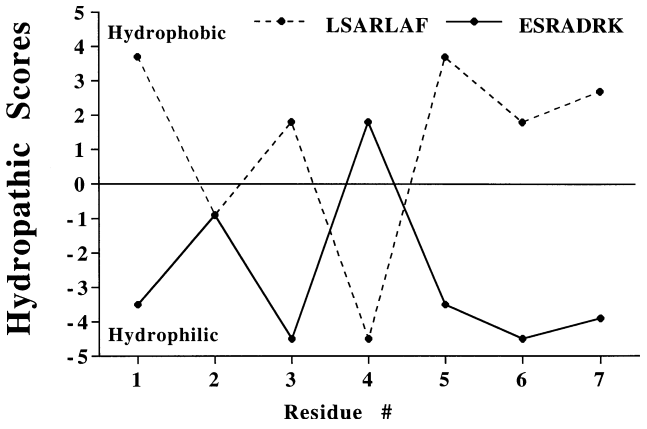

Figure 1 Plot of hydropathic scores for LSARLAF

The plot shows the potential for the peptide to bind to the ESR sequence of α_{IIb} and thereby affect fibrinogen-dependent platelet functions. Residues of a peptide are scored on a scale of 0–5 with regard to their relative hydrophobicity (positive numbers) or hydrophilicity (negative numbers). For best complementarity, one sequence should be approximately the inverse of the other. For further details see Markus et. al. [32].

gel-filtered platelets were in the presence of 1 μg/ml PGE_1. Mouse and rabbit antibodies, in these experiments, were added to platelets in plasma at final concentrations of 25 μg/ml and 0.4 mg/ml respectively, just before the cuvettes were placed in the aggregometer and approx. 1 min before the peptide was added.

Secretion studies

Platelet factor 4 (PF-4, a platelet α granule component) released from platelets in aggregation experiments was quantified by an anti-PF-4 antibody ELISA (Asserachrom® kit) using a modification of the manufacturer's instructions. Briefly, the platelets were stirred in the aggregometer at 800 rev/min for approx. 30 s at 37 °C, with or without inhibitors, before the addition of thrombin-receptor-activating peptide (TRAP) (50 μM), LSA (0.75 mM for platelets in plasma or 0.25 mM for gel-filtered platelets) or the control peptide, FRALASL (FRA) (0.4 ml final volumes). After stirring for 3 min, 0.1 ml samples of the platelets in plasma, gel-filtered platelets or resting gel-filtered platelets were removed and centrifuged briefly at $> 1000\ \boldsymbol{g}$ (micro-centrifuge 59A; Fisher Scientific). PF-4 release was estimated in 50 μl aliquots of the supernatant, diluted 1:500 in the kit dilution buffer. Developed PF-4 adsorption strips were read at A_{490} (Microplate Autoreader, model EL309; Bio-Tek Instruments). See the Aggregation section for antibody concentrations.

Receptor binding ELISA

The binding of $\alpha_{IIb}\beta_3$ to LSA- or FRA-coated wells was measured. The peptides LSA and FRA (5 μg/ml) were immobilized on XENOBIND™ covalent binding micro-well plates, which were post-coated with a solution of clarified BSA (35 mg/ml). Samples of purified [15], biotinylated [16] $\alpha_{IIb}\beta_3$ (10 μg/ml) were incubated for 10 min with or without excess (100 μg/ml) free LSA, or with 1 mg/ml of BSA, and then added to the LSA-coated wells. Incubation was continued overnight at 30 °C.

In a second set of cell-free assays, binding of fibrinogen to purifed $\alpha_{IIb}\beta_3$ was quantified as described previously [16]. Biotinylated fibrinogen (0.5 μg/well), in the presence or absence of increasing amounts of the designated peptide, was added to wells coated previously with $\alpha_{IIb}\beta_3$ (0.5 μg/well). Binding of $\alpha_{IIb}\beta_3$ to LSA- or FRA-coated wells and binding of fibrinogen to wells coated with $\alpha_{IIb}\beta_3$ was quantified using an anti-biotin-antibody ELISA [16], and plates were read at A_{405} (Microplate Autoreader; Bio-Tek).

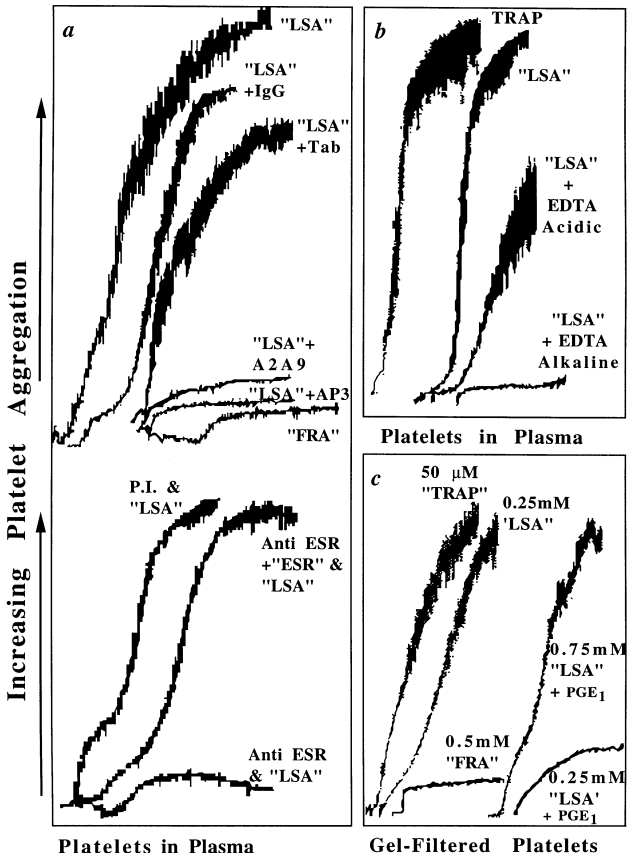

Figure 2 LSA induced platelet aggregation is (b) dependent on intact fibrinogen-receptor complexes and (c) occurs under conditions requiring secretion as a prerequisite to aggregation

(**a**) The anti-$\alpha_{IIb}\beta_3$ mAb A2A9, which blocks binding of fibrinogen to $\alpha_{IIb}\beta_3$, and the non-blocking anti-β_3 mAb AP3, but not the non-blocking anti-α_{IIb} mAb Tab or murine control IgG, inhibited LSA-induced aggregation. Platelet aggregation induced by ADP (30 μM) was not inhibited by either of the non-blocking antibodies (results not shown). Anti-ESR, but not PI, inhibited LSA-induced platelet aggregation in plasma. LSA-induced platelet aggregation was rescued from anti-ESR-IgG-imposed inhibition by preincubation of anti-ESR IgG with free ESR peptide (0.25 mM) for 30 min before addition of the mixture to the platelets in plasma. ADP-induced platelet aggregation was not inhibited by anti-ESR IgG (results not shown). (**b**) Platelets removed from EDTA acidic treatment aggregated in response to 1 mM LSA, whereas platelets removed from EDTA alkaline treatment, which dissociates $\alpha_{IIb}\beta_3$ complexes, did not. Control peptide was without effect at 1 mM (results not shown). The trace labelled LSA represents aggregation of non-EDTA-treated control platelets in replaced plasma induced by 0.75 mM LSA, and is compared with aggregation of platelets in plasma induced by TRAP (50 μM). (**c**) LSA induced the aggregation of non-resting gel-filtered platelets as extensively as did TRAP under conditions requiring secretion as a prerequisite to aggregation (first two traces). The control peptide FRA did not cause gel-filtered platelet aggregation (third trace). High doses of LSA (0.75 mM) overcame the inhibition of LSA (0.25 mM)-induced aggregation of resting gel-filtered platelets by 1 μg/ml PGE_1 (last two traces).

RESULTS AND DISCUSSION

The results reported here support the following model for the platelet agonist activity demonstrated by the peptide LSA. LSA interacts with and directly activates the fibrinogen receptor. LSA appears to mediate receptor activation by driving a con-

Table 1 LSA-induced secretion of α granule contents (measured by PF-4 release) from (a) platelets is dependent on (b) intact $\alpha_{IIb}\beta_3$ complexes but (c) is not aggregation dependent

Platelets in plasma (a and b) or gel-filtered platelets (c) were treated with (a) anti-$\alpha_{IIb}\beta_3$ mAbs, (**b**) the calcium chelator EDTA, or (c) 1 μg/ml of the adenylate cyclase agonist, PGE_1, before use in aggregation experiments. Supernatants from aggregation experiments were assayed for PF-4 secretion. (a) LSA-induced secretion was inhibited by two blocking mAbs and also, AP3, a mAb with a non-blocking function ($n \geqslant 9$). (b) LSA (1 mM) elicited PF-4 secretion in aggregation experiments in platelet-rich plasma pretreated with EDTA under acidic conditions but not in platelets pretreated with EDTA under alkaline conditions ($n = 9$). (c) LSA (0.25 mM), but not FRA, induced secretion from stirred gel-filtered platelets and gel-filtered platelets in the presence of 5 mM EDTA (not stirred) ($n = 4$), but not from stirred resting gel-filtered platelets ($n = 9$). Resting gel-filtered platelets were induced to secrete PF-4 when treated with 0.75 mM LSA ($n = 6$). The results presented are means $\pm$ S.D.

(a)

	Secretion from platelets in plasma (%)				
	Peptide only	With mouse IgG	With mAb Tab	With mAb AP3	With mAb A2A9
LSA	100.00	97.82 $\pm$ 4.85	78.75 $\pm$ 4.75	30.54 $\pm$ 1.57	31.92 $\pm$ 1.04
FRA	16.85 $\pm$ 1.00				
TRAP	144.50 $\pm$ 4.20				

(b)

	Secretion from platelets in reduced plasma (%)		
	Not pretreated	Pretreated with EDTA (acidic)	Pretreated with EDTA (alkaline)
LSA	100.00 $\pm$ 0.75	69.50 $\pm$ 2.32	24.95 $\pm$ 0.87
FRA	17.34 $\pm$ 0.30	12.94 $\pm$ 1.72	23.72 $\pm$ 1.08

(c)

	Secretion from gel-filtered (plasma free) platelets (%)		
	Platelets only	With PGE_1	With EDTA (not stirred)
LSA 0.25 mM	100.00 $\pm$ 8.00	1.57 $\pm$ 0.51	87.60 $\pm$ 7.10
LSA 0.75 mM		28.90 $\pm$ 2.40	
FRA 0.25 mM	6.90 $\pm$ 3.10	3.08 $\pm$ 3.63	
TRAP 50 μM	180.51 $\pm$ 4.32		

formational change in $\alpha_{IIb}\beta_3$. The LSA-activated receptors elicit platelet activation and secretion of PF-4. The conformationally active receptors can bind fibrinogen and thereby mediate activation-dependent platelet aggregation.

Testing of the peptide LSA in the aggregometer revealed that it behaved as a platelet agonist. The peptide induced both the aggregation of platelets (Figure 2) and platelet secretion (PF-4 release) (Table 1), whereas FRA did not elicit either response (Figure 2 and Table 1). The anti-$\alpha_{IIb}\beta_3$ murine mAbs 7E3 [17] (results not shown) and A2A9 [18] (each can block the binding of fibrinogen to $\alpha_{IIb}\beta_3$), but not mouse non-specific IgG control or mAb 6D1 [19] (anti-GPIb, results not shown) blocked LSA-induced platelet aggregation (Figure 2a) and secretion (Table 1a). These results indicate that LSA-induced platelet aggregation is dependent on the normal function of $\alpha_{IIb}\beta_3$ and does not simply represent platelet agglutination.

The demonstration that FRA did not induce platelet aggregation or secretion shows that the ability of LSA to induce platelet aggregation and secretion is dependent on the amino-acid sequence of the peptide. Therefore, the response of platelets to treatment with LSA is not due to non-specific effects of the peptide.

Antibodies raised against the presumed LSA binding site (anti-ESR), but not PI (Figure 2a), inhibited the LSA-induced aggregation of platelets in plasma. As a control for the specificity of the antibody, free peptide ESR incubated with anti-ESR before the addition of the antibodies to platelets in plasma, rescued LSA-induced aggregation from anti-ESR inhibition (Figure 2a). ADP-induced aggregation was not affected substantially by the presence of anti-ESR (results not shown). Although these results support the conclusion that LSA binds to the ESR region of α_{IIb}, they are also consistent with the alternative explanation that some antibodies that bind to $\alpha_{IIb}\beta_3$ can prevent LSA-induced activation of the receptor without blocking LSA binding to $\alpha_{IIb}\beta_3$.

The hypothesis that LSA mediates its effects specifically by binding to $\alpha_{IIb}\beta_3$ is supported by the following observations. Neither of the anti-$\alpha_{IIb}\beta_3$ mAbs, AP3 [20] nor Tab [21], inhibit the binding of fibrinogen to $\alpha_{IIb}\beta_3$ or platelet aggregation in response to physiological agonists. However, the mAb AP3 inhibited both LSA-induced platelet aggregation and PF-4 secretion (Figure 2a and Table 1a). In contrast, the mAb Tab was almost without inhibitory effect on LSA-induced secretion and platelet aggregation (Table 1a and Figure 2a). Although these results obscure the significance of the anti-ESR data, they support the hypothesis that LSA causes platelet aggregation by binding to the platelet fibrinogen receptor. This is evident since AP3 does not inhibit platelet aggregation in response to physiological agonists [20].

Further support for the model that LSA causes its effects by binding to $\alpha_{IIb}\beta_3$ comes from cell-free direct binding assays. Purified $\alpha_{IIb}\beta_3$ bound to immobilized LSA in an ELISA (Table 2a). Approximately four times more biotinylated $\alpha_{IIb}\beta_3$ bound to LSA immobilized in microtitre plates than to immobilized FRA. The binding of the receptor to LSA was inhibited by excess free LSA but not by excess BSA. These data support the conclusion that LSA can bind to $\alpha_{IIb}\beta_3$ in an amino-acid sequence specific manner. In a separate cell-free assay, LSA in solution activated immobilized receptors. LSA (at 250 μM) enhanced the binding of fibrinogen to immobilized $\alpha_{IIb}\beta_3$ by two and a half times relative to the binding of fibrinogen to $\alpha_{IIb}\beta_3$ in the absence of LSA, or in the presence of control peptide (Table 2b). Thus, activation of $\alpha_{IIb}\beta_3$ (at least the isolated form of the receptor) by LSA does not depend on signal transduction. These observations support the view that LSA exerts its effects on platelets by binding to $\alpha_{IIb}\beta_3$ and inducing an activated conformation in that receptor.

Other data reveal that LSA-elicited platelet functions are dependent on functional $\alpha_{IIb}\beta_3$ complexes. Platelets in plasma were treated both under conditions which cause and conditions which do not cause the irreversible dissociation of $\alpha_{IIb}\beta_3$ complexes (see the Materials and methods section) [22,23]. Both types of platelets were treated with LSA in an aggregometer and secreted PF-4 was measured. The results reveal that LSA-treated platelets with putatively dissociated receptors did not aggregate or secrete PF-4, whereas LSA treated platelets with intact receptors both aggregated and secreted PF-4 (Figure 2b and Table 1b). These results support the conclusion that LSA causes secretion by binding to $\alpha_{IIb}\beta_3$.

Presumably, plasma-free platelets (platelets removed from plasma by gel-filtration) must secrete the fibrinogen required for aggregation before aggregation can occur. The results shown in Figure 2(c) and Table 1(c) reveal that LSA acts as an $\alpha_{IIb}\beta_3$-dependent agonist which can cause secretion in the absence of both aggregation and the binding of fibrinogen to $\alpha_{IIb}\beta_3$ com-

Table 2 Purified $\alpha_{IIb}\beta_3$ (a) binds directly to and (b) is activated by LSA but not FRA in a cell-free system

(a) Solutions of purified $\alpha_{IIb}\beta_3$, with or without excess free LSA, added to LSA or FRA immobilized on XENOBIND™ covalent binding microwell plates, bound specifically to LSA ($n = 3$). (b) In separate experiments, LSA specifically activated the immobilized receptors to bind fibrinogen ($n = 4$). In addition, the binding of fibrinogen to $\alpha_{IIb}\beta_3$ (non-specific binding $< 15\%$) in the absence of LSA was inhibited by the peptide GRGDSP (results not shown). Similar results were obtained with biotinylated $\alpha_{IIb}\beta_3$ binding to immobilized fibrinogen (results not shown). The results presented are means ± S.D.

(a)

	$\alpha_{IIb}\beta_3$ binding to peptide (A_{405})		
	$\alpha_{IIb}\beta_3$/biotin	With 10 × free LSA	With 10 × BSA
Binding to LSA	0.780 ± 0.021	0.186 ± 0.020	0.629 ± 0.019
Binding to FRA	0.163 ± 0.014		

(b)

	Fibrinogen binding to $\alpha_{IIb}\beta_3$ (A_{405})				
Peptide added	No peptide	50 μM	100 μM	250 μM	500 μM
LSA	0.946	0.927	1.750	2.330	2.500
FRA	0.946	0.851	0.892	0.872	0.877

plexes. First, LSA elicited the secretion-dependent aggregation of plasma-free platelets, demonstrating that secretion can occur in the absence of prior aggregation (Figure 2c). Secondly, secretion was shown not to be dependent on binding of fibrinogen to $\alpha_{IIb}\beta_3$, since the plasma-free platelets in EDTA (binding of fibrinogen to $\alpha_{IIb}\beta_3$ is Ca^{2+} dependent) secreted PF-4 in response to LSA but not FRA (Table 1c).

The significance of the effects of LSA on platelets is a function of how LSA causes platelet activation. Although intact $\alpha_{IIb}\beta_3$ complexes on platelet surfaces are not necessary for platelets to secrete in response to thrombin (thrombasthenic platelets secrete, see [24]), intact $\alpha_{IIb}\beta_3$ complexes are necessary for platelets to secrete in response to LSA treatment. Furthermore, it is apparent that intact $\alpha_{IIb}\beta_3$ complex-dependent LSA-induced secretion does not require $\alpha_{IIb}\beta_3$–fibrinogen binding. That is, LSA stimulates platelet secretion in the absence of available fibrinogen (to mediate aggregation-driven secretion) under conditions eliminating the ability of $\alpha_{IIb}\beta_3$ to bind fibrinogen. Therefore LSA-induced platelet secretion is due to LSA–integrin interaction, and not to fibrinogen-mediated crosslinking. This explanation is supported by the results presented here. In summary, the integrin binds specifically to LSA in cell-free assays. Purified $\alpha_{IIb}\beta_3$ is activated in cell-free assays by LSA. A mAb specific for $\alpha_{IIb}\beta_3$, which cannot block $\alpha_{IIb}\beta_3$–fibrinogen interaction or ADP-driven platelet aggregation does block LSA-driven aggregation and secretion. This non-blocking mAb may prevent either the binding of LSA to the integrin or the conformational response of the integrin to LSA binding.

A strong agonist is defined as one which causes platelet secretion in a non-aggregation-dependent manner [25]. LSA treatment of plasma-free platelets caused aggregation to the same extent as TRAP (Figure 2c) under conditions which presumably precluded aggregation in the absence of prior secretion. The LSA-driven PF-4 secretion by these platelets was approximately half as extensive as TRAP-stimulated secretion (Table 1c). Furthermore, a high concentration of LSA (0.75 mM compared with 0.25 mM, which is usual for plasma free platelets) overcame the inhibitory effects of PGE_1 on LSA-induced secretion by, and the aggregation of, resting plasma-free platelets (Figure 2c and Table 1c). These effects are characteristic of a strong agonist. For example, a high concentration of thrombin (a strong agonist) can overcome the inhibitory effects of PGE_1, whereas a high concentration of ADP (a weak agonist) cannot overcome the inhibitory effects of that inhibitor [6].

Ligand-induced conformational changes in α_{IIb} are not unprecedented. For example, $\alpha_{IIb}\beta_3$ assumes a ligand-induced conformation as a consequence of fibrinogen binding [26–28]. Also, some mAbs specific for ligand-induced binding sites can drive conformational change in $\alpha_{IIb}\beta_3$ in the absence of ligand and activate the receptor directly, rather than by working through classical signal transduction [29,30]. Similarly, the peptide RGDS can drive a conformational change in the receptor but does not activate platelets [31] or drive secretion (results not shown). Despite these observations, the action of LSA is unprecedented in that LSA is the only peptide reported to elicit platelet secretion by binding to an integrin ($\alpha_{IIb}\beta_3$).

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REFERENCES

1 Phillips, D. R., Charo, I. F., Parise, L. V. and Fitzgerald, L. A. (1988) Blood **71**, 831–843
2 Ikeda, Y., Handa, M., Kawano, K., Kamata, T., Murata, M., Araki, Y., Anbo, H., Kawai, Y., Watanabe, K., Itagaki, I. et al. (1991) J. Clin. Invest. **87**, 1234–1240
3 D'Souza, S. E., Ginsberg, M. H., Burke, T. A. and Plow, E. F. (1990) J. Biol. Chem. **265**, 3440–3446
4 D'Souza, S. E., Ginsberg, M. H., Matsueda, G. R. and Plow, E. F. (1991) Nature (London) **350**, 66–68
5 Taylor, D. B. and Gartner, T. K. (1992) J. Biol. Chem. **267**, 11729–11733
6 Gartner, T. K. and Taylor, D. B. (1990) Thromb. Res. **60**, 291–309
7 Loftus, J. C., Halloran, C. E., Ginsberg, M. H., Feigen, L. P., Zablocki, J. A. and Smith, J. W. (1996) J. Biol. Chem. **271**, 2033–2039
8 Poncz, M., Eisman, R., Heidenreich, R., Silver, S. M., Vilaire, G., Surrey, S., Schwarttz, E. and Bennett, J. S. (1987) J. Biol. Chem. **262**, 8476–8482
9 Blalock, J. E. and Smith, E. M. (1984) Biochem. Biophys. Res. Commun. **121**, 203–207
10 Blalock, J. E. and Bost, K. L. (1986) Biochem. J. **243**, 679–683
11 Ruiz-Opazo, N., Akimoto, K. and Herrera, V. L. M. (1995) Nature Med. **1**, 1074–1081
12 Bennett, J. S., Shattil, S. J., Power, J. W. and Gartner, T. K. (1988) J. Biol. Chem. **263**, 12948–12953
13 Taylor, D. B., Derrick, J. M. and Gartner, T. K. (1994) Proc. Exp. Biol. Med. **20**, 35–43
14 Knutson, V. P. (1988) J. Biol. Chem. **263**, 14146–14151
15 Phillips, D. R., Fitzgerald, L., Parise, L. and Steiner, B. (1992) Methods Enzymol. **215**, 244–263
16 Charo, I. F., Nannizzi, L., Phillips, D. R., Hsu, M. A. and Scarborough, R. M. (1991) J. Biol. Chem. **266**, 1415–1421
17 Coller, B. S. (1985) J. Clin. Invest. **76**, 101–103
18 Bennett, J. S., Hoxie, J. A., Leitman, S. F., Vilaire, G. and Cines, D. B. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 2417–2421
19 Coller, B. S., Peerschke, E. I., Scudder, L. E. and Sullivan, C. A. (1983) Blood **61**, 99–110
20 Newman, P. J., Allen, R. W., Kahn, R. A. and Kunicki, T. J. (1985) Blood **65**, 227–232
21 McEver, R. P., Bennett, E. M. and Martin, N. M. (1983) J. Biol. Chem. **258**, 5269–5275
22 Zucker, M. B. and Grant, R. A. (1978) Blood **52**, 505–514
23 Brass, L. F., Shattil, S. J., Kunicki, T. J. and Bennett, J. S. (1985) J. Biol. Chem. **260**, 7875–7881

24 Weiss, H. J. and Rodgers, J. (1973) Proc. Soc. Exp. Biol. Med. **142**, 30–35
25 Charo, I. F., Feinman, R. D. and Detwiler, T. C. (1977) J. Clin. Invest. **60**, 866–873
26 Parise, L. V., Helgerson, S. L., Steiner, B., Nannizzi, L. and Phillips, D. R. (1987) J. Biol. Chem. **262**, 12597–12602
27 Du, X. P., Plow, E. F., Frelinger, A. L., O'Toole, T. E., Loftus, J. C. and Ginsberg, M. H. (1991) Cell **65**, 409–416
28 Frelinger, III, L. A., Lam, S. C.-T, Plow, E. F., Smith, M. A., Loftus, J. C. and Ginsberg, M. H. (1988) J. Biol. Chem. **263**, 12397–12402
29 Kouns, W. C., Wall, C. D., White, M. M., Fox, C. F. and Jennings, L. K. (1990) J. Biol. Chem. **265**, 20594–20601
30 O'Toole, T. E., Loftus, J. C., Du, X. P., Glass, A. A., Ruggeri, Z. M., Shattil, S. J., Plow, E. F. and Ginsberg, M. H. (1990) Cell Regul. **1**, 883–893
31 Huang, M.-M., Lipfert, L., Cunningham, M., Burgge, J. S., Ginsberg, M. H. and Shattil, S. J. (1993) J. Biol. Chem. **122**, 473–483
32 Markus, G., Tritsch, G. L. and Parthasarathy, R. (1989) Arch. Biochem. Biophys. **272**, 433–439

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