Ligand Inhibition of the Platelet Glycoprotein IIb-IIIa Complex Function as a Calcium Channel in Liposomes*

(Received for publication, December 20, 1988)

Mary Ellen M. Rybak* and Lori A. Rezulli
From the Division of Hematology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655

Platelet glycoproteins IIb and IIIa function as a fibrinogen receptor on the activated platelet. We have shown that these glycoproteins can be incorporated onto the surface of phosphatidylethanolamine vesicles with retention of fibrinogen and antibody binding properties and can permit fibrinogen and antibody binding properties and can permit Ca2+ transit across the phospholipid bilayer. In the current study we demonstrate that this apparent Ca2+ channel function is specifically inhibited by the synthetic analogue of the fibrinogen γ COOH-terminal peptide, His-His-Leu-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val (His-12-Val), but not by the adhesive protein sequence Arg-Gly-Asp-Ser (RGDS). Prior incubation of IIb-IIIa liposomes with RGDS prevented Ca2+ transit inhibition by 25 μM His-12-Val, analogous to RGDS inhibition of His-12-Val binding to platelets. His-12-Val inhibited a minor component of transmembrane Ca2+ influx into ADP and thrombin-activated human platelets but had no effect on steady-state platelet 45Ca flux. These data indicate that ligand binding may exert a regulatory influence on transmembrane Ca2+ influx into activated platelets. The difference in inhibitory potency of the peptides studied may be related to differences in conformational changes in the glycoprotein IIb-IIIa complex induced by His-12-Val and RGDS, steric considerations, or differences in interactions with glycoprotein IIb Ca2+ binding domains.

The platelet membrane glycoprotein IIb-IIIa (GP IIb-IIIa) complex is a member of a family of membrane glycoproteins involved in cellular adhesion, the integrins (1–3). During platelet activation these glycoproteins become a fibrinogen (Fgn) receptor and mediate platelet aggregate formation (4–7). The proposed sites on the Fgn molecule that interact with the GPIIb-IIIa complex are a dodecapeptide, His-His-Leu-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val (His-12-Val), residues 400–411 of the carboxyl terminus of the fibrinogen γ chain (8–10), and a tetrapeptide (RGDS) near the carboxyl terminus of the α chain or a tripeptide (RGD) near the amino terminus of the α chain (11–15). The synthetic peptide analogues of these regions inhibit platelet aggregation and Fgn binding to activated platelets and are mutually inhibitory in binding studies (11–16). The data suggest that these peptides share common or closely related binding sites on GPIIb-IIIa.

Ligand binding to GPIIb-IIIa may signal secondary events in the platelet and alter the conformation and distribution of the IIb-IIIa complex. Binding of Fgn, RGDS, or the γ dodecapeptide induces clustering of platelet GPIIb-IIIa (17), and occupancy of GPIIb-IIIa by Fgn is necessary to maintain Na+/H+ exchange in epinephrine-stimulated platelets (18). Parise et al. (19) have demonstrated that binding of LGGAKQAGDV or RGDS to soluble GPIIb-IIIa alters the hydrodynamic properties of the complex and renders GPIIb susceptible to thrombin hydrolysis. This suggests that binding of either the dodecapeptide or RGDS induces an unfolding of the GPIIb-IIIa complex.

In a previous study we reported that the purified GPIIb-IIIa complex but not the dissociated glycoproteins can facilitate calcium movement across a phospholipid membrane when the complex is inserted into the surface of liposomes (20). This apparent Ca2+ channel is inhibited by a monoclonal antibody to the GPIIb-IIIa complex. In studies of calcium homeostasis in normal and thrombathetic platelets, Brass (21) demonstrated that the GPIIb-IIIa complex may play a role in Ca2+ flux across the platelet plasma membrane. In experiments using a monoclonal antibody (TM83) to the GPIIb-IIIa complex and GRGDSP with aequorin-loaded platelets, Yamaguchi et al. (22) concluded that the IIb-IIIa complex was involved in Ca2+ influx during platelet activation with thrombin and collagen but not phorbol 12-myristate 13-acetate. In the current study, we investigated whether binding of His-12-Val or RGDS alters the apparent calcium channel function of GPIIb-IIIa in this liposome model.

EXPERIMENTAL PROCEDURES

Synthetic peptides HHLGGAKQAGDV and RGDS were purchased from Peninsula Laboratories, Inc. (Belmont, CA). The synthetic peptide HHLGGAKQAGDV was a generous gift of Dr. Jack Hawiger, Harvard Medical School. Purified fibrinogen was a gift from Dr. Leslie Parise, University of North Carolina, Chapel Hill; 45Ca was purchased from Amersham Corp.; leupetin from Vega Biotechnologies, Inc. (Tucson, AZ); Triton X-114 from Sigma; phosphatidylcholine (PC) type IIE, 99% pure from Sigma; and Pura-2 from Molecular Probes Inc. (Eugene, OR). Plateletpheresis units were obtained from the University of Massachusetts Medical Center Blood Bank (Worcester, MA).

GPIIb-IIIa Preparation—GPIIb and IIIa were prepared as previously reported (20, 21, 22–24) from Triton X-114-solubilized platelet membranes from clinically outdated washed platelet concentrates. Membranes were suspended in 1% (v/v) precondensed Triton X-114, 10 mM Tris, 0.15 M NaCl, pH 7.4, with 0.4 mM phenylmethylsulfonyl fluoride, 100 μg/ml leupetin, incubated overnight at 4 °C, and centrifuged at 78,000 × g for 1 h at 4 °C. The detergent phase was applied to a 6% sucrose cushion, heated at 37 °C for 5 min, centrifuged at 1500 × g for 5 min, and the detergent micelle layer removed. Further purification of proteins was achieved by lentil-lectin Sepharose chromatography with 10% α-methyl-D-mannoside elution. Detergent was partially removed with Bio-Beads SM-2. Each GPIIb-IIIa preparation was assayed for total protein by a modified Lowry procedure (25) and for residual Triton X-114 by spectrophotometric assay (26). This preparative method yielded GPIIb-IIIa in complex as determined by

*This work was supported by United States Army Research and Development Command Grant DAMD 17-87-C-7172. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom reprint requests should be addressed.
GPIIb resistance to thrombin hydrolysis (28, 29). Preparations maximally contained 0.27 mg of Triton/mg of protein. Previous data demonstrated that this residual detergent did not qualitatively alter the experimental results. Proteins were analyzed by SDS-PAGE, reduced and nonreduced, using 7.5% gels; only two bands with M, consistent with GPIIb and GPIIIa, were detectable by combined silver and Coomassie Blue stains of gels of the pelleted preparation. GPIIb-IIIa preparations were detectable by combined silver and Coomassie Blue stains of gels of the pelleted preparation. 

**Proteoliposome Preparation.—** Large unilamellar PC vesicles were prepared by reverse phase as previously described (20-27). PC (25 mg in hexane, 100 mg/ml) was dried to a thin film in a 50-ml round-bottom flask in vacuo for 3 h. The lipid film was resuspended in 1.5 ml of ether, then in 0.25 ml of 10 mM HEPES pH 7.40, 0.15 M NaCl, and the fluorescent Ca2+ indicator, Fura-2, as pentapotassium salt (30 μM). This mixture was sonicated for 2 min in a bath sonicator. The ether was then removed by rotary evaporation to form a lipidic gel. This gel was sonicated for 1 min with 1 ml of GPIIb-IIIa (200 μg/ml in 10-25 mM) and Fura-2 (30 μM). Liposomes were washed two times by centrifugation at 14,000 × g for 10 min at 4°C. Liposomes were relatively homogeneous in size and entrapped volume with a mean diameter of 0.1 μm by laser light scattering with a Coulter N4 submicron particle size. On average, 70% of added protein was incorporated, with 48.6 ± 0.8% of protein in an outside-out orientation determined by neuraminidase digestion of sialic acid residues (28). As previously reported, the GPIIb-IIIa complex incorporated onto the PC liposomes bound Fg with approximate Kd = 10-7 M (20, 28, 29) and bound monoclonal antibodies against GPIIb-IIIa (20). SDS-PAGE of proteins associated with SDS-solubilized liposome preparations revealed only two bands consistent with GPIIb and GPIIIa (20). Gel filtration studies confirmed that Fura-2 remained within the liposomes (20). 

**Calcium Transit Studies.—** Liposomes were utilized within 1 h of preparation and kept on ice until use. Temperature and pH were rigorously controlled. Double-distilled deionized 18 milliohm water was utilized in all buffers. Fura-2-loaded liposomes (10-4 M intravesicular Ca2+) were injected into Tris buffer (0.15 M NaCl, 10-25 mM Ca2+), 20 μl of liposome suspension, to 980 μl of buffer. This approximate infinite cis-entry conditions (saturating external calcium). The Fura-2 fluorescence was measured (λex 345 nm, λem 510 nm, slit width 10-25 nm, respectively) in a Fluoromax Elmer 650-10S fluorescence spectrophotometer. These emission and excitation wavelengths minimized the contribution from intrinsic liposomal fluorescence and beam dispersion. This system measures only Ca2+ which actually enters the liposome, not surface-associated ion. 

**Peptide Effects.—** Prior to Ca2+ transit studies, GPIIb-IIIa liposomes were incubated with His-12-Val (0-200 μM) or RGDS (0-200 μM) for 20 min at 25°C; liposomes were then injected into Ca2+-containing buffer as described above. The peptides were homogeneous by high pressure liquid chromatography and thin layer chromatography. Amino acid analysis of the peptides was consistent with their predicted composition. The ability of these peptides to inhibit platelet aggregation was demonstrated by standard methods. To assess the ability of RGDS binding to alter the effect of the His-12-Val on calcium entry, the liposomes were incubated with RGDS (170 μM) prior to the addition of dodecapeptide (10-25 μM). Parallel incubations were performed on PC (nonprotein) and GPIIb-IIIa liposomes without peptide as controls. Experiments were performed six times in quadruplicate. 

**Calcium Movement into Platelets.—** This was performed by a modification of the method of Brass (21). Gel-filtered platelets (GFP) were prepared by standard methods (30) from volunteer donors into 4 ml of S-MAX, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 1 mg/ml bovine serum albumin, 3.3 mM Na2HPO4, pH 7.40. For steady-state studies, GFP (109/μl) were incubated with 0.2 mM CaCl2 for 30 min at 25°C and followed by His-12-Val (30) for 30 min (25°C and 5 μCi/ml [35S]Ca2+); 10-15 aliquots were obtained for total counts. Platelets were then counted for 10 min. Aliquots (200 μl) were rapidly washed on 0.45-μm filters (Millipore, Bedford, MA) with 4°C HEPES buffer and 5 mM EGTA to remove surface-associated Ca2+. Filters with retained platelet [35S]Ca2+ were counted. For non-steady-state studies of activated platelets, GFP (1 ml, 109 platelets/μl) were incubated with His-12-Val, 25-170 μM; HLRGDS, 50 μM; HHLGGAQRAGDVQ or buffer, and 10-25 μM Ca2+ for 30 min at 25°C. Platelets were then counted for 10 min and aliquots washed on Millipore filters with 4°C HEPES buffer, 5 mM EGTA. Filters with retained platelets were then counted for retained [35S]Ca2+. 

Ca2+ influx into Fura-2-loaded GFP was quantitated by the method of Zavoico and Cragoe (31). Washed platelets were loaded with 4 μM Fura-2, gel-filtered into 10 μM HEPES buffer with 0.2 mg/ml bovine serum albumin and 1 mM CaCl2. The platelet count was adjusted to 2-4 × 1010/ml, and platelets were incubated with buffer control, 50-200 μM His-12-Val, or 50-125 μM RGDS. Aliquots of platelets were diluted to 0.32 × 1010/ml in buffer and stirred continuously at 37°C. Human thrombin (0.05 unit/ml) was added immediately and Ca2+ was monitored as the change in Fura-2 fluorescence by a SPEX Fluorolog-2 spectrofluorometer (Edison, NJ), excitation 340 nm, 380 nm; emission 510 nm. 

**RESULTS** 

**Ca2+ Movement into Vesicles—** Ca2+ entrance was monitored by absolute fluorescence (λem 345 nm, λex 510 nm) of Fura-2-loaded vesicles incubated in Ca2+-free buffers. As previously reported (19), kinetic studies performed from 5 s to 15 min demonstrated that the onset of influx was rapid, reaching 83.6% of maximum by 5 s with a minimal, but detectable, additional increase over the subsequent 10 s (p = 0.0013, 15 s compared with 5 s). No further influx occurred after 15 min. 

**Dodecapeptide Inhibition of Ca2+ Entrance into Vesicles—** The dodecapeptide, His-12-Val, inhibited Ca2+ entrance into the vesicles in a dose-dependent manner (Fig. 1). The apparent IC50 was 15 μM for inhibition of influx for 10-20 μM external Ca2+. At concentrations of dodecapeptide >30 μM, inhibition of Ca2+ influx was so complete as to permit no net detectable Ca2+ influx into vesicles after 5 min. His-12-Val had no effect on the intrinsic fluorescence of Fura-2 within liposomes. This was determined by increasing intraliposomal Ca2+ with 1 μM ionomycin in Ca2+-free buffers in the presence and absence of His-12-Val. In contrast to His-12-Val, RGDS had no effect on Ca2+ entrance into vesicles; the data for RGDS were identical to GPIIb-IIIa liposomes incubated with buffer (Fig. 1). PAC-1 antibody also demonstrated no inhibition of Ca2+ influx. When GPIIb-IIIa liposomes were incubated with 76 μg/ml RGDS for 20 min prior to incubation with 10-45 μM His-12-Val, no inhibition of Ca2+ movement across the phospholipid membrane by His-12-Val was observed (Table I). Higher concentrations of dodecapeptide could overcome the RGDS blockade, and inhibition was observed. This block-
Table I

| Peptide incubation prior to addition | % Control Ca\(^{2+}\) entry into liposomes |
|-------------------------------------|-----------------------------------------|
| Buffer                              | 100                                     |
| RGDS, 170 \(\mu\)M                  | 100 ± 0.2\(^*\)                        |
| His-12-Val, 20 \(\mu\)M              | 60 ± 2                                  |
| His-12-Val, 50 \(\mu\)M              | 0 ± 3.1                                 |
| RGDS, 170 \(\mu\)M; His-12-Val, 20 \(\mu\)M | 100 ± 3.2                              |
| RGDS, 170 \(\mu\)M; His-12-Val, 25 \(\mu\)M | 100 ± 0.5                              |
| Fibrinogen, 1 \(\mu\)M               | 0 ± 0.2                                 |

\(^*\)X ± S.D.; N = 24.

Table II

| Peptide inhibition of \(4^{\text{Ca}}\) movement into steady-state and ADP-activated GFP |
|-----------------------------------------------|
| Platelets                                     |
| \([^4\text{Ca}]\)                             |
| cpm/platelet at 10 min                       |
| Steady state                                  |
| Control                                      | 0.0026 ± 0.0005 |
| His-12-Val                                   | 0.0025 ± 0.0005 |
| RGDS                                         | 0.0027 ± 0.0006 |
| Activated (32 \(\mu\)M ADP)                  |
| Control                                      | 0.022 ± 0.002 |
| His-12-Val                                   | 0.017 ± 0.001 |
| RGDS                                         | 0.024 ± 0.002 |
| HHLGGRQAGDVG                                  | 0.023 ± 0.002 |

\(^*\)X ± S.D.; n = 6.

The effect of HHLGGRQAGDVG, a dodecapeptide, on platelet aggregation is analogous to inhibition of His-12-Val binding to intact platelets by RGDS.

Intact Fgn was extremely potent as an inhibitor of transmembrane Ca\(^{2+}\) movement into GPIIb-III\(a\) liposomes; a total inhibition of Ca\(^{2+}\) entrance into proteoliposomes was observed at 1 \(\mu\)M fibrinogen (Table I).

Calcium Transit into Intact Platelets—His-12-Val and RGDS in concentrations up to 100 \(\mu\)M had no effect on steady-state Ca\(^{2+}\) transit across the platelet membrane as determined by \(4^{\text{Ca}}\) exchange. His-12-Val inhibited the entrance of \(4^{\text{Ca}}\) into ADP-activated gel-filtered platelets. Results from a 10-min incubation with \(4^{\text{Ca}}\) and ADP at 25 °C after incubation with His-12-Val are shown in Table II. Similar results are obtained with a 1-min incubation. This inhibition represented only a 10.06 ± 0.06% decrease in total platelet Ca\(^{2+}\) influx in this system. RGDS had no effect on \(4^{\text{Ca}}\) influx at 1 or 10 min. To determine if Ca\(^{2+}\) influx inhibition was a function of peptide specificity and size (i.e., dodecapeptide versus tetrapeptide), the effect of HHLGGRQAGDVG, a dodecapeptide, was assessed in activated platelets.

This peptide had no effect on \(4^{\text{Ca}}\) entrance at concentrations up to 100 \(\mu\)M.

With Fura-2-loaded thrombin-stimulated platelets, His-12-Val effected a dose-dependent decrease in the change in Fura-2 fluorescence 30 s following activation; 50 \(\mu\)M His-12-Val caused a 25% decrease and 200 \(\mu\)M His-12-Val a 50% decrease. The final increase in Fura-2 fluorescence at 5 min was not changed. In contrast, preincubation with La\(^{3+}\) caused an 83% decrease in change in Fura-2 fluorescence. RGDS (25-125 \(\mu\)M) had no effect on the thrombin-induced Ca\(^{2+}\) increase.

Discussion

The present study demonstrates that binding of an analogue of the Fgn γ carboxyl-terminal dodecapeptide, His-12-Val, to GPIIb-III\(a\) liposomes decreases the GPIIb-III\(a\)-mediated Ca\(^{2+}\) entry into these liposomes. At concentrations of His-12-Val ≥30 \(\mu\)M, Ca\(^{2+}\) entry was profoundly inhibited, with no Ca\(^{2+}\) influx after 5 min of incubation. A related dodecapeptide with an arginine substitution at position 7, which is relatively ineffective in inhibition of platelet aggregation, failed to inhibit Ca\(^{2+}\) transit. The synthetic peptide, RGDS, in concentrations up to 125 \(\mu\)M and a monoclonal antibody to the RGDS binding site had no effect on Ca\(^{2+}\) influx into liposomes. Binding of RGDS to GPIIb-III\(a\) liposomes prevented the His-12-Val inhibition of Ca\(^{2+}\) transit across the liposome membrane; therefore, the difference in inhibitory capacity between the peptides is not due to failure of RGDS to bind to GPIIb-III\(a\) molecules on the surface of the liposomes. The ability of Fgn as well as His-12-Val to inhibit this Ca\(^{2+}\) channel function suggests that the GPIIb-III\(a\) complex on the surface of the liposome in the activated configuration facilitates Ca\(^{2+}\) influx.

Potential mechanisms for His-12-Val inhibition of Ca\(^{2+}\) transit include His-12-Val-induced conformational change in the GPIIb-III\(a\) complex, change in topographical distribution of GPIIb-III\(a\) on the surface of the liposomes, or His-12-Val-induced changes in the four potential Ca\(^{2+}\) binding regions on the GPIIb molecule (32, 33). Tsein et al. (34) have proposed that such Ca\(^{2+}\) binding domains provide selectivity to Ca\(^{2+}\) "trigger proteins." The difference in inhibitory capacity between dodecapeptide and RGDS may be related to nonideality of binding sites of these two peptides or differences in conformational changes associated with ligand-receptor interaction. While both peptides induce conformational changes in the soluble GPIIb-III\(a\) complex (19), subtle differences in these changes may account for differences in Ca\(^{2+}\) channel inhibition. While data from a number of studies suggest that His-12-Val and RGDS bind to related sites on the GPIIb-III\(a\) complex, these sites may not be identical. Despite the mutually inhibitory nature of RGDS and LGGAKQAGDVG binding, Santoro and Lawing (35) with affinity labeling, Williams and Granick (15) with binding competition, and Bennett et al. (16) have hypothesized that the binding sites for these peptides are not identical but that ligand-induced conformational changes may be responsible for mutually exclusive binding.

Our studies with His-12-Val and the intact platelet extend and are in general accord with those of Brass (21), Yamaguchi et al. (22), and Sinigaglia et al. (36). Their data suggest that GPIIb-III\(a\) may play a role in calcium flux in the intact platelet. The lack of inhibition with RGDS in the current study is in contrast to the inhibition of Ca\(^{2+}\) influx by GRGDSP observed by Yamaguchi et al. (22) and by RGDS observed by Sinigaglia et al. (36) in stimulated platelets. This difference may be due to differences in methodology, i.e. dimethyl sulfoxide-treated platelets in the Yamaguchi study.
following thrombin and collagen activation versus intact, ADP, and thrombin-activated platelets, or from different time points chosen for analysis. The 10-min time point in the current 45Ca study follows the completion of transmembrane Ca2+ influx (37, 38), and both control and peptide-treated platelet data may include a component of efflux. This efflux would actually result in an underestimate of His-12-Val inhibition. Differences among these three studies should not arise from differences among RGDS, GRGDSP, and GRGDS since the IC50 values of these peptides for Fgn γ chain binding to activated platelets are similar (16). The Sinigaglia study (36) measured the total increase in platelet Ca2+, while the current 45Ca study examines transmembrane Ca2+ movement. The Fura-2 platelet data include a significant component of calcium release from intracellular sites; however, the La3+ inhibition suggests a significant contribution from extracellular Ca2+ to [Ca2+] at 30 s. The complex nature of changes in Ca2+ concentration in the activated platelet with its multiple sources of Ca2+ in contrast to the liposome model render the former a more difficult system to interpret. The observation that ADP-induced activation and secretion by human platelets proceeds normally in the absence of external calcium indicates that the Ca2+ influx mediated by GPIIb-IIIa may play only a subsidiary role in platelet activation. The current platelet data indicate that His-12-Val inhibits only a minor component of Ca2+ movement into the platelets. This may explain the apparent discrepancy between these data and those of Powling and Hardisty (39) who were able to demonstrate normal calcium influx into ADP-activated quin-2-loaded thrombasthenic platelets. Our platelet data, in concert with the liposome model data, suggest that GPIIb-IIIa may play a role in platelet activation.

REFERENCES

1. Phillips, D. R., Charo, I. F., Parise, L. V., and Fitzgerald, L. A. (1988) Blood 71, 831-843
2. Pytel, R., Pierschbacher, M. D., Ginsberg, M. H., Plow, E. F., and Ruoslahti, E. (1986) Science 231, 1559-1561
3. Hynes, R. O. (1987) Cell 48, 549-554
4. Bennett, J. S., and Vilaire, G. (1979) J. Clin. Invest. 64, 1395-1401
5. Marguerie, G. A., Plow, E. F., and Edgington, T. S. (1979) J. Biol. Chem. 254, 5557-5563
6. Bennett, J. S., Vilaire, G., and Cines, D. B. (1982) J. Biol. Chem. 257, 8049-8054
7. Nachman, R. L., and Leung, L. K. (1982) J. Clin. Invest. 69, 263-269
8. Kloczewiak, M., Timmons, S., and Hawiger, J. (1982) Biochem. Biophys. Res. Commun. 107, 181-187
9. Kloczewiak, M., Timmons, S., Lukas, T. J., and Hawiger, J. (1984) Biochemistry 23, 1767-1774
10. Ruggeri, Z. M., Houghten, R. A., Russel, S. R., and Zimmerman, T. S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5708-5712
11. Haverstick, D. M., Cowan, J. F., Yamada, K. M., and Santoro, S. A. (1985) Blood 66, 946-952
12. Peerschke, E. I. B., and Galanakis, D. K. (1987) Blood 69, 950-952
13. Lam, S. C. T., Plow, E. F., Smith, M. A., Andreix, A., Ryckvaert, J., Marguerie, G., and Ginsberg, M. H. (1987) J. Biol. Chem. 262, 947-950
14. Plow, E. F., Pierschbacher, M. D., Ruoslahti, E., Marguerie, G., and Ginsberg, M. H. (1987) Blood 70, 110-115
15. Williams, S., and Gralnick, H. (1987) Thromb. Res. 46, 467-471
16. Bennett, J. S., Shattil, S. J., Power, J. W., and Gartner, T. K. (1988) J. Biol. Chem. 263, 12948-12953
17. Isenberg, W. M., McEver, R. P., Phillips, D. R., Shuman, M. A., and Bainton, D. F. (1987) J. Cell Biol. 104, 1655-1663
18. Banga, H. S., Simons, E. R., Brass, L. F., and Rittenhouse, S. E. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9197-9201
19. Parise, L. V., Helgerson, S. L., Steiner, B., Nannizzi, L., and Phillips, D. R. (1987) J. Biol. Chem. 262, 12597-12602
20. Rybak, M. E., Renzulli, L., Bruns, M., and Cahaly, D. (1988) Blood 72, 714-721
21. Brass, L. F. (1985) J. Biol. Chem. 260, 2231-2236
22. Yamazuchi, A., Yamamoto, N., Kitagawa, H., Tanoue, K., and Yamazaki, H. (1987) FEBS Lett. 225, 228-232
23. Rybak, M. E. (1986) Thromb. Res. 35, 240-247
24. Newman, P. J., Knipp, M. A., and Kahn, R. A. (1982) Thromb. Res. 27, 221-224
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
26. Garrewal, H. S. (1985) Anal. Chem. 51, 319-324
27. Duangnes, N., Wilschut, J., Hong, K., Fraley, R., Perry, C., Friend, D. S., James, T. L., and Papahadjopoulos D. (1983) Biochim. Biophys. Acta 732, 289-299
28. Parise, L. V., and Phillips, D. R. (1985) J. Biol. Chem. 260, 10968-10707
29. Balthassare, J. J., Kahn, R. A., Knipp, M. A., and Newman, P. J. (1985) J. Clin. Invest. 75, 35-39
30. Tangen, O., Berman, H. J., and Marfoy, P. (1971) Thromb. Diath. Haemorrh. 25, 268-278
31. Zavoico, G. B., and Cragoe, E. J. (1988) J. Biol. Chem. 263, 9635-9689
32. Fitzgerald, L. A., Poncz, M., Steiner, B., Rall, S. C., Jr., Bennett, J. S., and Phillips, D. (1987) Biochemistry 26, 8158-8165
33. Poncz, M., Eisnam, R., Heidenreich, R., Silver, S. M., Vilaire, G., Surrey, S., Schwartz, E., and Bennett, J. S. (1987) J. Biol. Chem. 262, 5476-5482
34. Tein, R. W., Hess, P., McCleskey, E. W., and Rosenberg, R. L. (1987) Annu. Rev. Biochem. Biophys. Chem. 16, 16, 265-290
35. Santoro, S. A., and Lawing, W. J., Jr. (1987) Cell 48, 867-873
36. Sinigaglia, F., Bisio, A., Torti, M., Baldini, C. L., Bertolino, G., and Baldini, C. (1988) Biochem. Biophys. Res. Commun. 154, 258-264
37. Sage, S. O., and Rink, T. J. (1987) J. Biol. Chem. 262, 16364-16369
38. Jones, G. D., and Gear, A. R. (1988) Blood 71, 1539-1543
39. Powling, M. J., and Hardisty, R. M. (1985) Blood 66, 731-734