Structural Determinants of the Factor IX Molecule Mediating Interaction with the Endothelial Cell Binding Site Are Distinct from Those Involved in Phospholipid Binding*

(Received for publication, August 1, 1989)

Jane Ryan*, Barry Wolitzky*, Edgar Heimer*, Theodore Lambrose*, Arthur Felix*, James P. Tam*, Linda H. Huang*, Peter Nawroth*, George Wilner*, Walter Kiesiel*, Gary L. Neelsetuen*, and David M. Stern*

From the *Department of Physiology and Cellular Biophysics, Columbia University, New York, New York 10032,
§Department of Molecular Genetics and *Department of Peptide Chemistry, Hoffmann-La Roche, Nutley, New Jersey 07110,
&The Rockefeller University, New York, New York 10021, *Hematology Research, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104, 'Department of Pathology, Washington University, St. Louis, Missouri 63110,
†Department of Pathology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131, and
*Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108

Previous studies have indicated that Factor IXa interacts in a specific and high affinity manner with a binding site on the endothelial cell surface. In this study, the contributions of the γ-carboxyglutamic acid-containing (GLA) and growth factor domains to the finding of Factor IX to the endothelium were assessed. While GLA-containing peptides from Factors IX, X, and prothrombin were inhibitors of 125I-Factor IX-endothelial cell binding, the GLA peptide from Factor IX was about 250-800-fold more effective than those from prothrombin and Factor X, respectively. In contrast to its relative efficacy as an inhibitor of Factor IX-cell surface interaction, the Factor IX-GLA peptide neither bound to lipid vesicles nor inhibited Factor IX-lipid interaction. A synthetic peptide comprising the entire first epidermal growth factor (EGF) domain and the first loop of the second EGF domain was also an inhibitor of 125I-Factor IX-endothelial cell binding, although it did not interact with lipid vesicles. Experiments with synthetic peptides comprising each of the three loops of the first EGF domain or the entire first EGF region with specific substitutions indicated the importance of determinants in both the first and probably third loops for Factor IX-endothelial interaction. In contrast, the second loop of the first EGF domain and the first loop of the second EGF exon are probably not involved in Factor IX-endothelial interaction based on their inability to block 125I-Factor IX binding to cells. These results indicate that determinants in both the GLA and the first EGF domain contribute to the specific binding of Factor IX to the endothelial cell surface and that structural requirements for Factor IX-cell surface interaction are distinct from those for Factor IX binding to lipids.

Factor IX/Xa binds to the endothelial cell surface with high affinity (apparent Kd ≈ 2 nM) and specificity, as other vitamin K-dependent coagulation factors (such as Factor X and prothrombin) do not compete. This contrasts with the lower affinity interaction of Factor IX with phospholipids and the ability of one vitamin K-dependent coagulation factor to displace another from the lipid surface (4-6). In this context, cross-linking studies have implicated the involvement of a cell surface protein, M, ≈ 140,000, in mediating, at least in part, the binding of Factor IX to endothelium (7).

These considerations have led us to examine domains of the Factor IX molecule involved in recognizing the endothelial binding site (8, 9) and to compare their role in Factor IX-cell surface interaction with their effects on Factor IX binding to lipid vesicles. The results indicate that both the γ-carboxyglutamic acid (GLA)-containing and first epidermal growth factor (EGF) domains are involved in Factor IX-endothelial interaction and highlight the differences between the structural requirements for the binding of Factor IX to cellular surfaces and synthetic phospholipids.

MATERIALS AND METHODS

Preparation of Coagulation Proteins and GLA Peptides—Bovine Factors IX (230 units/mg) and X (100 units/mg) and human prothrombin fragment 1 were purified to homogeneity by previously described methods (10-12). Factor IX was radiolabeled by the lactoperoxidase method (13) using Enzymebeads (Bio-Rad) and 125I-Factor IX isolated as described previously (14). The specific radioactivity of these preparations was 4.6 x 10^5 cpm/μg over 15 iodinations, and the radioactivity profile of reduced 125I-Factor IX on sodium dodecyl sulfate-PAGE showed a single peak at mass of ≈60 kDa. Factor IX modified by cleavage of the GLA domain, GLA-domainless Factor IX, was prepared by limited proteolysis with lysyl endopeptidase as described by Morita et al. (15, 16).

GLA peptides were prepared from Factors IX, X, and prothrombin fragment 1 by incubating with Nα-p-tosyl-L-lysine chloromethyl ketone-treated α-chymotrypsin (Sigma) at an enzyme-to-substrate ratio of 1:200 in the absence of calcium followed by chromatography on QAE-Sephadex to separate the peptides from the other components of the reaction mixtures, as described previously (15-19). In the case of Factor X, this produces a peptide comprising residues 1-44. For human prothrombin fragment 1, a mixture of two peptides (by analogy with bovine prothrombin fragment 1 (20), one including residues 1-41 and the other residues 1-44, could be produced. By using the results of amino acid analysis and protein determination (21), as described by Pollock et al. (20), the latter two peptides were identified. Human prothrombin fragment comprising residues 1-44 accounted

*This work was supported by grants from the United States Public Health Service (HL 34625, HL 41935, and HL 28433), the Council for Tobacco Research (CTR 1971), and Hoffmann-La Roche. 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.

†Recipient of a Genentech-American Heart Association Established Investigator Award during tenure of which this work was done.
for 40% of the material present in fractions pooled from the QAE-Sephadex column, the remainder being the peptide derived from residues 1–41. A similar analysis of material eluted in peak fractions from the Factor IX-chymotrypsin cleavage mixture indicated that the Factor IX-GLA peptide consisted predominantly of the fragment corresponding to residues 1–42.

Preparation and Characterization of Synthetic Peptides—The human Factor IX-EGF domain (residues 45–87) (22) was synthesized manually by the solid-phase method (24). Details of the synthesis, refolding, and characterization of the latter peptide and related peptide derivatives containing amino acid substitutions have been reported (25). These peptides have been found to be appropriately refolded and have recently been shown to be a suitable substrate to assess the β-hydroxylation of Asp-64 by a 2-oxoglutarate-dependent dioxygenase (25).

Synthetic peptides comprising each of the loops of the first Factor IX-EGF exon (corresponding to residues 45–64, 55–73, and 72–85 from the bovine molecule) (25) and the first loop of the second Factor IX-EGF exon (residues 85–101) were prepared by solid-state methodology (manual method) (24). Peptides were purified to homogeneity by HPLC and characterized by amino acid analysis, and the molecular weight was confirmed by Fast Atom Bombardment (FAB/mass spectroscopy). For cyclization, linear peptides (100 μg/ml) were dissolved in 8 M urea containing KFe(CN)₆ (0.18 mM). The solution was stirred at room temperature for 260 h, and cyclization was monitored by analytical HPLC. Then the solution was acidified with 40% aqueous trifluoroacetic acid (final pH 2.5) and purified on a Waters Bondapak column (1.9 × 50 cm). Fractions containing the desired product were cooled, evaporated, and lyophilized. The product was shown to be homogeneous by analytical HPLC and gave a negative Ellman test (for free sulfhydryls).

Human and murine EGF were purchased from Calbiochem and Sigma, respectively.

Cell Culture and Binding Assays—Bovine aortic endothelial cells were grown from aortae of newborn calves as described (26) and were used from passages 3–26. Experiments were carried out within 24 h after the cells achieved confluence using 0.32-cm² wells. At confluence, there were 1.0–1.5 × 10⁴ cells/cm². The procedure for carrying out Factor IX binding assays has been described previously (2, 14). Previous studies from our (21) and other laboratories (3) have shown that human and bovine Factor IX interact with endothelium in a very similar manner. Thus, use of peptides with sequences from the human and bovine molecules should not introduce an additional variable with respect to cell surface interaction. Data showing inhibition of [³²P]-Factor IX binding to endothelium by peptides was fit to the equation (27):

\[ b = B_0[A]/(K_0 + K[X] + K[A]) \]

where \( b \) = bound radioligand (³²P-Factor IX), \( [A] \) = concentration of radioligand, \( K_0 \) = \( K \) in the absence of inhibitor, \( [X] \) = concentration of inhibitor (peptides), \( B_0 \) = maximal binding of radioligand in the absence of inhibitor. This representation of binding data is shown in the insets to Figs. 1 and 2. The inhibition studies shown in the figures employed lower concentrations of [³²P]-Factor IX (0.87 nM and below), but in a limited number of pilot studies higher concentrations of radioligand were used (2–6 nM), and similar results were obtained.

RESULTS

To investigate the role of Factor IX-GLA domain in the binding of this conglutination factor to the endothelial surface, peptides comprising GLA domains were prepared from Factors IX, X, and prothrombin by limited proteolysis. When these peptides were added to mixtures containing [³²P]-Factor IX and cultured endothelial cells, only the GLA peptide from Factor IX was highly effective as an inhibitor of binding (Fig. 1A). In an attempt to determine if this binding and inhibition represented equilibrium measurements, inhibition of Factor IX binding at three concentrations of [³²P]-Factor IX was employed. Although the relationship fits closely to a model of competitive inhibition (27) with \( K_i \approx 0.06 \mu M \) (Fig. 1A, inset), this value is viewed as approximate. The GLA peptides from Factor X and prothrombin were also inhibitors of [³²P]-Factor IX binding to endothelium. However, they were less effective (Fig. 1, B and C). While the data employing the Factor X and prothrombin-GLA peptides did not extend to full inhibition, the partial inhibition was highly reproducible, suggesting that the observed inhibition was of significance. When analyzed by equilibrium binding inhibition, the data suggested competitive inhibition with \( K_i \) values of ≈50 μM and ≈15 μM, for the Factor X and prothrombin-GLA peptides, respectively (Fig. 1, B and C). Consistent with an important role for the GLA domain in mediating the interaction of Factor IX with endothelium, GLA-domainless Factor IX neither bound to endothelium nor competed with [³²P]-Factor IX for the cell surface sites up to a concentration of ≈1 μM (data not shown).

Our previous studies suggested a role for the EGF domain in Factor IX-endothelial interaction (8, 9). Other studies have suggested the involvement of the EGF domain in the interaction of other proteins, such as urokinase, with their cellular receptors (28). We therefore performed experiments with synthetic peptides spanning the entire first EGF exon of Factor IX (residues 45–87). This peptide appeared to inhibit binding of [³²P]-Factor IX to endothelium, and the data obtained (Fig. 24) fit to a competitive inhibition model with \( K_i \approx 3 \mu M \). More extensive displacement measurements were precluded due to the limited quantities of this material. Nevertheless, more complete displacement of [³²P]-Factor IX was achieved with higher concentrations of a smaller peptide from the EGF domain (loop 1, see below). In contrast to its effect on Factor IX-cell surface interaction, the full length EGF peptide neither bound to lipid vesicles nor altered the binding of Factor IX to these vesicles (32).

The preliminary findings with the entire EGF domain led us to further examine portions of this structure. Two approaches were employed for these studies: 1) experiments with synthetic peptides comprising each of the loops of the first EGF domain; and 2) experiments with synthetic peptides comprising the whole first EGF exon substituted in specific areas. For the first approach, the effect of cyclized peptides corresponding to the first, second, and third loops of the first EGF domain on [³²P]-Factor IX-endothelial interaction was examined. The peptide corresponding to the first loop was the best inhibitor (Fig. 2B and Table I, line 1), and the data in Fig. 2B fit to a model of competitive inhibition with \( K_i = 10 \) μM. Neither of the peptides comprising the second or third loop of the first EGF exon nor a peptide comprising the first loop of the second EGF exon was an inhibitor of [³²P]-Factor IX-endothelial interaction (Table I, lines 2–4) up to the highest concentrations examined (≈100 μM).

Our other approach for assessing determinants of the first EGF domain mediating the interaction of Factor IX with endothelium led us to use peptides spanning the entire first EGF domain with specific substitutions in the first and third loops (Table I, lines 5–10). The latter substitutions were selected since these regions of EGF may be involved in its binding to the EGF receptor. A peptide comprising the first EGF domain with substitution of residues from the sequence of murine EGF for those in Factor IX in the first loop (see Table I legend) failed to inhibit the binding of [³²P]-Factor IX to endothelium (line 6). Similarly, substitution of the residues from the sequence of human EGF for those from Factor IX in the first loop and the region just before results in an inactive peptide (line 7). Substitution of residues from the third loop

1 J. P. Tam, unpublished observation.
of the first EGF exon with those from human EGF or the laminin binding domain also rendered the peptide inactive as an inhibitor of Factor IX binding to endothelium (lines 8 and 9, respectively). If only the Asn at position 81 was replaced by an Arg (this Asn is highly conserved in native EGF), the peptide retained its ability to inhibit Factor IX binding (line 10 and Fig. 2C). The latter peptide's ability to inhibit $^{125}$I-Factor IX-endothelial binding fit to a model of competitive inhibition (27) with $K_i \approx 12 \mu M$. The specificity of the peptides derived from the EGF domain of Factor IX for inhibition of $^{125}$I-Factor IX-endothelial binding was further confirmed by the finding that neither murine nor human EGF had any measurable effect on Factor IX-cell surface binding (lines 11 and 12).

**DISCUSSION**

Studies in our and other laboratories (1-3) have defined the presence of a selective binding site for Factor IX on endothelium. These observations have led to efforts to define the nature of the determinants mediating the interaction of Factor IX with the endothelial cell surface and how they may differ from those structures involved in Factor IX-phospholipid interaction. The experiments presented here demonstrate that the interaction of Factor IX with its binding site...
on endothelium involves structural features present in the GLA and first EGF domain of the molecule. Our data, however, do not rule out that other regions of the molecule may also be involved.

The GLA domain has an important role in mediating the interaction of Factor IX with endothelium. In our preliminary report (9), we showed that the GLA-peptide derived from Factor IX was an inhibitor of 125I-Factor IX binding to endothelium. These data could be confirmed by Derian et al. (3), who noted an even higher inhibitory potency of the GLA peptide. Furthermore, the latter work demonstrated that recombinant decarboxy-Factor IX did not bind to endothelium. In the present study, the GLA peptide derived from Factor IX inhibits the binding of 125I-Factor IX to endothelium considerably more effectively than similar peptides derived from Factor X or prothrombin (Fig. 1). Although initially we reported in a pilot study that Factor IX modified by limited proteolysis to cleave the GLA domain retained some (but considerably reduced) binding activity (8), this was found to be due to a small amount of contaminating native Factor IX, and our results now agree with those recently reported by Derian et al. (3), i.e. Factor IX lacking the GLA domain does not bind to endothelium.

The GLA domain is traditionally thought of as mediating Ca2+-dependent interaction of vitamin K-dependent coagulation proteins with phospholipid surfaces (4–6). The GLA peptide from Factor IX (residues 1–42) employed in our study did not bind to phospholipids and did not inhibit the interaction of Factor IX with phospholipid (32). Lack of phospholipid binding by Factor IX GLA peptide from which residues 43–45 were removed supports the observations of Pollock et al. (20) that GLA peptides from bovine prothrombin spanning

---

**TABLE I**

Binding of Factor IX to endothelium: effect of peptides derived from the EGF domain

Binding studies to assess inhibition of 125I-Factor IX binding by the peptides were carried out by incubating confluent monolayers of endothelium at 4 °C for 2 h with 125I-Factor IX at two or three different concentrations (0.13, 0.32, and 0.87 nM) in the presence of a range of peptide concentrations (0.5 to 250 μM). Cultures were then washed, and bound radioactivity was eluted with EDTA-containing buffer. This protocol for radioligand binding assays followed that referred to in the text. Each experiment was repeated at least four times.

| Peptidea | Inhibitor of bindingb |  |
|----------|----------------------|---|
| 1. Tyr-Val-Asp-Gly-Asp-Gln-Cys-Glu-Ser-Asn-Pro-Ala-Leu-Asn-Gly-Gly-Ser-Cys-Lys-Asp | + |  |
| 2. Pro-Cys-Leu-Asn-Gly-Gly-Ser-Ala-Lys-Asp-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Ala | - |  |
| 3. Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp | - |  |
| 4. Asp-Val-Thr-Cys-Asn-Ile-Lys-Glu-Asn-Ala-Glu-Gly-Arg-Cys-Lys-Asn | - |  |
| 5. Tyr-Val-Asp-Gly-Asp-Gln-Cys-Glu-Ser-Asn-Pro-Cys-Leu-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp-Val-Thr | + |  |
| 6. Tyr-Val-Asp-Gly-Asp-Gln-Cys-Pro-Ser-Ser-Tyr-Asp-Gly-Tyr-Cys-Leu-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp-Val-Thr | - |  |
| 7. Asn-Ser-Arg-Ser-Glu-Cys-Pro-Leu-Ser-His-Asp-Gly-Tyr-Cys-Leu-His-Gly-Gly-Ser-Cys-Lys-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp-Val-Thr | - |  |
| 8. Tyr-Val-Asp-Gly-Asp-Gln-Cys-Glu-Ser-Asn-Pro-Cys-Leu-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp-Val-Thr | - |  |
| 9. Tyr-Val-Asp-Gly-Asp-Gln-Cys-Glu-Ser-Asn-Pro-Cys-Leu-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp-Val-Thr | - |  |
| 10. Tyr-Val-Asp-Gly-Asp-Gln-Cys-Glu-Ser-Asn-Pro-Cys-Leu-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Ile-Asn-Ser-Tyr-Glu-Cys-Trp-Cys-Pro-Phe-Gly-Phe-Glu-Gly-Lys-Asn-Cys-Glu-Leu-Asp-Val-Thr | + |  |
| 11. Murine EGF | - |  |
| 12. Human EGF | - |  |

a Peptides correspond to cyclized loops of the Factor IX EGF domain (lines 1–4) or peptides with/without substitutions from the full first exon of the EGF domain (lines 5–10). Lines 1–4, peptides 1–4 correspond to loops from the bovine Factor IX (22) EGF domain as follows: peptide 1 (residues 45–64, loop 1, disulfide formed between Cys 51 and 59) to close the loop, Cys at 56 (underlined) replaced by Ala; peptide 2 (residues 55–73, loop 2, disulfide formed between Cys at 56 and 71, Cys at 73 (replaced) replaced by Ala); peptide 3 (residues 72–85, loop 3, disulfide formed between Cys at 73 and 82); peptide 4 (residues 85–101, loop 1 of second EGF exon, disulfide formed between Cys at 88 and 90, Cys at 95 (underlined) replaced by Ala). Lines 5–10, peptide 5 is composed of residues 45–87 from human Factor IX (23) and the reformed disulfide bonds link Cys at residues 41 to 62, 56 to 71, and 73 to 82 to form the three loops. The substituted amino acids in the other peptides (peptides 2–6) are underlined and derived from human/murine EGF (28), or the laminin binding domain (30), as follows (amino acid numbers in parentheses refer to location of that amino acid in murine/human EGF or Factor IX, and Cys residues linked by reformed disulfides are numbered based on arbitrarily assigning the number 45 to the first residue of the EGF domain or chimeric peptides): peptide 6, chimeras of murine EGF (residues 6–18, underlined and constituting loop 1) and human Factor IX EGF domain (residues 45–50 and 61–87) with reformed disulfides linking Cys residues at 50 to 64, 58 to 73, and 75 to 84; peptide 7, chimeras of human EGF (residues 1–16, underlined and constituting loop 1) and human Factor IX (residues 59–87) with reformed disulfides linking Cys residues at 50 to 64; peptide 8, chimeras of human EGF (residues 34–74, underlined and comprising loop 3) and human Factor IX (residues 73–82 and 82–95); peptide 9, a chimera of the laminin binding domain (from the laminin B1 chain, Tyr-Ile-Gly-Ser-Arg, underlined and substituted into the loop 3) and human Factor IX (residues 45–76 and 82–87); peptide 10 is the same as peptide 1 except that Asn (residue 81) has been substituted by Arg.

b If a peptide inhibited 125I-Factor IX binding to endothelium, a (+) follows that entry and the actual data are shown in the figure which is stated in the brackets. If a peptide had no effect on 125I-Factor IX binding, then a (−) follows that entry. The limit of detection in the binding assay was less than 10% displacement.
residues 1–42 do not have phospholipid binding properties. The ability of our GLA peptide (residues 1–42) to block Factor IX-endothelial interaction (although it did not block Factor IX-phospholipid interaction (32)) with greater potency than the GLA peptides from Factor X and prothrombin (both of which do bind to phospholipid (32)) emphasizes that different structural features in the GLA domain are important in mediating interaction of Factor IX with the cellular binding site versus a lipid surface.

The first EGF domain of Factor IX also appears to contribute to the interaction of this coagulation factor with the cell surface. The full length EGF peptide failed to interact with synthetic phospholipids and did not affect the binding of Factor IX to lipids (32). The results in Fig. 2 and Table I suggest that amino acid residues, especially in the first and possibly in the third loops of the first EGF exon, are involved in binding of Factor IX to endothelium. However, Derian et al. (3) found that recombinant decarboxy-Factor IX did not bind to endothelium. On the one hand, this result would appear to contradict our findings, that the first EGF domain or portions of this domain are capable of inhibiting Factor IX-endothelial cell binding, since these portions of the molecule should be intact in the recombinant protein. Nevertheless, lack of inhibition by the recombinant protein might arise from any of several factors, such as interference by the unconjugated GLA region, or by its effect on secondary/tertiary structure. A requirement for interaction of two or more domains of Factor IX (such as the EGF and GLA domains) for the molecule to bind to the cell surface might also underlie the failure of GLA-domainless Factor IX to bind to endothelium. In the case of GLA-domainless Factor IX, the modified molecule has an additional internal cleavage (residue 142/143) (16), and this may affect recognition of the cellular binding site. Further studies will be needed to determine the basis for failure of recombinant Factor IX and GLA-domainless Factor IX to inhibit binding of Factor IX to endothelium.

In relation to the high affinity binding constant of Factor IX for the endothelium ($K_d \approx 2 \text{ nM}$), the relatively high concentrations of Factor IX GLA-peptide and synthetic peptides necessary to effect inhibition of $^{125}$I-Factor IX binding to endothelium in all likelihood reflects the fact that Factor IX interaction with the endothelial cell surface involves the cooperative effects of several determinants. In addition, these peptides probably exist in solution as random conformers in equilibrium with a conformer that assumes the "native" conformation of that segment of the Factor IX molecule (31). Presumably, it is this "native" conformer that associates with the cell surface Factor IX binding site to result in the inhibition of $^{125}$I-Factor IX binding, and, accordingly, this interaction may be very weak in the absence of other determinants that contribute to the overall energy of binding.

Overall, the studies reported here are consistent with a model in which binding of Factor IX to endothelium involves contributions by several domains of the molecule. Because of this, it is difficult to localize with confidence the binding region of the Factor IX molecule, and there are probably multiple, complex structural features involving more than one loop which enable Factor IX to recognize the endothelial binding site. In addition, our results emphasize differences in determinants mediating interaction of Factor IX with lipids versus interaction with the endothelial cell binding site.

Acknowledgments—We are grateful to Carmen Viana for her efforts on behalf of our laboratory and Samuel Rover for his generous contribution.

REFERENCES

1. Heimark, R. L., and Schwartz, S. M. (1983) Biochem. Biophys. Res. Commun. 111, 723–731
2. Stern, D., Drilling, M., Nossel, H., Hurlet-Jensen, A., Lamanna, K., and Owen, J. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4119–4123
3. Derian, C., VanDusen, W., Przyziecki, C., Walsh, P., Berkner, K., Kaufman, R., and Friedman, P. (1989) J. Biol. Chem. 264, 6615–6618
4. Esmun, C. T., Suttie, J. W., and Jackson, C. M. (1975) J. Biol. Chem. 250, 4095–4099
5. Nelsestuen, G. L., Kisiel, W., and Di Scipio, R. G. (1978) Biochemistry 17, 2138–2138
6. Beals, J., and Castellino, F. (1986) J. Biochem. (Tokyo) 236, 861–869
7. Rimon, S., Melamed, R., Savion, N., Scott, T., Nawrath, P. P., and Stern, D. M. (1987) J. Biol. Chem. 262, 6023–6031
8. Nawroth, P., Wilner, G., and Stern, D. (1986) Circulation 74 (Suppl. II), 233 (abstr.)
9. Ryan, J., Wolitzky, B., Heimer, E., Felix, A., Huang, L., Tam, J., Kisiel, W., Nawrath, P., and Stern, D. (1989) FASEB J. 3, A1050
10. Fujikawa, K., Legaz, M. E., Kato, H., and Davie, E. W. (1974) Biochemistry 13, 4508–4516
11. Fujikawa, K., Legaz, M., and Davie, E. W. (1972) Biochemistry 11, 4892–4905
12. Downing, M. R., Butkowski, R. J., Clark, M. M., and Mann, K. G. (1975) J. Biol. Chem. 250, 8897–8906
13. David, G., and Reisfeld, R. (1974) Biochemistry 13, 1014–1021
14. Stern, D. M., Nawroth, P. P., Kisiel, W., Vehar, G., and Esmon, C. T. (1986) J. Biol. Chem. 260, 6717–6722
15. Morita, T., and Kisiel, W. (1985) Biochem. Biophys. Res. Commun. 130, 841–847
16. Morita, T., Isacis, B. S., Esmon, C. T., and Johnson, A. E. (1984) J. Biol. Chem. 259, 5968–5974
17. Morita, T., and Jackson, C. (1980) in Vitamin K Metabolism and Vitamin K-Dependent Proteins (Sutte, J., ed) pp. 124–128, University Park Press, Baltimore, MD
18. Skogen, W. F., Esmon, C. T., and Cox, A. C. (1984) J. Biol. Chem. 259, 2306–2310
19. Nawroth, P., Kisiel, W., and Stern, D. (1986) Thromb. Res. 44, 625–637
20. Pollock, J. S., Shepard, A. J., Weber, D. J., Olson, D. L., Klapper, D. G., Pedersen, L. G., and Hisky, R. G. (1988) J. Biol. Chem. 263, 14216–14223
21. Smith, P., Krohn, R., Hermanson, G., Mallia, A., Gartner, F., Provenzano, M., Fujimoto, E., Boeke, N., Olson, B., and Klenk, D. (1985) Anal. Biochem. 150, 76–85
22. Katayama, K., Ericsson, L., Enfield, D., Walsh, K., Neurath, H., Davie, E., and Titani, K. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4990–4994
23. Kurihara, K., and Davie, E. W. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6461–6465
24. Merrifield, R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6367–6371
25. Stenflo, J., Holme, E., Lindstedt, S., Chandramouli, N., Huang, L., Tam, J. P., and Merrifield, R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 444–447
26. Schwartz, S. (1978) In Vitro 14, 966–968
27. Segel, I. (1975) Enzyme Kinetics, pp. 100–106, John Wiley & Sons, New York
28. Appella, E., Weber, I., and Blasi, F. (1988) FEBS Lett. 231, 1-4
29. Carpenter, G., and Zendegui, J. (1986) Exp. Cell Res. 164, 1–10
30. Graf, J., Ogle, R., Robey, F., Sasaki, M., Martin, G., Yoshiko, Y., and Kleinman, H. (1987) Biochemistry 26, 6896–6900
31. Berzofsky, J., and Berkower, I. (1984) in Fundamental Immunology, Paul, W., ed. 1st Ed. pp. 355–644, Raven Press, New York
32. Schwab, R. A., Ryan, J., Stern, D. M., Kisiel, W., Dahlback, B., and Nelsestuen, G. L. (1989) J. Biol. Chem. 264, 20285–20296