Factor VIII, a cofactor of the intrinsic clotting pathway, is proteolytically inactivated by the vitamin K-dependent serine protease, activated protein C in a reaction requiring Ca++ and a phospholipid surface. Factor VIII was inactivated 15 times faster than factor VIII in complex with either von Willebrand factor (vWF) or the large homodimeric fragment, SP III (vWF residues 1-1365). Free factor VIII or factor VIII in complex with a smaller fragment, SP III-T4 (vWF residues 1-272), were inactivated at the same rate, suggesting that this effect was dependent upon the size of factor VIII-vWF complex rather than changes in factor VIII brought about by occupancy of the vWF-binding site. Thrombin cleavage of the factor VIII light chain to remove the vWF-binding site eliminated the protective effects of vWF. In the absence of phospholipid, high levels of the protease inactivated both free and vWF-bound factor VIII at equivalent rates. Using the same conditions, isolated heavy chains and the heavy chains of factor VIII were proteolyzed at similar rates. Taken together, these results suggested that, in the absence of phospholipid, inactivation of factor VIII is independent of factor VIII light chain and further suggest that vWF did not mask susceptible cleavage sites in the cofactor. Solution studies employing fluorescence energy transfer using coumarin-labeled factor VIII (fluorescence donor) and synthetic phospholipid vesicles labeled with octadecyl rhodamine (fluorescence acceptor) indicated saturable binding and equivalent extents of donor fluorescence quenching for factor VIII alone or when complexed with SP III-T4. However, complexing of factor VIII with either vWF or SP III eliminated its binding to the phospholipid. Since a phospholipid surface is required for efficient catalysis by the protease, these results suggest that vWF protects factor VIII by inhibiting cofactor-phospholipid interactions.

Factor VIII, a plasma protein absent or defective in individuals with hemophilia A, circulates as a series of Me++-linked heterodimers (1-3) in noncovalent association with vWF (4, 5). Activation of factor VIII by thrombin dissociates factor VIII from vWF, thus allowing its participation as a cofactor in the membrane-dependent activation of factor X by the vitamin K-dependent serine protease factor IXa (see Ref. 6 for review). Precedence for vitamin K-dependent plasma protein (7), when activated to a serine protease (activated protein C), becomes a potent inhibitor of blood coagulation (8, 9). The basis of this anticoagulant activity is the phospholipid-dependent proteolytic inactivation of factors Va (10-12) and VIIIa (12-15).

Factor VIII is synthesized as a single-chain precursor represented by the domain structure A1-A2-B-A3-C1-C2 with heterodimers formed as a result of proteolysis at the B-A3 junction plus additional cleavages within the B domain (16). The factor VIII heavy chain is minimally represented by the A1-A2 domains but exhibits significant size heterogeneity resulting from the presence of some or all of the contiguous B domain, whereas the light chain corresponds to the A3-C1-C2 domains derived from the COOH-terminal end of the precursor. The intact heterodimeric structure is essential for cofactor function in that the subunits of factor VIII are dissociated by chelating reagents resulting in loss of clotting activity (1, 17). While little is known of the role of the heavy chain in factor VIII function, the light chain has been observed to contain sites for binding of vWF (1), activated protein C (18), and phospholipid (19).

Recent studies have indicated that the association of factor VIII with vWF protects the cofactor from activated protein C (20, 21). In this report we have examined the effects of multimeric vWF and vWF fragments containing the factor VIII binding site on the interactions of factor VIII with activated protein C and phospholipid vesicles. Our results are consistent with a mechanism where protection of the cofactor when bound to vWF results from a reduced affinity of the complex for the phospholipid surface.

MATERIALS AND METHODS

Reagents—Human factor VIII concentrate (Kontes™) was a generous gift from the Cutter Division of Miles Laboratories. L-1-3,5-diaryl-2-phenylethyl chloromethyl ketone-treated trypsin (bovine pancreas) was purchased from Sigma and further purified by reverse phase high performance liquid chromatography using a Vydac C4 column (5 μm, 0.45 × 25 cm) developed with a linear gradient from 20-50% acetonitrile in 0.1% trifluoroacetic acid. Staphylococcus aureus V8 protease was purchased from ICN ImmunoBioscience. Human α thrombin was obtained from Enzyme Research Laboratories. The fluorescent probes CPM and OR were purchased from Molecular Probes.

The abbreviations used are: vWF, von Willebrand factor; CPM, 7-dimethylamino-3-[4'-maleimidophenyl]-4-methylcoumarin; OR, octadecyl rhodamine B; Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; PC, Palmitoyl-oleoyl phosphatidylcholine; PS, Palmitoyl-oleoyl phosphatidyl-L-serine; SDS, sodium dodecyl sulfate.
Fluorescent Labeling of Factor VIII—Factor VIII (90-240 µg/ml) in 0.01 M histidine (pH 6.0), 0.05 M NaCl, 0.005 M CaCl₂, 0.01% Tween-20, and 50% (v/v) ethylene glycol was reacted with the sulphydryl-specific fluorophore CPM. Reactions contained a 20-50-fold molar excess of CPM, assuming a mean molecular mass of 220 kDa (28). These fragments, which retain the factor VIII-binding site (29), form stoichiometric complexes with factor VIII (24). A similar protective effect from activated protein C action to the factor VIII-vWF complex was observed for factor VIII bound to the SPIII homodimer. Here again, the rate of inactivation of the factor VIII-SPIII complex was approximately 15-fold slower compared with that observed for free factor VIII. However, complexing of the smaller SPIII-T4 with factor VIII offered no protection from activated protein C in that essentially similar reaction rates were observed for free factor VIII and the factor VIII-SPIII-T4 complex. These results suggested that the protection from activated protein C observed for factor VIII complexed with either intact vWF or SPIII did not result from either occupancy of the vWF-binding site or potential conformational changes induced in factor VIII following binding, but instead suggested a mechanism dependent upon the size of the ligand that occupies the vWF-binding site.

Results shown in Fig. 2 indicated that when the vWF-

![Fig. 1. Effect of vWF (-derived fragments) on activated protein C-catalyzed inactivation of factor VII. Panel A, energy transition times were measured in buffer containing 0.02 M Hepes (pH 7.2), 0.15 M NaCl, 0.003 M CaCl₂, and 0.01 M lysine HCl. For reactions containing vWF or vWF-derived fragments, (CPM- factor VIII was first preincubated with the vWF for 1 h at 22 C in the reaction buffer prior to the addition of (OR-) PCPs vesicles.](image-url)
VI11 light chain was reconstituted with a 2-fold molar excess of native VI11 and was abolished. For this analysis, factor VI11 was treated with thrombin to effect removal of the NH2-terminal peptide (residues 1649-1689) (30) containing the vWf-binding site or mask the cleavage sites located on the heavy chain.

The first possibility is that vWf can sterically block access to the activated protein C binding site or mask the cleavage sites located on the heavy chain. The second possibility is that vWf can block the interaction of factor VIII with membranes and prevent formation of catalytic complex which would include protease, Ca2+, phospholipid, and factor VIII.

The requirement for phospholipid in the formation of the catalytic complex can be overcome by high concentrations of activated protein C (Fig. 3). In the absence of phospholipid, an approximate 15-fold molar excess of enzyme relative to factor VIII resulted in a rate of inactivation similar to that observed using catalytic levels of enzyme in the presence of phospholipid (see Fig. 1). Further, under these conditions of a high ratio of enzyme to substrate and no phospholipid, the rate of factor VIII inactivation either in the presence or absence of vWf were equivalent. This experiment suggested that vWf did not mask the cleavage sites in the heavy chain from activated protein C.

In the absence of phospholipid, we observed that cleavage of the heavy chain was also light chain-independent. High concentrations of activated protein C resulted in proteolysis of the isolated factor VIII heavy chains (Fig. 4). The apparent rate of degradation, as judged by disappearance of heavy chains and appearance of terminal fragments of 48 and 23 kDa, was independent of the presence of phospholipid. Furthermore, for reactions run in the absence of phospholipid, the rate of cleavage of isolated heavy chains was similar to that observed for the heavy chains of intact factor VIII. This result suggested that inactivation of factor VIII by activated protein C, in the absence of phospholipid, occurs by a mechanism independent of factor VIII light chain. These experiments suggest that the effect of vWf on activated protein C-catalyzed inactivation of factor VIII is to alter a parameter that is dependent upon the presence of the light chain.

Effects of vWf on Factor VIII-Phospholipid Interactions—Fluorescence energy transfer techniques were applied to determine if the results obtained from clotting assays, namely

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*Fig. 2. Effect of vWf on inactivation of a modified factor VIII lacking the vWf binding domain.* Thrombin-cleaved factor VIII light chain was reconstituted with a 2-fold molar excess of native heavy chains as described under "Materials and Methods." This modified factor VIII (20 µg/ml in total factor VIII protein; approximately 25 units/ml) was reacted with either buffer alone (●) or vWf (146 µg/ml; ■) for 1 h at room temperature followed by reaction with activated protein C (1.2 µg/ml). Aliquots were removed at the indicated times following protease addition and assayed for factor VIII activity. Data points represent the mean of two experiments.

*Fig. 3. Effect of vWf on factor VIII inactivation in the absence of phospholipid.* Factor VIII (13.6 µg/ml, approximately 70 units/ml) was reacted for 1 h at room temperature with either buffer (●) or vWf (149 µg/ml; ■). Samples were then treated with activated protein C (46 µg/ml) in the standard assay buffer but lacking phospholipid. Aliquots were assayed at the indicated times. Data points represent the mean of three separate experiments.

*Fig. 4. SDS-polyacrylamide gel electrophoresis of isolated factor VIII heavy chains and intact factor VIII following interaction with activated protein C.* Reaction mixtures (120 µl) contained either factor VIII heavy chains (15 µg/ml), heavy chains (15 µg/ml) plus 100 µg/ml phospholipid, or intact factor VIII (20 µg/ml). Activated protein C (50 µg/ml) was added to each reaction and incubated at 37°C. Aliquots were removed at the indicated times, denatured, subjected to electrophoresis, and the gel stained with silver nitrate. Lanes 1, 5, and 9 represent heavy chains, heavy chains plus phospholipid, and intact factor VIII, respectively, prior to addition of protease. Lanes 2–4, 6–8, and 10–12 represent 5-, 15-, and 60-min time points following protease addition to reactions shown in lanes 1, 5, and 9, respectively. Lane 13 shows activated protein C alone. M, ×10^{-4}.
protection of factor VIII from activated protein C when bound to vWF and SPIII, but not to SPIII-T4, were compatible with the effects of these substrates on the interaction between factor VIII and phospholipid vesicles. For these experiments, factor VIII was modified with the sulfhydryl-specific fluorophore, CPM (fluorescence donor). We have previously used this probe to modify factor VIII subunits and have shown incorporation into residues in both the heavy chain (Cys$^{259}$) and the light chain (Cys$^{1858}$) (23). Intact factor VIII incorporated on average about 2 mol of probe per mol of protein, suggesting sites on both subunits were modified. Clotting activity of the CPM-modified protein was similar to the native material (data not shown). Phospholipid vesicles (4:1 PCPS) were modified with OR at a ratio of approximately 1 mol of probe per 300 mol of phospholipid monomer for use as the fluorescence acceptor. Substitution of OR-phospholipid for unmodified phospholipid used in assays monitoring the activated protein C-catalyzed inactivation of factor VIII yielded equivalent results (data not shown) indicating that presence of the probe did not alter the interactions of the lipid with factor VIII or activated protein C. The OR-PCPS had an excitation spectrum that overlapped the emission spectrum of CPM-factor VIII (Fig. 5). Thus the fluorescence of CPM-factor VIII should be quenched upon binding to the labeled phospholipid vesicles. However, no donor fluorescence quenching would be observed if prior complexing of CPM-factor VIII with vWF (or a vWF-derived fragment) prevented its association with the phospholipid surface.

CPM-factor VIII was titrated with OR-PCPS (Fig. 6). The acceptor quenched the fluorescence of CPM-factor VIII approximately 12% and this effect was saturable. Incubation of CPM-factor VIII with vWF or SPIII blocked subsequent donor quenching following addition of OR-PCPS, whereas prior complexing of CPM-factor VIII with SPIII-T4 yielded a result equivalent to that observed for free factor VIII. These data suggested that prior complexing of factor VIII with either vWF or SPIII prevented factor VIII from binding the phospholipid vesicles. The above results, taken together with the results indicating protection of factor VIII from the protease when the cofactor was complexed with vWF or SPIII but not SPIII-T4, support a model where vWF-mediated protection from activated protein C results from inhibition of the factor VIII-phospholipid interaction.

**DISCUSSION**

Limited proteolysis of factor VIII by activated protein C correlates with the observed inactivation of cofactor function (13-15, 30). This proteolysis is restricted to the heavy chain subunit which is cleaved to several intermediate and terminal digest products (15). However, our earlier observations (18) that (i) heavy chain alone was not a substrate for activated protein C, (ii) isolated light chain inhibited inactivation of factor VIII, and (iii) a (phospholipid-dependent) binding of light chain and the enzyme suggested an integral role for the light chain in this catalytic mechanism. This paper further emphasizes the importance of the light chain in the catalytic mechanism. In this paper we observe that association of the light chain with phospholipid membranes is an essential step in activated protein C-catalyzed inactivation of factor VIII. In addition, we have observed that cleavage of the isolated heavy chain does not appear to be accelerated by these membranes, suggesting that an activated protein C-phospholipid interaction is not sufficient for the rapid cleavage of substrate. Recently we have localized an activated protein C-binding region to light chain residues 2009-2018 (33) located near the COOH-terminal end of the A3 domain.

Also contained within the factor VIII light chain (A3-C1-C2 domainal structure; residues 1649-2332) (16) are sites for binding vWF and phospholipid. vWF binds very near the NH$_2$ terminus of this subunit in that thrombin cleavage (at residue 1689) dissociates factor VIII (or light chain) from vWF (34, 35). Results of Foster et al. (31) have further localized the vWF-binding region to residues 1670-1684. Recently, these investigators have suggested that residues 2303-2332 mediate the binding of factor VIII to phospholipid (32). It is not known how these regions that bind protease, vWF and phospholipid are spatially oriented in the folded protein.

As a result of the multiple macromolecular interactions attributed to the light chain, one can envision several alternative mechanisms for the vWF-dependent protection of factor VIII from activated protein C-catalyzed inactivation. Protection of factor VIII was observed when the cofactor was complexed with multimeric vWF or the homodimeric SPIII but not with the smaller monomer, SPIII-T4. This result indicated a size dependence of the binding substrate with respect to protection, thus excluding the possibilities that occupancy of the vWF-binding site would either induce a conformational change or in itself preclude interaction of factor VIII with protease. Instead, the above result was compatible with the larger vWF substrates either sterically blocking access to the activated protein C-binding sites and/or masking the cleavage sites present in the heavy chain. This latter alternative was suggested by our previous studies which

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**Fig. 5.** Spectral relationship between CPM-factor VIII and OR-PCPS. Corrected emission spectrum for CPM-factor VIII excited at 387 nm (-----) and excitation spectrum for OR-PCPS measured at 650 nm (——) were determined at 1-nm increments. Fluorescence intensity is in arbitrary units.

**Fig. 6.** Effect of vWF (-derived fragments) on the CPM-factor VIII/OR-PCPS pairing. (CPM-) factor VIII (0.5 μg) was incubated with either buffer (C), 6.1 μg of vWF (●), 3.9 μg of SPIII (○), or 0.8 μg of SPIII-T4 (●) for 1 h at room temperature. The indicated amounts of (OR-) PCPS were then added and the reaction mixtures (0.3 ml) were subjected to fluorescence analysis. Relative fluorescence was determined as described under "Materials and Methods." Data points represent the mean of at least two determinations.
indicated activated protein C initially cleaves factor VIII at site(s) within the A2 domain of the heavy chain (15) and a close spatial separation of approximately 30 Å between the NH₂-terminal region of vWF and this domain in the reconstituted factor VIII-vWF complex (24). Our experiments do not allow us to exclude the possibility that vWF sterically blocks the interaction between activated protein C and the light chain. Experimentally, this would be difficult to prove since binding of activated protein C to the light chain is lipiddependent and vWF inhibits the light chain interaction with lipid, it is not possible to set up an assay in which a lipid-bound light chain-vWF complex could be used as a ligand for protein C binding. However, vWF does not appear to mask bonds in the factor VIII heavy chain from cleavage by activated protein C. In the absence of a phospholipid surface and at high protease levels, we observed similar rates of inactivation of factor VIII and factor VIII complexed with vWF (Fig. 3) and similar rates of proteolysis of free heavy chain and the heavy chain of intact factor VIII (Fig. 4). These results suggested a surface-independent mechanism for cofactor inactivation that was also independent of an activated protein C-light chain interaction. These results suggest that the solution phase inactivation of factor VIII, which is slow, is independent of light chain.

Factor VIII binds tightly and reversibly to phospholipid vesicles (36) and platelets (37) and it is when factor VIII is surface-bound that it is an optimal substrate for activated protein C. Earlier results showed that high concentrations of phospholipid (above 250 μg/ml) dissociated factor VIII from vWF (38), indicating an antagonistic relationship between vWF and phospholipid for factor VIII binding. Thus vWF, by interfering with the factor VIII-phospholipid interaction, could potentially reduce the rate of cofactor inactivation by activated protein C.

We have employed fluorescence energy transfer techniques to assess phospholipid binding of free factor VIII and factor VIII complexed to vWF, SPIII or SPIII-T4. Fluorescence data indicated saturable binding of both free factor VIII and factor VIII complexed with SPIII-T4 to the phospholipid surface. Levels of fluorescence quenching at saturating levels of phospholipid, an indicator of the distance separating donor and acceptor fluorophores, were equivalent for factor VIII and factor VIII/SPIII-T4. Thus occupancy of the vWF-binding site by the 34-kDa SPIII-T4 fragment did not perturb the factor VIII-phospholipid interaction, suggesting that the phospholipid and vWF binding sites within the light chain were not juxtaposed so closely that they were mutually exclusive. However, little if any donor fluorescence quenching was observed for factor VIII in complex with vWF or SPIII, indicating no interaction of these complexes with the phospholipid surface. Thus the effects of vWF, SPIII, and SPIII-T4 on the factor VIII-phospholipid interaction paralleled their effects in the factor VIII-activated protein C system, suggesting that protection from protease resulted from inhibition of cofactor binding to phospholipid.

The protection offered factor VIII by vWF from activated protein C-catalyzed inactivation has been the subject of two recent reports. Rick et al. (21) showed that vWF modestly decreased the level of factor VIII inactivation by about 20–30%. These studies used factor VIII levels of 0.6–0.8 unit/ml and protease concentrations of up to 2.3 μg/ml. Assuming a specific activity for factor VIII of 5 units/μg, this would indicate a (weight) ratio of protease:substrate = 16. Thus, the observed lack of significant protection was likely attributed to the high levels of enzyme used and reflected a phospholipid-independent inactivation of the cofactor. Koedam et al. (20) have observed partial protection of factor VIII (1.2 nm) from the protease (4 nM) by vWF, whereas no protection was observed following thrombin activation of factor VIII, the latter result consistent with protection requiring a physical association between factor VIII and vWF.

Similar to thrombin-activated factor VIII (factor VIIIa), the proteolytically modified factor VIII employed in this study, composed of a thrombin-cleaved light chain bound to a native heavy chain was inactivated by the protease at a rate independent of the presence of vWF. Furthermore, in the absence of vWF, the rate of inactivation of this form of factor VIII was similar to that observed for native factor VIII (see Figs. 1 and 2). Although factor VIII is a good substrate for activated protein C (15), Marlar et al. (12) reported that factor VIIIa was inactivated 30-fold faster by the protease. Since this value was determined from comparison with the inactivation of factor VIII/vWF, the rate enhancement observed for the activated cofactor probably resulted, in part, from its dissociation from vWF. However, preliminary data from our laboratory suggest a significant increase in the rate of factor VIIIa inactivation by the protease. Since thrombin cleaves both factor VIII subunits during activation, one or both cleavage events must result in increasing the reactivity of the activated cofactor for the enzyme. The above result suggests that it is thrombin cleavage of the heavy chain, not light chain, that disposes factor VIIIa to rapid inactivation by activated protein C.

It is not known what function(s) are impaired in activated protein C-cleaved factor VIII. Since the light chain subunit is not covalently altered it is unlikely that inactivation results from altered phospholipid binding. Activated protein C cleavage of factor Va reduces the affinity of the cofactor for both prothrombin and factor Xa (39). By analogy, inactivated factor VIII would show reduced affinity for factor X and factor Xa. Studies on the macromolecular interactions among components of the factor Xase enzyme complex and alterations produced by activated protein C are currently in progress.

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REFERENCES

1. Fass, D. N., Knutson, G. J., and Katzmann, J. (1982) Blood 59, 594–600
2. Andersson, L. O., Forsman, N., Huang, K., Larsen, K., Lundin, A., Pavlu, B., Sandberg, H., Sewerin, K., and Smart, J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2979–2983
3. Fay, P. J., Anderson, M. T., Chavin, S. I., and Marder, V. J. (1986) Biochim. Biophys. Acta 871, 268–278
4. Weiss, H. J., Sussman, I. I., and Hoyer, L. W. (1977) J. Clin. Invest. 60, 390–404
5. Owen, W. G., and Wagner, R. H. (1982) Thromb. Diath. Haemorrh. 27, 502–515
6. Kane, W. H., and Davie, E. W. (1988) Blood 71, 539–555
7. Stenflo, J. (1976) J. Biol. Chem. 251, 355–363
8. Seegers, W., McCoy, L. E., Groben, H. D., Sakuragawa, N., and Agrawal, B. B. L. (1972) Thromb. Res. 1, 443–456
9. Kissel, W., Ericsson, L. H., and Davie, E. W. (1976) Biochemistry 15, 4983–4990
10. Kissel, W., Canfield, W. M., Ericsson, L. H., and Davie, E. W. (1977) Biochemistry 16, 5824–5831
11. Walker, F. J., Sexton, P. W., and Esmon, C. T. (1979) Biochim. Biophys. Acta 571, 333–342
12. Marlar, R. W., Kleiss, A. J., and Griffin, J. H. (1982) Blood 59, 1067–1072
13. Vehar, G. A., and Davie, E. W. (1980) Biochemistry 19, 401–410
14. Fulcher, C. A., Gardiner, J. E., Griffin, J. H. and Zimmerman, T. S. (1984) Blood 63, 486–489
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15. Walker, F. J., Chavin, S. I., and Fay, P. J. (1987) Arch. Biochem. Biophys. 252, 322–328
16. Vehar, G. A., Keyt, B., Eaton, D., Rodriguez, H., O’Brien, D. P., Rotblat, F., Oppermann, H., Keck, R., Wood, W. I., Harkins, R. N., Tuddenham, E. G. D., and Capon, D. J. (1984) Nature 312, 337–342
17. Fay, P. J. (1988) Arch. Biochem. Biophys. 262, 525–531
18. Fay, P. J., and Walker, F. J. (1989) Biochim. Biophys. Acta 994, 142–148
19. Bloom, J. W. (1987) Thromb. Res. 48, 439–448
20. Koedam, J. A., Meijers, J. C. M., Sixma, J. J., and Bouma, B. (1988) J. Clin. Invest. 82, 1236–1243
21. Rick, M. E., Esmon, N. L., and Krizek, D. M. (1990) J. Lab. Clin. Med. 115, 415–421
22. Walker, F. J. (1980) J. Biol. Chem. 255, 5521–5524
23. Fay, P. J., and Smudzin, T. M. (1989) J. Biol. Chem. 264, 14005–14010
24. Fay, P. J., and Smudzin, T. M. (1990) J. Biol. Chem. 265, 6197–6202
25. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
26. Husten, E. J., Esmon, C. T., and Johnson, A. E. (1987) J. Biol. Chem. 262, 12953–12962
27. Skipski, V., Peterson, R., and Barclay, M. (1964) Biochem. J. 90, 374–378
28. Marti, T., Rössle, S. J., Titani, K., and Walsh, K. A. (1987) Biochemistry 26, 8009–8109
29. Bahou, W. F., Ginsburg, D., Sikkink, R., Litwiller, R., and Fass, D. N. (1989) J. Clin. Invest. 84, 56–61
30. Eaton, D., Rodriguez, H., and Vehar, G. A. (1986) Biochemistry 25, 505–512
31. Foster, P. A., Fulcher, C. A., Houghten, R. A., and Zimmerman, T. S. (1988) J. Biol. Chem. 263, 5230–5234
32. Foster, P. A., Fulcher, C. A., Houghten, R. A., and Zimmerman, T. S. (1990) Blood 75, 1999–2004
33. Walker, F. J., Scandella, D., and Fay, P. J. (1990) J. Biol. Chem. 265, 1484–1489
34. Hamer, R. J., Koedam, J. A., Besser-Visser, N. H., and Sixma, J. J. (1987) Eur. J. Biochem. 167, 253–259
35. Lollar, P., Hill-Eubanks, D. C., and Parker, C. G. (1988) J. Biol. Chem. 263, 10451–10455
36. Gilbert, G. E., Furie, B. C., and Furie, B. (1990) J. Biol. Chem. 265, 815–822
37. Nesheim, M. E., Pittman, D. D., Wang, J. H., Slonosky, D., Giles, A. R., and Kaufman, R. J. (1988) J. Biol. Chem. 263, 16467–16470
38. Anderson, L-O., and Brown, J. E. (1981) Biochem. J. 196, 161–167
39. Guinto, E. R., and Esmon, C. T. (1984) J. Biol. Chem. 259, 19886–19892