Human Factor Va_1 and Factor Va_2: Properties in the Procoagulant and Anticoagulant Pathways†

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ABSTRACT: Human plasma factor V is heterogeneous and yields two forms of activated factor V that bind with low (factor Va_1) and high affinity (factor Va_2) to phospholipids. The properties of factor Va_1 and factor Va_2 in the anticoagulant and procoagulant pathways were evaluated by comparing their sensitivity for inactivation by APC and their ability to act as cofactor in prothrombin activation. At low phospholipid concentrations and on membranes containing low amounts of phosphatidylserine (PS), factor Va_1 was inactivated by APC at 15-fold lower rates than factor Va_2, both in the absence and in the presence of protein S. At high phospholipid concentrations and on membranes with more than 15 mol % PS, factor Va_1 and factor Va_2 were inactivated with equal efficiency. Differences between cofactor activities of factor Va_1 and factor Va_2 in prothrombin activation were only observed on membranes with less than 7.5 mol % PS. Due to the different phospholipid requirements of APC-catalyzed factor Va inactivation and of expression of factor Va cofactor activity in prothrombin activation, the thrombin-forming capacity of factor V_1 was 7-fold higher than that of factor V_2 in a reaction system containing factor Xa, prothrombin, APC, protein S, vesicles with a phospholipid composition resembling that of activated platelets, and traces of thrombin to initiate prothrombin activation. This shows that in the process of generation, expression, and down-regulation of factor Va cofactor activity on physiological membranes, the overall procoagulant activity of factor V_1 can considerably exceed that of factor V_2.

Inactivation of factor Va by activated protein C (APC)[1] plays an important role in the down-regulation of thrombin formation (Esmon, 1989). Factor Va loses its cofactor activity in prothrombin activation after APC-catalyzed cleavage in its heavy-chain domain at positions Arg^{506} and Arg^{306} (Kalafatis *et al.*, 1994, 1995; Nicolaes *et al.*, 1995). Factor Va inactivation by APC is stimulated by negatively charged phospholipids (Walker *et al.*, 1979; Bakker *et al.*, 1992) and protein S (Bakker *et al.*, 1992; Walker, 1980; Solymoss *et al.*, 1988). Recently, we reported that protein S promotes factor Va inactivation by specifically accelerating the slow peptide bond cleavage at Arg^{306} (Rosing *et al.*, 1995).

The physiological importance of APC and protein S is demonstrated by the association of venous thrombosis with hereditary deficiencies in protein C (Griffin *et al.*, 1981) or protein S (Schwarz *et al.*, 1984; Comp *et al.*, 1984) and by the occurrence of familial thrombophilia in individuals that have a hereditary defect (replacement of Arg^{506} by Gln) that makes factor Va insensitive to proteolytic inactivation by APC (APC resistance, Dahlbäck *et al.*, 1993; Bertina *et al.*, 1994).

In 1993 we reported (Rosing *et al.*, 1993) that plasma contains two forms of factor V which likely differ in the degree of glycosylation in the carboxy-terminal C2 domain (Ortel *et al.*, 1994a). Activation by thrombin yields two forms of factor Va (factor Va_1 and factor Va_2) with different affinities for procoagulant membrane surfaces and which exhibit different cofactor activities in prothrombin activation. Considering the physiological importance of down-regulation of factor Va activity via the anticoagulant pathway, we have compared the procoagulant and anticoagulant properties of factors Va_1 and Va_2 in order to get insight in their contributions to *in vivo* thrombin formation. We report that under several conditions factors Va_1 and Va_2 have similar procoagulant activity whereas they considerably differ in their sensitivity for APC. In these situations, the overall procoagulant activity of factor V_1 is higher than that of factor V_2.

EXPERIMENTAL PROCEDURES

Materials. Phospholipids were obtained from Avanti Polar Lipids, Pelham, AL. Small unilamellar phospholipid vesicles were prepared as described before (Rosing *et al.*, 1993). S2238, S2366, and I2581 were supplied by Chromogenix, Mölndal, Sweden. Materials used for protein purification were purchased from Pharmacia. Human prothrombin, thrombin, and factor Xa were prepared by earlier described procedures (DiScipio *et al.*, 1977; Pletcher & Nelsestuen, 1982; Bock *et al.*, 1989). Human protein C was prepared and activated as reported by Gruber *et al.* (1989). Human protein S was obtained from Enzyme Research Laboratories Inc. (Swansea, U.K.). Human factor V_1, factor V_2, factor Va_1, and factor Va_2 were purified as described previously (Rosing *et al.*, 1993).

Protein Concentrations. Molar prothrombin, thrombin, and factor Xa concentrations were determined as described

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[1] Abbreviations: APC, activated protein C; BSA, bovine serum albumin; I2581, *N*-dansyl-(*p*-guanidino)phenylalaninepiperidide hydrochloride; S2238, D-Phe-(pipecolyl)-Arg-pNA; S2366, L-pyroGlu-Pro-Arg-pNA; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phosphoserine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine.

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before (Chase & Shaw, 1969; Smith, 1973). APC concentrations were determined with S2366 using kinetic parameters reported by Sala *et al.* (1984). Protein S concentrations were calculated from the A_{280} using an $A_{280}^{1\%}$ of 9.5 and M_r = 70 000 for protein S (DiScipio & Davie, 1979). Factor Va was quantitated as described below.

Factor Va Assay. Cofactor activity of factor Va was quantitated by determining the rate of factor Xa-catalyzed prothrombin activation in reaction mixtures that contained a limiting amount of factor Va, 5 nM factor Xa, 50 μM phospholipid vesicles (DOPS/DOPC, 10/90, mol/mol), and 0.5 μM prothrombin (Tans *et al.*, 1991a). Rates of prothrombin activation were determined with S2238 (Rosing *et al.*, 1980). The molar factor Va concentration in the assay mixture was calculated from the rate of prothrombin activation using a turnover number of 6000 mol of prothrombin activated per minute per mole of factor Xa−Va complex (Tans *et al.*, 1991b).

Inactivation of Factor Va by APC. Factor Va_1 or factor Va_2 was incubated at 37 °C with phospholipid vesicles in the absence or presence of protein S in a reaction mixture containing 25 mM Hepes (pH 7.5), 175 mM NaCl, 3 mM $CaCl_2$, and 5 mg/mL BSA, and its concentration was determined in triplicate to obtain $[\text{factor Va}]_{t=0}$. Factor Va inactivation was started by addition of APC, and the time-dependent loss of factor Va cofactor activity was followed as described in the previous paragraph.

Curve Fitting of Pseudo-First Order Time Courses of Factor Va Inactivation. Time courses of factor Va_1 or factor Va_2 inactivation by APC were determined by monitoring the loss of cofactor activity of factor Va as a function of time. Data reported in the literature (Kalafatis *et al.*, 1994, 1995; Nicolaes *et al.*, 1995; Rosing *et al.*, 1995) demonstrate that the loss of factor Va cofactor activity can proceed via two pathways:

$$\text{factor Va} \xrightarrow{k_{506}} \text{factor Va}_{\text{int}} \xrightarrow{k_{306}} \text{factor Va}_{\text{i}} \quad (1)$$

$$\text{factor Va} \xrightarrow{k'_{306}} \text{Va}_{\text{i}} \quad (2)$$

in which factor Va_{int} is a reaction intermediate with partial cofactor activity, factor Va_i is a form of factor Va that has completely lost its cofactor activity, k_{506} is the second-order rate constant for cleavage at Arg^{506}, k_{306} is the second-order rate constant for cleavage at Arg^{306} in factor Va_{int}, and k'_{306} is the second-order rate constant for cleavage at Arg^{306} in native factor Va. The rate constants k_{506}, k_{306}, and k'_{306} and the cofactor activity of factor Va_{int} were obtained by fitting time courses of factor Va inactivation to eq 3 using nonlinear least-squares regression analysis (Nicolaes *et al.*, 1995; Rosing *et al.*, 1995).

$$[\text{Va}]_t = [\text{Va}]_o e^{-(k_{506}+k'_{306})t} + B[\text{Va}]_o \frac{k_{506} e^{-k_{306}t}}{k_{506} + k'_{306} - k_{306}} \times [1 - e^{-(k_{506}+k'_{306}-k_{306})t}] \quad (3)$$

In this equation, k_{306}, k'_{306}, and k_{506} are the rate constants for peptide bond cleavages defined above and B represents the residual cofactor activity (percent of native factor Va) of the reaction intermediate factor Va_{int}.

Cofactor Activity of Factor Va in Prothrombin Activation. Factor Va_1 or factor Va_2 was incubated with 2.5 pM factor Xa and phospholipid vesicles at 37 °C in 25 mM Hepes (pH 7.5), 175 mM NaCl, 3 mM $CaCl_2$, and 5 mg/mL BSA. After 5 min, prothrombin was added, and 2 and 4 min later, the amount of thrombin formed was quantitated as described before (Rosing *et al.*, 1980).

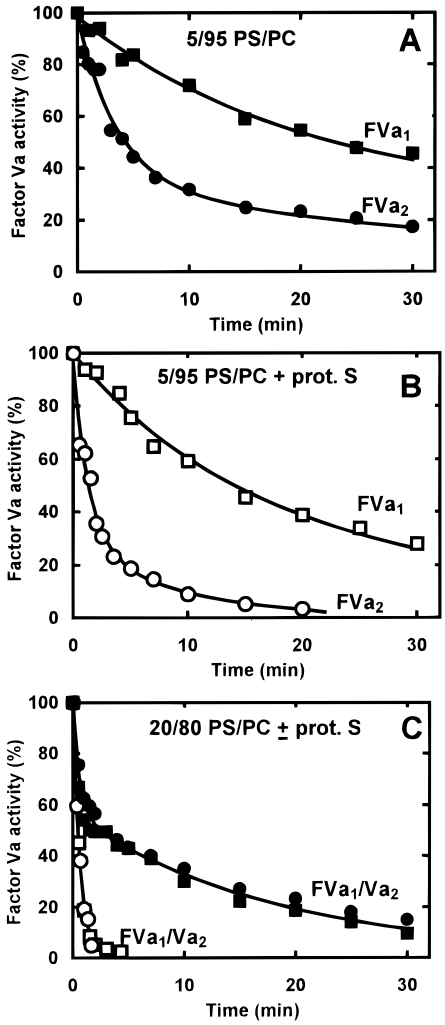

FIGURE 1: Inactivation of factor Va_1 and factor Va_2 by APC. 1.5 nM purified human factor Va_1 (■,□) or factor Va_2 (●,○) was incubated at 37 °C with 1 nM APC (A, B) or 0.12 nM APC (C) without (■,●) or with (□,○) 200 nM protein S in 25 mM Hepes (pH 7.5), 175 mM NaCl, 3 mM $CaCl_2$, 5 mg/mL BSA, and (A,B) 5 μM PS/PC (5/95, mol/mol) or (C) 50 μM DOPS/DOPC (20/80, mol/mol). At the indicated time points, factor Va activity was determined as described under Experimental Procedures. The solid lines represent a best fit of the data to eq 3 given under Experimental Procedures using nonlinear least-squares regression analysis.

RESULTS

Effect of Phospholipid on Inactivation of Factor Va_1 and Factor Va_2 by APC in the Absence and Presence of Protein S. Recently, we have shown (Nicolaes *et al.*, 1995) that inactivation of factor Va by APC proceeds via a biphasic reaction (*cf.* Figure 1) in which fast cleavage at Arg^{506} in the heavy chain of factor Va results in the formation of an intermediate with partial cofactor activity in prothrombin activation that is fully inactivated by APC after slow cleavage at Arg^{306}. We subsequently reported that protein S promotes factor Va inactivation by specifically accelerating cleavage at Arg^{306} (Rosing *et al.*, 1995).

It was also reported (Rosing *et al.*, 1993) that plasma factor V is heterogeneous and yields two forms of factor Va (Va_1 and Va_2) after activation that bind with different affinities to negatively charged membranes.

Table 1: Rate Constants (M^{-1} s^{-1}) for APC-Catalyzed Peptide Bond Cleavages in Factor Va_1 and Factor Va_2[a]

	no protein S		with protein S	
	k_{506}	k_{306}	k_{506}	k_{306}
5 μM PS/PC (5/95)				
factor Va_1	8.3×10^5	3.4×10^4	1.1×10^6	5.7×10^5
factor Va_2	7.0×10^6	5.1×10^5	1.6×10^7	3.3×10^6
50 μM PS/PC (20/80)				
Factor Va_1	2.1×10^8	6.4×10^6	2.2×10^8	1.2×10^8
Factor Va_2	1.2×10^8	6.6×10^6	1.2×10^8	1.4×10^8

[a] The rate constants of APC-catalyzed inactivation of factor Va were obtained by fitting the time courses of factor Va inactivation (Figure 1) using the equation for a biphasic inactivation reaction described under Experimental Procedures.

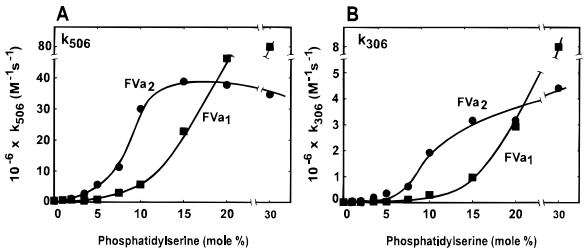

FIGURE 2: Effect of phosphatidylserine on rate constants of APC-catalyzed cleavage at Arg^{506} and Arg^{306} in factor Va_1 and factor Va_2. 1.5 nM purified human factor Va_1 (■) or factor Va_2 (●) was incubated at 37 °C with varying amounts (0.4−60 nM) of APC in 25 mM Hepes (pH 7.5), 175 mM NaCl, 3 mM $CaCl_2$, 5 mg/mL BSA, and 5 μM DOPS/DOPC vesicles with the mole percentage phosphatidylserine (DOPS) indicated in the figure. Rate constants for APC-catalyzed cleavage at Arg^{506} (k_{506}) and Arg^{306} (k_{306}) were obtained from the best fit of the time courses of factor Va inactivation (cf. Figure 1) to eq 3 given under Experimental Procedures using nonlinear least-squares regression analysis. Plotted are k_{506} (A) and k_{306} (B) as a function of the phosphatidylserine content of the membrane.

In Figure 1, it is shown that on membranes with 5 mol % phosphatidylserine, factor Va_1 was inactivated by APC at considerably lower rates than factor Va_2, both in the absence (Figure 1A) and in the presence of protein S (Figure 1B). The rate constants for APC-catalyzed cleavage at Arg^{506} and Arg^{306} (k_{506} and k_{306}) were about 15 times lower for factor Va_1 than for factor Va_2 (Table 1). When the same experiment was performed in the presence of a 10-fold higher concentration of phospholipid vesicles containing 20 mol % phosphatidylserine, inactivation was much faster, and factor Va_1 and factor Va_2 were inactivated by APC at the same rate, both in the absence and in the presence of protein S (Figure 1C).

APC-Catalyzed Inactivation of Factor Va_1 and Factor Va_2 on Membranes Containing Varying Amounts of Phosphatidylserine. Time courses of factor Va_1 and factor Va_2 inactivation by APC (*cf.* Figure 1) were determined on 5 μM phospholipid vesicles containing varying amounts of phosphatidylserine. Since protein S had similar effects on the inactivation of both forms of factor Va, we have performed these experiments in the absence of protein S. The time courses of factor Va inactivation were fitted using an equation for a biphasic inactivation reaction [eq 3; *cf.* Nicolaes *et al.* (1995) and Rosing *et al.* (1995)] which yielded the rate constants, k_{506} and k_{306}, for APC-catalyzed cleavage at Arg^{506} and Arg^{306} in the heavy chain of factor Va. Increasing the amount of phosphatidylserine in the membrane surface resulted in an increase of both k_{506} (Figure 2A) and k_{306} (Figure 2B) for each form of factor Va. This indicates

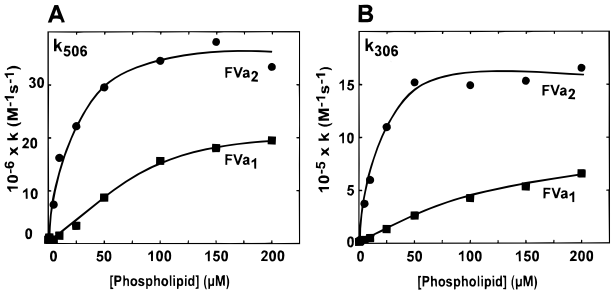

FIGURE 3: Effect of the phospholipid concentration on rate constants of APC-catalyzed cleavage at Arg^{506} and Arg^{306} in factor Va_1 and factor Va_2. 1.5 nM purified human factor Va_1 (■) or factor Va_2 (●) was incubated at 37 °C with varying amounts (2.6−46 nM) of APC in 25 mM Hepes (pH 7.5), 175 mM NaCl, 3 mM $CaCl_2$, 5 mg/mL BSA, and varying amounts of DOPS/DOPC (5/95, mol/mol). Rate constants for APC-catalyzed cleavage at Arg^{506} (k_{506}) and Arg^{306} (k_{306}) were obtained from the best fit of the time courses of factor Va inactivation (cf. Figure 1) to eq 3 given under Experimental Procedures using nonlinear least-squares regression analysis. Plotted are k_{506} (A) and k_{306} (B) as a function of the phospholipid concentration.

that the two cleavages required for full inactivation of factor Va are equally affected by variation of the phosphatidylserine content of the membrane. The data further show that factor Va_1 was inactivated at considerably lower rates than factor Va_2 on membranes with less than 15 mol % phosphatidylserine. At a higher phosphatidylserine content, both forms of factor Va were inactivated with similar rates, and at 30 mol % phosphatidylserine, factor Va_1 was even activated about 2 times faster than factor Va_2.

Effect of Variation of the Phospholipid Concentration on APC-Catalyzed Inactivation of Factor Va_1 and Factor Va_2. The previous experiments have shown that factor Va_1 was inactivated by APC at considerably lower rates than factor Va_2 on 5 μM phospholipid vesicles containing 5 mol % phosphatidylserine. Since this likely reflects differences in the binding affinities of factors Va_1 and Va_2 for membrane surfaces (Rosing *et al.*, 1993), it is expected that the difference in sensitivity of both forms of factor Va for APC will annihilate at higher phospholipid concentrations. The experiment presented in Figure 3 shows that this indeed occurs. At low phospholipid concentrations, the rate constants, k_{506} (Figure 3A) and k_{306} (Figure 3B), for factor Va_1 inactivation were ∼15-fold lower than those for factor Va_2. At increasing phospholipid concentrations, the rates of factor Va inactivation gradually converged, and at 200 μM phospholipid, the difference between the rates of factor Va_1 and Va_2 inactivation was about 2-fold.

Effect of Phospholipid Composition and Concentration on the Cofactor Activity of Factor Va_1 and Factor Va_2 in Prothrombin Activation. In a previous paper (Rosing *et al.*, 1993), we reported that at low concentrations of phospholipid vesicles with a low phosphatidylserine content factor Va_2 exhibits a higher cofactor activity in prothrombin activation than factor Va_1. In Figure 4, it is shown, however, that optimal expression of factor Va cofactor activity in prothrombin activation required much less phospholipid and phosphatidylserine than APC-catalyzed factor Va inactivation (*cf.* Figures 2 and 3). Although at low phospholipid concentrations and on membranes with a low phosphatidylserine content factor Va_2 was a more effective cofactor than factor Va_1, the cofactor activities of both forms of factor Va were optimal and similar at 5 μM phospholipid vesicles containing 10 mol % phosphatidylserine (Figure 4A) and

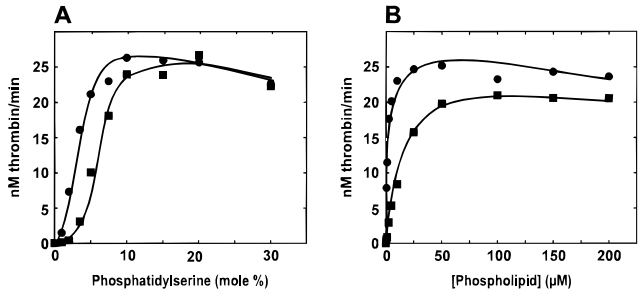

FIGURE 4: Effect of the phospholipid composition and concentration on the cofactor activity of factor Va_1 and factor Va_2 in prothrombin activation. 1.5 nM purified human factor Va_1 (■) or factor Va_2 (●) was incubated at 37 °C with 2.5 pM factor Xa in 25 mM Hepes (pH 7.5), 175 mM NaCl, 3 mM $CaCl_2$, 5 mg/mL BSA, and (A) 5 μM DOPS/DOPC vesicles with varying mole percentages of phosphatidylserine (DOPS) or (B) varying amounts of DOPS/DOPC (5/95, mol/mol) vesicles. After 5 min, prothrombin was added to a final concentration of 1 μM. The rate of prothrombin activation was determined as described under Experimental Procedures. Plotted are the rate of prothrombin activation as a function of the phosphatidylserine content of the membranes (A) or as a function of the phospholipid concentration (B).

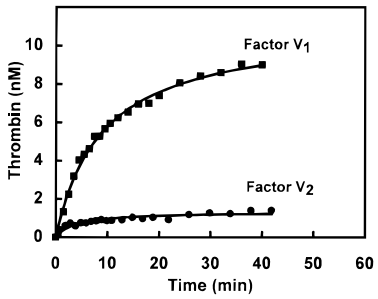

FIGURE 5: Comparison of procoagulant activities of factor V_1 and factor V_2 in a reaction system containing prothrombinase components, APC, and protein S. 1.5 nM purified human factor V_1 (■) or factor V_2 (●) was incubated at 37 °C with 0.1 nM thrombin, 4 pM factor Xa, 1 μM prothrombin, 2 nM APC, 200 nM protein S, and 12.5 μM vesicles containing 10% brain PS, 39% brain PC, 27% brain PE, 19% brain sphingomyelin, and 5% liver PI on a molar basis in 25 mM Hepes (pH 7.5), 127 mM NaCl, 3 mM $CaCl_2$, and 5 mg/mL BSA. The amounts of thrombin formed at the indicated time points were determined as described under Experimental Procedures.

on 50 μM phospholipid vesicles with 5 mol % phosphatidylserine (Figure 4B).

Comparison of the Procoagulant Activities of Factor V_1 and Factor V_2. The experiments presented in the previous paragraphs show that under several conditions (*e.g.*, 20 μM phospholipid vesicles containing 10 mol % phosphatidylserine) the cofactor activities of factor Va_1 and factor Va_2 in prothrombin activation were approximately similar (Figure 4) while their sensitivities for APC were considerably different (Figures 2 and 3). This means that in a system, in which factor V is activated by factor Xa/thrombin and in which the factor Va that is formed is allowed to express its cofactor activity in prothrombin activation in the presence of APC and protein S, factor V_1 will exhibit a higher overall procoagulant activity (*i.e.*, will yield more thrombin) than factor V_2. This was confirmed by the experiment presented in Figure 5. In this experiment, equal amounts of factor V_1 or factor V_2 were activated by factor Xa and thrombin in reaction mixtures containing 12.5 μM vesicles with a phospholipid composition identical to that of activated platelet membranes (Bevers *et al.*, 1983), 1 μM prothrombin, 4 pM factor Xa , 2 nM APC, and 200 nM protein S at physiological ionic strength. In the case of factor V_1, this indeed resulted in the formation of much more thrombin than in the case of factor V_2 (Figure 5). From the fact that the final level of thrombin formed with factor V_2 is ~14% of that of factor V_1, we conclude that the overall procoagulant activity of factor V_1 under these conditions was 7-fold higher than that of factor V_2.

DISCUSSION

Efficient inactivation of factor Va by APC and protein S requires the presence of negatively charged membranes (Walker *et al.*, 1979; Bakker *et al.*, 1992). The membrane presumably acts as a collecting device that binds factor Va, APC, and protein S and that promotes the assembly of an anticoagulant complex, thus accelerating factor Va inactivation by APC. In this paper, we show that one of the plasma forms of factor Va (*i.e.*, factor Va_1) is inactivated by APC at considerably lower rates than the other form of factor Va (factor Va_2). Factor Va_1 and factor Va_2 have different light-chain domains (Rosing *et al.*, 1993; Ortel *et al.*, 1994a,b) and bind with different affinities to negatively charged membranes (Rosing *et al.*, 1993). In agreement with this phenomenon, we observed that differences in the rates of APC-catalyzed inactivation of factors Va_1 and Va_2 are especially observed at low membrane concentrations and on membranes that contain less than 15 mol % of the anionic phospholipid phosphatidylserine. At high phospholipid and phosphatidylserine concentrations, factor Va_1 and factor Va_2 were inactivated at similar rates. Under these conditions, the number of available binding sites for factor Va on the membrane likely exceeds the dissociation constants of the factor Va−membrane complex of both factor Va_1 and factor Va_2. This results in a situation in which almost all factor Va_1 and Va_2 will be bound to the membrane and hence will be inactivated by APC at comparable rates.

Similar differences between APC-catalyzed inactivation of factor Va_1 and factor Va_2 were observed in the presence of the APC cofactor protein S. This indicates that, at the chosen reaction conditions, protein S does not affect the assembly of the factor Va−APC−membrane complex. Our data confirm that protein S specifically accelerates APC-catalyzed factor Va_1 and Va_2 inactivation by increasing the rate constant of cleavage at Arg^{306} (Rosing *et al.*, 1995).

Recently, we reported (Nicolaes *et al.*, 1995; Rosing *et al.*, 1995) a kinetic analysis of APC-catalyzed inactivation of a factor Va preparation that contained both factor Va_1 and factor Va_2. Careful assessment of cofactor activity in prothrombin activation showed that this preparation contained ~10% factor Va_1. Since the experiments reported in these papers were performed on 25 μM PS/PC (10/90, mol/mol) vesicles, factor Va_1 negligibly contributed to factor Va inactivation, and the given rate constants were actually those for factor Va_2.

Factor Va_1 and factor Va_2 also exhibited different cofactor activities in prothrombin activation. However, full expression of factor Va cofactor activity required much less phospholipid and phosphatidylserine than APC-catalyzed factor Va inactivation. This results in a situation in which at intermediate phospholipid conditions (e.g., 20 μM phospholipid with 10 mol % phosphatidylserine) both forms of factor Va have the same cofactor activity in prothrombin activation but are inactivated by APC at considerably different rates (see also below).

When the properties of factor Va_1 and factor Va_2 are compared, it appears that their functional difference dimin-

ishes in the following order: binding affinity for negatively charged membranes (Rosing *et al.*, 1993), sensitivity for APC (this paper), and cofactor activity in prothrombin activation (Rosing *et al.*, 1993; this paper). This can be explained by the fact (1) that binding experiments yield true affinity differences of factor Va_1 and factor Va_2 for membranes independent of the phospholipid concentration, (2) that in APC-catalyzed factor Va inactivation this difference may be (partially) compensated at higher phospholipid concentrations (binding sites $\geq K_d$), and (3) that in prothrombinase measurements the presence of factor Xa (Rosing *et al.*, 1980; Nesheim *et al.*, 1980; Lindhout *et al.*, 1982) and prothrombin (Billy *et al.*, 1996) additionally contributes to the binding of factor Va to the membrane.

Our observation that much less phospholipid and phosphatidylserine are required for full expression of cofactor activity of factor Va in prothrombin activation than for regulation of factor Va cofactor activity by APC may have physiological implications. On membranes with 5−10 mol % phosphatidylserine, which likely simulate the *in vivo* situation (Bevers *et al.*, 1983), factor Va_1 and factor Va_2 have similar cofactor activities in prothrombin activation but exhibit considerable differences in their sensitivity for APC. This means that, in contrast to the expectation, the overall procoagulant activity of factor V_1 will exceed that of factor V_2 in reaction systems containing membranes with physiologic phospholipid compositions, factor V, factor Xa, prothrombin, APC, protein S, and traces of thrombin to initiate factor Va formation. The experiment presented in Figure 5, which was performed on membranes with the phospholipid composition of activated platelets, shows that this is indeed the case. The total amount of thrombin formed in the reaction system in which factor V was activated by factor Xa and thrombin and in which the factor Va formed was simultaneously allowed to express cofactor activity in prothrombin activation and to be down-regulated by APC and protein S was 7-fold higher with factor V_1 than with factor V_2. This shows that, due to differences in sensitivity for APC, the overall procoagulant activity of the form of factor V that has the lowest affinity for membranes (factor V_1) was at least 7 times higher than that of the form of factor V which has the highest membrane affinity (factor V_2).

In a recent paper, Zöller *et al.* (1994) reported that in approximately 5% of the APC-resistant families the Arg^{506}-Gln mutation (factor V_{Leiden}), that is usually associated with APC resistance (Dahlbäck *et al.*, 1993; Bertina *et al.*, 1994), is not found and suggested that apart from this mutation other, as yet unknown, genetic defects may also cause inherited APC resistance. In addition, Zöller *et al.* (1994) decribed that heterozygous carriers of the factor V_{Leiden} mutation with a history of thrombosis had lower APC sensitivity ratios than heterozygous family members without thrombosis. It is conceivable, that individuals with low APC sensitivities in APTT-based assays may have ratios of circulating factor V_1 and factor V_2 that differ from that of normal individuals (normal plasma contains ∼30% factor V_1; Rosing *et al.*, 1993). Based on the observations reported in the present paper, we are currently developing a method to quantitate the amounts of factor V_1 and factor V_2 in plasma. With such a procedure, we will investigate whether there is a relation between non-factor V_{Leiden} related APC resistance, plasma levels of factor V_1 and V_2, and a possible risk for venous thrombosis.

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