Binding of Vascular Anticoagulant α (VACα) to Planar Phospholipid Bilayers*

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Vascular anticoagulant α (VACα, annexin V) is a member of the family of calcium and phospholipid binding proteins, the annexins. The binding properties of VACα to phospholipid bilayers were studied by ellipsometry. Adsorption was calcium-dependent and completely reversible upon calcium depletion. Half-maximal adsorptions to phospholipid bilayers consisting of 100, 20, 5, and 1% dioleoyl-phosphatidylserine (DOPS) supplemented with dioleoyl-phosphatidylcholine (DOPC) were reached at Ca²⁺ concentrations of 0.04, 0.22, 1.5, and 8.6 mM. These surfaces all showed the same maximal adsorption of 0.22 ± 0.01 μg of VACα/cm² (mean ± S.D.). The adsorption to bilayers containing more than 10% DOPS was independent of VACα concentrations in the range of 0.5–100 mM. Dissociation constants for VACα binding to these surfaces were estimated to be below 2 × 10⁻¹⁵ M. No adsorption was observed on pure DOPC bilayers at a Ca²⁺ concentration of 3 mM.

The ability to mediate VACα binding to 20% DOPS/80% DOPC bilayers was highly specific for Ca²⁺. The use of other divalent cations resulted in decreased binding in the order Cd²⁺ > Zn²⁺ > Mn²⁺ > Co²⁺ > Ba²⁺ > Mg²⁺. Zinc ions had a synergistic effect on Ca²⁺-dependent VACα binding. The Ca²⁺ concentration needed for half-maximal binding to cardiolipin, dioleoyl-phosphatidylglycerol, DOPS, phosphatidylinositol, phosphatidic acid, dioleoyl-phosphatidylethanolamine, and sphingomyelin increased in that order. Adsorption was independent of the overall surface charge of the phospholipid membrane.

Blood coagulation consists of a cascade of enzymatic reactions, resulting in the formation of thrombin, which finally cleaves fibrinogen into fibrin. Several procoagulant reactions, like the activation of prothrombin by factors X, and V, are catalyzed by phospholipid surfaces to which the coagulation proteins bind (1–3). Recently we have described the presence in vascular tissue of an anticoagulant protein VACα (formerly VAC), which binds to phospholipids (4, 5). Protein and cDNA sequence information (6) showed VACα to be a member of the Ca²⁺/phospholipid binding protein family including plasmid pHL291 and purified as described before (6). The preparation was more than 99% pure. The only difference detected between recombinant and natural VACα was an unblocked N-terminal alanine in rVACα, resulting in a slightly higher pI (4.9 versus 4.8).

Lipids—Dioleoyl-phosphatidylcholine (DOPC, no. P-1013) and dioleoyl-phosphatidylethanolamine (DOPA, no. P-0510) cardiolipin (no. C-5546), dioleoyl-phosphatidylglycerol (DOPG, no. P-9694), phosphatidylinositol (PI, no. P-0639), dioleoyl-phosphatidic acid (DOPA, no. P-2767), stearylamine (SA, S-6755), and egg yolk sphingomyelin (S-0756) were purchased from Sigma. Purity of DOPC and DOPE was confirmed by thin layer chromatography (26). Dioleoyl-phosphatidylserine (DOPS) was prepared by enzymatic conversion of DOPC (27). ¹⁴C-Labeled DOPS (specific activity = 100,000 dpm/μg) was purchased from Amersham Corp.

Preparation of Phospholipid Bilayers on Silicon Slides—Phospho-

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lipid bilayers were stacked on silicon slides using a Langmuir film balance (Lauda type FW-1) as described by Corsell et al. (28). Hydrophilic silicon slides (Wacker Chemie) treated for 24 h with 30% chromic sulfuric acid and water were stored in 50% ethanol and water. They were thoroughly cleaned with detergent and water before use. The film balance was filled with deionized water (Millipore) and 50 μM CaCl₂. A solution containing 2 g/liter phospholipid in chloroform was spread on this subphase. The DOPS fractions in stacked bilayers were checked with [³¹C]DOPS in mixtures with DOPC. The stacked bilayers were removed from the silicon slide with a scintillation detergent (Du Pont Formul-a-990) and total radioactivity was measured in a Beckmann LS 5801 scintillation counter. Stable bilayers of pure DOPG, stearylamine, and PI could not be obtained with the above-mentioned procedure.

**Binding Measurements by Ellipsometry—** Adsorption of rVACA to phospholipid bilayers was studied using an automated ellipsometer (28, 29). Binding experiments were performed in a hydrophilic cuvette containing a final volume of 5 ml of stirred buffer (0.05 M Tris/HCl, 0.1 M NaCl, pH = 7.5, T = 20 °C). Divalent cations were added step by step as chloride salts.

A continuous infusion of buffer, containing the specific rVACA concentration, was used in order to avoid rVACA depletion of the buffer at concentrations of rVACA < 0.1 μg/ml.

The refractive index n and thickness d (∼10⁻⁶ m) of the adsorbed film were calculated from combined polarizer and analyzer readings (29). The mass (Γ) of the adsorbed protein layer, expressed as micrograms/cm², was calculated from refractive index and thickness by a modified Lorentz-Lorenz equation (Equation 1) (29, 31):

\[ \Gamma = 3d(n² - n₀²)/(n² + 2) - (n²)/2 - (n² - 1)) \]

where \( n₀ \) is the refractive index of the buffer. Values \( r = 0.254 \) ml/g were used for the specific molar refractivity and \( u = 0.71 \) ml/g were used for the specific molar refractivity and \( nb \) is the refractive index of the buffer. Values \( r = 0.254 \) ml/g were used for the specific molar refractivity and \( u = 0.71 \) ml/g were used for the specific molar refractivity and \( nb \) is the refractive index of the buffer.

**Analysis of Calcium Titration Curves—** The maximal adsorption of rVACA to phospholipid bilayers was studied using an automated ellipsometer (Equation 1) (29, 31):

\[ \Gamma = 3d(n² - n₀²)/(n² + 2) - (n²)/2 - (n² - 1)) \]

where \( n₀ \) is the refractive index of the buffer. Values \( r = 0.254 \) ml/g and \( u = 0.71 \) ml/g were used for the specific molar refractivity and the partial specific volume (28) of the protein.

**RESULTS**

**Stacking of Bilayers—** When a mixture of phospholipids is stacked on the slide it may differ from the surface composition on the trough, due to selective adherence. In order to check lipid composition on the silicon slide various mixtures of [¹⁴C]DOPS and DOPC were added to the film balance and subsequently stacked on the slides. The mass of the adsorbed phospholipids was measured by ellipsometry. The composition was determined after subsequent removal of the phospholipid with detergent by measuring the amount of [¹⁴C] label. The mass of the stacked double layers was approximately 0.4 μg/cm², corresponding with 0.65 nm² surface/molecule. Table I shows that the phospholipid composition on the silicon slide was in good agreement with the composition of the mixture spread on the Langmuir trough, which excludes a selective stacking behavior of DOPS.

**Effect of Divalent Cations on the Binding of rVACA to Phospholipids—** rVACA bound calcium dependently to phospholipid membranes consisting of 20% DOPS/80% DOPC. Addition of EDTA produced instantaneous and complete desorption (Fig. 1). The adsorption could be repeated several times by varying the free Ca²⁺ concentration without substantial changes in adsorbed mass (S.D. = 0.001 μg/cm²) or adsorption rate (C.V. = 2%). The subsequent free Ca²⁺ concentrations in the experiments shown in Fig. 1 amounted to 3, 3.5, and 5 mM. Similar repeated adsorptions were observed for a series of decreasing Ca²⁺ concentrations 3, 2.5, and 2 mM (data not shown). This relative independence of rVACA adsorptions on the Ca²⁺ concentration in this range is consistent with data presented in Fig. 2. Irreversible changes in the rVACA molecule or the phospholipid bilayer due to the adsorption or desorption are thus unlikely. The binding of rVACA was also completely reversible if the cuvette was rinsed with Ca²⁺-free buffer.

Fig. 2 shows the effect of calcium and phospholipid composition on rVACA binding. These Ca²⁺ dose response curves are characterized by the Ca²⁺ concentration ([Ca⁺⁺]) at which half-maximal rVACA adsorption was reached. For phospholipid bilayers consisting of 100, 20, 5, and 1% DOPS, ([Ca⁺⁺]) values of 36 μM, 220 μM, 1.5 mM, and 8.6 mM respectively, were measured (Table II). This is in good agreement with the ([Ca⁺⁺]), of 53 μM measured for endonexin II (rVACA) binding to an equimolar mixture of PS/PC vesicles (19). The maximal adsorbed mass (Γ_max) in Table II was determined by extrapolation of the calcium titration curve and was independent of the DOPS fraction in the membrane and amounted to 0.22 ± 0.01 μg/cm² (mean ± S.D.). The shape of the curve (an offset is indicated by t and V, respectively).

**Evaluation of the composition of a phospholipid bilayer stacked on a silicon slide**

<table>
<thead>
<tr>
<th>[¹⁴C]DOPS on film balance</th>
<th>Mass phospholipid ellipsometry</th>
<th>Activity</th>
<th>DOPS fraction measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>mol %</td>
<td>μg/cm²</td>
<td>dpm</td>
<td>%</td>
</tr>
<tr>
<td>2</td>
<td>0.386</td>
<td>460</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>0.409</td>
<td>1,130</td>
<td>4.5</td>
</tr>
<tr>
<td>20</td>
<td>0.401</td>
<td>5,000</td>
<td>20</td>
</tr>
<tr>
<td>100</td>
<td>0.442</td>
<td>27,170</td>
<td>99</td>
</tr>
</tbody>
</table>

**Fig. 1.** Repeated adsorption and desorption of rVACA. Adsorption of rVACA (1 μg/ml) to a 20% DOPS/80% DOPC phospholipid bilayer. Addition of Ca²⁺ (3, 4, and 6 mM) resulting in free Ca²⁺ concentrations of 3, 3.5, and 5 mM, and EDTA (3.5, 4.5, 10 mM) is indicated by t and V, respectively.

**Fig. 2.** The influence of phospholipid composition and Ca²⁺ concentration on the adsorption of rVACA. ●, 100% DOPS; ○, 20% DOPS; □, 5% DOPS; △, 1% DOPS; ●, 100% DOPC. All mixtures were supplemented with DOPC: [rVACA] = 1 μg/ml.
TABLE II
Maximal adsorption and calcium requirement for the binding of VACo to various phospholipid surfaces

Maximal binding (Γmax) of rVACo to the indicated phospholipid surface together with the calcium concentration that results in half maximal binding [Ca²⁺]₁/₂ were calculated from calcium titration curves as described in "Methods." Mean values ± S.D. of at least three separate experiments are presented. ND, not determined.

<table>
<thead>
<tr>
<th>Lipid (mol %/mol %)</th>
<th>Γmax (μg/cm²)</th>
<th>[Ca²⁺]₁/₂ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPS (100)</td>
<td>0.195 ± 0.025</td>
<td>0.036 ± 0.013</td>
</tr>
<tr>
<td>DOPS/DOPC (20/80)</td>
<td>0.222 ± 0.024</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td>DOPS/DOPC (5/95)</td>
<td>0.228 ± 0.004</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>DOPS/DOPC (1/99)</td>
<td>0.234 ± 0.007</td>
<td>8.6 ± 2.5</td>
</tr>
<tr>
<td>Cardiolipin/DOPC (20/80)</td>
<td>0.209 ± 0.011</td>
<td>0.039 ± 0.022</td>
</tr>
<tr>
<td>DOPE/DOPC (20/80)</td>
<td>0.212 ± 0.003</td>
<td>0.155 ± 0.027</td>
</tr>
<tr>
<td>PI/DOPC (20/80)</td>
<td>0.221 ± 0.005</td>
<td>0.47 ± 0.06</td>
</tr>
<tr>
<td>DOPA/DOPC (20/80)</td>
<td>0.207 ± 0.006</td>
<td>0.75 ± 0.26</td>
</tr>
<tr>
<td>DOPE/DOPC (20/80)</td>
<td>0.213 ± 0.003</td>
<td>0.86 ± 0.21</td>
</tr>
<tr>
<td>Sphingomyelin/DOPC (20/80)</td>
<td>0.295 ± 0.014</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>DOPC (100)</td>
<td>ND</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of divalent ions on the adsorption of rVACo. rVACo adsorption to 20% DOPS and 80% DOPC bilayers. [rVACo] = 1 μg/ml.

is strikingly different from values reported by Schlaepfer et al. (19) for endonexin II (=VACo).

The cation requirement was highly specific for Ca²⁺ (Fig. 3). Binding was only marginally promoted by Cd²⁺, Zn²⁺, Mn²⁺, and Co²⁺ and not by Ba²⁺ and Mg²⁺. The binding promoting capacity of the cation was slightly stronger if the ionic radius resembled the radius of the calcium ion (0.99 Å). This trend did not apply for zinc ions which have a relatively small ionic radius (0.74 Å). The addition of zinc resulted in comparatively large adsorption.

Zinc Synergism—High concentrations of zinc ions (1 mM) promote only little rVACo adsorption (Fig. 3), and a concentration of 50 μM did not result in any adsorption. This concentration, however, strongly affected the binding in the presence of Ca²⁺. The value of [Ca²⁺]₁/₂ dropped from 8.6 to 2.7 mM for bilayers with 1% DOPS (Fig. 4). Analysis of the calcium titration curves by the modified method of Hill resulted in similar curves as obtained in the absence of zinc ions. The slope at [Ca²⁺]₁/₂ of 1.6 ± 0.25 (mean ± S.D.) was not different from the slope in the absence of Zn²⁺.

High Affinity of rVACo for phospholipids—Binding of rVACo to a 20% DOPS/80% DOPC and 100% DOPS surfaces were measured at various rVACo concentrations in the presence of 3 mM CaCl₂. As is shown in Fig. 5 the quantity of rVACo bound exceeded 80% of maximal Γ at the lowest concentration tested (0.02 μg of rVACo/ml), indicating a Kd value lower than 5 × 10⁻⁹ M. Further lowering of rVACo concentrations produced practical problems since adsorption times were excessively prolonged. An estimation of the Kd by extrapolation of the binding isotherms indicated a Kd of approximately 10⁻¹⁰ M.

The binding of rVACo to a phospholipid surface was governed by the transport rate limit as revealed by analysis of binding kinetics (not shown). The rate of adsorption, therefore, was directly proportional to rVACo concentration. Remarkably, this turned out to be the case until a surface coverage of 90% of the maximal Γ was reached. This binding behavior clearly contrasts with the kinetics observed for e.g. prothrombin, albumin and fibrinogen, which show already a decreased absorption rate when about 30% of maximal surface coverage (28) is reached. This lack of effect of surface coverage on the rate of adsorption indicates high affinity of rVACo for phospholipids.

Maximal Binding of rVACo—The maximal bound mass (Γmax) of rVACo was 0.22 μg/cm² (Fig. 5). One adsorbed molecule of rVACo will occupy 27.4 nm² and cover 42 molecules of phospholipid. If the molecules are spherical and are adsorbed as a closed monolayer of protein a mean molecular radius of 2.6 nm in the plane of adsorption can be calculated.

Effect of Phospholipid Composition on the Binding of rVACo—Although the binding of rVACo is stimulated by the presence of DOPS (Fig. 2), the rVACo binding is not specific for this phospholipid. Fig. 6 presents the binding of rVACo to cardiolipin, DOPE, PI, DOPA, DOPG, PI, DOPA, DOPG, or sphingomyelin are used. Schlaepfer et al. (19) found high maximal binding of endonexin II (=VACo) at calcium concentrations of 40, 53, and 300 μM for mixtures of 50% phosphatidylethanolamine, 50% PS, and 50% PI supplemented with 50% PC, respectively. We found half-maximal binding to 50% DOPE/50% DOPC at [Ca²⁺]₁/₂ = 500 μM (not shown) which does not correspond to their findings. This could be due to differences in the acyl side chain of the phospholipid.

The difference in binding to DOPC and DOPE is striking. At physiological plasma calcium concentrations no binding of
rVACα to pure DOPC was observed. DOPE is neutral or partially negatively charged at pH = 7.5 and differs from phospholipids (TLC).

The importance of the electrical charge of the surface was further investigated by addition of SA to the phospholipid mixture. Only little adsorption to the positively charged lipid SA was observed (Fig. 6). Rosing et al. (32) showed that SA is distributed homogeneously in vesicles and can neutralize the negative charge of the surface. The procoagulant activities of these surfaces were not changed (32). As can be seen in Fig. 7 rVACα binds with unchanged Γ explaining to layers with a positive net charge containing 20% DOPS and 40% SA, and to charged layers with 20% PI and 20% SA. Only partial inhibition was observed when PI was mixed with a 2-fold molar excess of SA.

DISCUSSION

The amino acid sequence of VACα determines it as a member of the protein family of annexins (6). These proteins have closely related structures and they all show a 4-fold or, in the case of p67-calelectrin (14, 15) 8-fold, amino acid sequence repetition in the core, which is preceded by a variable N-terminal tail. Calpactin I exists as a tetramer of two heavy chains (p36) and two light chains (p11) (33, 34). These proteins are very well preserved in various species, indicating a high evolutionary conservation. The amino acid sequence of VACα determines it as a member of the protein family of annexins (6). These proteins have closely related structures and they all show a 4-fold or, in the case of p67-calelectrin (14, 15) 8-fold, amino acid sequence repetition in the core, which is preceded by a variable N-terminal tail. Calpactin I exists as a tetramer of two heavy chains (p36) and two light chains (p11) (33, 34). These proteins are very well preserved in various species, indicating a high evolutionary conservation.

One important feature is the Ca"²⁺-phospholipid binding ability. Although some in vitro activities of annexins are found, their true physiological functions are not yet known, but they are probably related to membrane-associated processes.

Possibility of Intracellular, Ca"²⁺-dependent Binding of VACα to Phospholipids—If phospholipid binding is important to the intracellular function of VACα, binding has to occur with calcium concentrations ranging from 0.1 to 10 μM. In contrast to e.g. lipocortin I (21) and calpactin I (22, 35), we found no phospholipid binding of rVACα in this range even to membranes consisting of 100% DOPS. With [Ca"²⁺]₀ as an indicator for the affinity of VACα binding, cardiolipin shows the strongest binding. Cardiolipin is an important compound of the mitochondrial inner membrane, but the calcium concentration required for binding to 20% cardiolipin still exceeds normal intracellular levels.

These data do not prove that intracellular VACα binding does not occur. Binding of other annexins can be modulated in various ways. Phosphorylation of the Tyr-21 residue of lipocortin I by epidermal growth factor receptor associated kinase reduces Ca"²⁺ requirement from 22 to 4 μM and as a result may play a part in the regulation of the intracellular function of this protein (21). Monoclonal antibodies directed against the N-terminal tail of the calpactin II interfere with the binding properties of the core (36). Interaction of calpactin I heavy chain with light chain increases affinity toward PS, whereas phosphorylation of the heavy chain results in the opposite effect (35). Limited proteolysis of lipocortin I (21, 37) and calpactin I (34) indicates that the core is important for Ca"²⁺ and phospholipid binding but that the N-terminal tail also affects the Ca"²⁺ requirement.

It is unknown whether one of these modulating mechanisms exists for the VACα molecule. Phosphorylation of the N-terminal tail of VACα has not yet been found. Purified antibodies against the N-terminal tail could not be obtained nor could limited proteolysis of VACα. It is yet unknown whether there exist a protein that associates with VACα. The binding of VACα could, however, be influenced by the addition of small amounts of zinc. Since the zinc concentrations used in this study are within the range of normal serum concentrations (38) this synergistic effect may have physiological significance.

Extracellular Binding of rVACα to Phospholipid Surfaces—Pepinsky et al. (13) demonstrated the extracellular appearance of lipocortin V (VACα) and Grundmann et al. (18) reported the presence of placentin protein 4 (=VACα) in peripheral blood. Since VACα lacks the classical signal sequence required for secretion it is not known whether the extracellular localization is caused by cell death or by a secretion process. The latter possibility would implicate a novel mechanism of secretion, as is believed to be true for certain growth factors (39).

Phospholipids which promote VACα binding most strongly are located in the intracellular leaflet of the plasma membrane (40). Activation of platelets or endothelial cells, or cell damage, will expose these phospholipids to the coagulation factors in plasma. These factors may be activated and thus start the coagulation cascade. The present study shows that VACα concentrations exceeding 10⁻¹⁰ M could cover these structures at the prevailing Ca"²⁺ concentrations and thus prevent this activation. In good agreement with this finding, VACα inhibits the in vitro procoagulant activity of platelets by binding to the platelets' membrane.

High Affinity of VACα for Phospholipid Surfaces—Displacement of coagulation factors at the phospholipid surface by VACα will occur if the Kd values of VACα are considerably lower than the Kd values of the coagulation factors. For prothrombin a Kd of 1.7 × 10⁻⁸ M was reported to 20% DOPS/80% DOPC (31). This Kd is at least three orders of magnitude higher than the Kd of VACα. The Kd of coagulation factor Va is estimated ranging from 10⁻⁴ to 10⁻² M (41, 42). The latter value is possibly in the range of the Kd of VACα. Future

\(^2\) C. P. M. Reutelingsperger, manuscript in preparation.
research will have to clarify whether an interaction between VACα and factor Va can be demonstrated. The displacement of factors Xa and II on large volume vesicles by bovine VACα was already shown (5). The $K_d$ of factor Xa is strongly dependent, upon the presence of factor Va (1, 43). Therefore, the displacement of factor Xa will probably be related to factor Va binding.

For bovine VACα we earlier reported a $K_d$ of $6 \times 10^{-6}$ M (5). This $K_d$ is at least 1 order of magnitude higher than found in the present study for human VACα obtained by recombinant DNA technique, possibly reflecting species differences. Models—For the annexins lipocortin I, calpactin I (heavy chain), and protein II, a phospholipid binding model has been proposed (44) based on the amino acid sequence and a comparison with crystallographic data on calmodulin, which does not belong to the family of annexins. In this model the phosphate group of the phospholipid molecule binds via calcium to negatively charged amino acids in a cleft in the annexin molecule. The amine group, present in e.g. PS and phosphatidylethanolamine, could support this binding by interaction with other negatively charged amino acids. When this model was applied to VACα, it turned out that VACα binding is not specific for the head of the phospholipid. Taylor and Geisow (44) already noticed that the hydrophilic head of the PI molecule is too large to fit in the cleft. The phosphate group of the phospholipid is best available if the size of the head is small. If the half-maximal calcium concentration is a good indicator of VACα binding, this concentration is expected to increase with the increasing size of the head of the phospholipids like in e.g. DOPA, DOPG, and PI. In contrast to this hypothesis, however, VACα needed the lowest Ca$^{2+}$ concentration to bind to DOPG, then to PI, and finally to DOPA. This theory of the Ca$^{2+}$ binding cleft, on the other hand, could explain the specificity of the binding for Ca$^{2+}$ if the size of the cleft determines the size of the binding divalent ion.

The binding of VACα as function of the calcium concentration showed dependence on the lipid used only with regard to the calcium concentration needed for half-maximal occupation. The curves were similar apart from a shift along the log[Ca$^{2+}$] axis. The curves in this study when analyzed according to Hill were non-linear, which contrasts with findings of Schlaepfer et al. (19). This difference could be caused by the large excess of protein binding sites present in the latter study, which limits the influence of protein-protein interactions on the total binding measurements. A comparable effect of protein surface coverage on the binding affinity and kinetics of prothrombin was shown earlier (98). The slope of the calcium titration curve in the presence of zinc was similar to the slope in the absence of zinc. This indicates that zinc does not occupy a calcium binding site. This hypothesis is confirmed by binding studies of a VACα preparation with deleted zinc binding site.2

One molecule of rVACα occupies 27.4 nm$^2$ of surface. More than 2 molecules of rVACα adsorb to each molecule DOPS in bilayers with only 1% DOPS. Selective stacking was ruled out with [14C]DOPS (Table 1) and one may propose three possible mechanisms to explain this observation.

First, VACα molecules may bind to PS and then interact with new VACα molecules. Such polymerization must occur in the plane of adsorption, otherwise it would not stop when the surface is covered with a monolayer VACα. High affinity of VACα to phospholipids is explained in this model because the VACα-VACα complex has multiple binding sites to the phospholipid surface. Polymerization other than intermolecular disulfide dimerization, has never been reported for VACα. Near the surface, however, high protein concentrations and favorable orientation may occur, or the VACα conformation may change due to adsorption.

Second, VACα molecules may bind to PS and then be transferred to PC, i.e. PS could catalyze the binding to PC. Binding to PC is still calcium-dependent and completely reversible upon EDTA addition. No irreversible hydrophobic interaction between VACα and the phospholipid layer is required.

Another possibility is that VACα binds directly to PC. This would explain the observed binding to layers of pure PC (Fig. 6). Binding to PC has not yet been described in the literature.

During preparation of this manuscript Tait et al. (45) reported a $K_d$ of $<1 \times 10^{-10}$ M for PAP-I to vesicles with 20% PS and 80% PC at ionic strength of 0.15 M. This value obtained by using fluorescence quenching measurements is consistent with our findings, however, they report a maximal binding ratio of 1 molecule of PAP-I to 550 molecules of phospholipid at 0.5 M NaCl. This difference in maximal binding could be a result of differences between planar phospholipid bilayers and sharply curved sonicated vesicles. The results of Tait et al. (45) support the postulated anticoagulant mechanism of VACα (5).

General Conclusions—The data of the present study make binding of VACα unlikely under intracellular conditions. However, exposition of the phospholipids located in the intracellular membrane leaflet to extracellular conditions, also important for inducing procoagulant activity, results in VACα binding under physiological conditions. If polymerization occurs, the protein-protein-phospholipid complex will cover the processes of other proteins (e.g. blood coagulation). More research is needed to prove this hypothesis.

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Phospholipid Binding of Vascular Anticoagulant α


