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Immobilized transition metals stimulate contact activation and drive factor XII-mediated coagulation

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Summary

Background—Upon contact with an appropriate surface, factor XII (FXII) undergoes autoactivation or cleavage by kallikrein. Zn^{2+} is known to facilitate binding of FXII and the cofactor, high molecular weight kininogen (HK), to anionic surfaces.

Objectives—To investigate whether transition metals immobilized on liposome surfaces can initiate coagulation via the contact pathway.

Methods & Results—Liposomes containing a metal ion-chelating lipid (DOGS-NTA) were prepared by membrane extrusion (20% DOGS-NTA, 40% phosphatidylcholine, 10% phosphatidylserine, and 30% phosphatidylethanolamine). Ni^{2+} immobilized on such liposomes accelerated clotting in normal, but not FXI- or FXII-deficient plasma. Results were comparable to a commercial aPTT reagent. Charging such liposomes with other transition metals revealed differences in their procoagulant capacity, with Ni^{2+}> Cu^{2+}> Co^{2+} and Zn^{2+}. Plasma could be depleted of FXI, FXII and HK by adsorption with Ni^{2+}-containing beads, resulting in delayed clot times. Consistent with this, FXI, FXII and HK bound to immobilized Ni^{2+} or Cu^{2+} with high affinity as determined by surface plasmon resonance. In the presence of Ni^{2+}-bearing liposomes, K_m and k_{cat} values derived for autoactivation of FXII and prekallikrein, as well as for activation of FXII by kallikrein or prekallikrein by FXIIa, were similar to literature values in the presence of dextran sulfate.

Conclusions—Immobilized Ni^{2+} and Cu^{2+} bind FXII, FXI and HK with high affinity and stimulate activation of the contact pathway, driving FXII-mediated coagulation. Activation of the contact system by immobilized transition metals may have implications during pathogenic infection or in individuals exposed to high levels of pollution.

Keywords

Contact pathway; Factor XII; Factor XI; Metal ions; Coagulation; Thrombin

Introduction

In this study we investigate the role of immobilized transition metal ions in triggering the contact pathway of blood clotting, with possible pathophysiologic consequences.

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Initiation of the contact pathway involves reciprocal proteolytic activation of prekallikrein (PK) and factor XII (FXII) to the active serine proteases, kallikrein and factor XIIa (FXIIa). FXIIa then activates factor XI (FXI) by limited proteolysis. The non-enzymatic cofactor, high molecular weight kininogen (HK), facilitates the initiation of the contact pathway by assembling PK and FXI on the activating surface. The product of these surface-bound reactions, factor XIa (FXIa), propagates the clotting cascade, ultimately leading to thrombin generation and fibrin formation.

Activators of the contact pathway commonly feature an anionic polymer or surface to which FXII and HK bind directly and PK indirectly. *In vitro*, the contact pathway is triggered when plasma is exposed to artificial surfaces like kaolin or glass [1,2], artificial anionic polymers such as dextran sulfate, or liposomes containing high levels of sulfatide or phosphatidylinositol phosphate [3-7]. Recent studies have identified potential physiological activators of this pathway including RNA [8], collagen [9] and platelet polyphosphate [10,11]. Activation of portions of the contact pathway can occur on cell surfaces as part of the kallikrein/kinin system [12].

Certain transition metal ions—most particularly Zn^{2+}—are reported to participate in activation of the contact pathway. Zn^{2+} and Cu^{2+}, but not Co^{2+}, accelerates FXII activation induced by dextran sulfate [13], while sulfatide-mediated activation of FXII and prekallikrein in the presence of HK is markedly accelerated by Zn^{2+} [14]. Zn^{2+} also enhances binding of HK to platelet surfaces [15] and has been reported to induce a conformational change in both HK and FXII [14,16,17], potentially altering their susceptibility to cleavage. The low molecular weight compound, ellagic acid, was originally thought to be the exception to the rule that potent contact activators are anionic polymers or surfaces. However, it was subsequently discovered that only insoluble complexes of ellagic acid are capable of triggering the contact pathway [18]. Furthermore, these insoluble complexes must include transition metal ions in order to exhibit procoagulant activity, with Cu^{2+} being more effective than Zn^{2+}, Co^{2+} or Fe^{3+} (Ni^{2+} was not tested) [18].

In this study we report that transition metals in addition to Zn^{2+} (most particularly, Ni^{2+} and Cu^{2+}) interact strongly with FXI, FXII and HK. Furthermore, immobilizing Ni^{2+}, Cu^{2+}, Co^{2+} or Zn^{2+} on the surface of phospholipid bilayers via metal-chelating lipids potently stimulates FXII-mediated coagulation.

Materials & Methods

Reagents

Reagents were from the following suppliers: citrated pooled normal human plasma (PNP) and factor-deficient plasmas, George King Bio-Medical (Overland Park, KS); HK-deficient plasma, Affinity Biologicals (Ancaster, Ontario, Canada); egg phosphatidylcholine (PC), porcine brain phosphatidylserine (PS), bovine liver phosphatidylethanolamine (PE), 1,2-dioleoyl-sn-glycero-3-[(N(5-amino-1-carboxypentyl) iminodiacetic acid) succinyl] ammonium salt (DOGS-NTA), and the same lipid as the nickel salt (DOGS-NTA-Ni), Avanti Polar Lipids (Alabaster, AL); D-Pro-Phe-Arg-*p*-nitroanilide, Bachem (Torrance, CA); STA-PTT-Automate 5, Diagnostica Stago (Parsippany, NJ); human FXII, α-FXIIa, PK, kallikrein and HK, Enzyme Research Laboratories (South Bend, IN); FXI and corn trypsin inhibitor, Haematologic Technologies (Essex Junction, VT); soybean trypsin inhibitor and polybrene (hexadimethrine bromide), Sigma-Aldrich; dextran sulfate (average MW, 500,000), Fisher; bovine serum albumin (BSA), Calbiochem; Ni Sepharose™ 6 Fast Flow, Amersham Biosciences; and NTA (nitrilotriacetic acid) sensor chips, BIAcore (GE Healthcare, Piscataway, NJ).

Preparation of Liposomes

Liposomes were prepared via membrane extrusion as described [19] but without BSA. Some liposomes were prepared using Bio-Beads [20]; both methods gave comparable results. Unless otherwise stated, "Ni-NTA-liposomes" contained 20% DOGS-NTA-Ni, 40% PC, 10% PS, and 30% PE; and "NTA-liposomes" had the same lipid composition except that DOGS-NTA (no Ni^{2+}) replaced DOGS-NTA-Ni. Lipid compositions are reported as mol% and liposome concentrations as molar concentration of total lipid.

Clotting Assays

Activated partial thromboplastin time (aPTT) clotting assays were performed in an ST4 coagulometer (Diagnostica Stago). Plasma (50 μl) was incubated at 37°C with 50 μl liposomes (in 20 mM HEPES-NaOH pH 7.5, 0.1% BSA, 0.1% NaN_3) for various times, followed by 25 mM $CaCl_2$ (50 μl). Some assays used a commercial aPTT reagent (STA-PTT-Automate 5) according to the manufacturer's instructions. Prothrombin time (PT) clotting assays were performed by incubating plasma (50 μl) with 0.1 ng/ml relipidated tissue factor (50 μl) [20] for 2 min at 37°C before adding 25 mM $CaCl_2$ (50 μl).

Depletion of Contact Factors from Plasma

Ni Sepharose™ 6 Fast Flow beads were washed thrice in H_2O and resuspended in 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.02 % NaN_3. Beads were collected in a Zebra™ spin column (Pierce) and resuspended in 730 μl pooled normal plasma before incubating on a rotator for 30 min at ambient temperature. Plasma was collected by centrifugation.

Chromogenic Activity Assays

FXII activation by kallikrein—Initial rates of FXII activation were quantified at 37°C by incubating FXII (200 nM), kallikrein (200 pM), ± HK (600 nM) and either dextran sulfate (25 μg/ml) or liposomes (100 μM) in 20 mM HEPES-NaOH pH 7.5, 150 mM NaCl, 0.01% NaN_3, 0.1% BSA (HBSA). At various times, 10 μl aliquots were removed to wells of a ultra-low binding polystyrene plate (Corning) containing 90 μl 1.1% Triton X-100, 5.5 μg/ml polybrene, 55.5 μg/ml soybean trypsin inhibitor, 50 mM HEPES-NaOH pH 7.5, 50 mM NaCl, 0.1% BSA. 1 mM D-Pro-Phe-Arg-*p*-nitroanilide (100 μl) was added, and change in A_{405} was monitored in a multiwell spectrophotometer.

FXII autoactivation—FXII autoactivation was analyzed as described [6] with the following changes. Varying concentrations of FXII (0.25 to 2 μM) were incubated with 100 μM liposomes in HBSA. At various times, 10 μl aliquots were mixed with 90 μl 1.1 % Triton X-100, 50 mM HEPES-NaOH pH 7.5, 50 mM NaCl, 0.1% BSA. Chromogenic substrate hydrolysis was quantified as for FXII activation. Data were analyzed as described [6] to determine K_m and k_{cat}.

PK activation by FXIIa—Initial rates of PK activation were quantified at 37°C by incubating PK (50 nM), FXIIa (20 pM), ± HK (600 nM) and either dextran sulfate (25 μg/ml) or liposomes (100 μM) in HBSA. At various times, 10 μl aliquots were mixed with 90 μl 1.1% Triton X-100, 5.5 μg/ml polybrene, 14 μg/ml corn trypsin inhibitor (CTI), 50 mM HEPES-NaOH pH 7.5, 50 mM NaCl, 0.1% BSA. Chromogenic substrate hydrolysis was quantified as for FXII activation.

PK autoactivation—Experiments were performed as described for PK activation by FXIIa, but without FXIIa. Data were analyzed as described [7] to derive apparent second-order rate constants.

Surface Plasmon Resonance

Binding studies were conducted in a BIAcore 3000. NTA sensor chips were treated with 300 mM EDTA pH 8 for 1 min at 20 μl/min to remove bound metal ions, after which the surface was charged with $NiSO_4$ or $CuSO_4$ at 25 μM for 1 min at 20 μl/min, until a response of approximately 50 resonance units (RU) was achieved. An uncharged NTA channel was the control surface. Various concentrations of FXI, FXII, PK or HK in 10 mM HEPES-NaOH pH 7.4, 150 mM NaCl, 0.005% P-20 were flowed over chips at 10 μl/min until a steady-state was reached. A positive control, soluble tissue factor with a hexahistidine tag [21] was included. Maximal (steady-state) RU levels were plotted versus protein concentration, to which the single-site ligand binding equation was fitted. NTA chips were regenerated with 300 mM EDTA (20 μl/min for 1 min).

Results

Liposomes containing Ni^{2+} are strongly procoagulant & stimulate contact activation

We prepared liposomes containing Ni^{2+} bound to the nickel-chelating lipid, NTA-DOGS, along with PC, PS and PE (termed Ni-NTA-liposomes), and also liposomes with the same composition but without bound Ni^{2+} (NTA-liposomes). Recalcifying citrated PNP in the presence of NTA-liposomes resulted in clot times of >300 s, but these clot times were dramatically shortened with Ni-NTA-liposomes (30 s, $P<0.001$). We obtained the shortest clotting times when liposomes contained 20% DOGS-NTA-Ni, 40% PC, 10% PS, and 30% PE (although PE was not an absolute requirement; data not shown). These data suggest that Ni^{2+} bound to NTA-liposomes triggers clotting in plasma, potentially via the contact pathway. The initial stages of contact activation are relatively slow and calcium-independent, so aPTT assays are typically conducted by preincubating plasma with a contact activator for 2 to 10 min before adding $CaCl_2$. Preincubating Ni-NTA-liposomes with citrated normal plasma prior to addition of $CaCl_2$ dramatically shortened the clotting time, with a maximal effect at 4-5 min (Fig. 1). In contrast, no change in clot time was observed in FXI- or FXII-deficient plasma (Fig. 1), consistent with Ni-NTA-liposomes acting via the contact pathway.

Clot times with Ni-NTA-liposomes were compared to a commercial aPTT reagent using a panel of factor-deficient plasmas (Fig. 2). Ni-NTA-liposomes behaved similarly to the commercial aPTT reagent, with prolonged clotting times evident in plasma deficient in the intrinsic pathway (FVIII, FIX, FXI, FXII, PK and HK), and the common pathway (FV, FX). Clot times were not prolonged with FVII-deficient plasma, as FVII functions exclusively via the tissue factor pathway.

Immobilized Cu^{2+}, Co^{2+} and Zn^{2+} also promote contact activation

NTA-liposomes supported a plasma clot time of 423 s, similar to that with liposomes lacking NTA-DOGS altogether (> 300 s), indicating that the NTA moiety itself does not initiate clotting via the contact pathway. NTA-liposomes were then loaded with different metal ions, including Cu^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+}, Cr^{2+}, Ag^{+}, or Mn^{2+}, to examine whether they could trigger contact activation. Ni^{2+}-containing liposomes were the most procoagulant; however, Cu^{2+}, Co^{2+} and Zn^{2+} also significantly shortened clot times of normal plasma (Fig. 3). The other metal salts analyzed ($FeSO_4$, $CdCl_2$, $CrCl_2$, $AgNO_3$ or $MnCl_2$) yielded clot times in excess of 300 s (data not shown), indicating little or procoagulant activity.

Binding of contact pathway proteins to immobilized transition metals

We hypothesized that Ni-NTA-liposomes must bind to one or more proteins in the contact pathway to promote reciprocal activation of FXII and PK. We investigated the ability of

immobilized Ni^{2+} to bind clotting factors by incubating PNP with Ni-Sepharose beads, removing the beads by centrifugation, and analyzing the plasma in PT and aPTT clotting assays. PT clot times were similar in untreated and treated PNP, indicating that the factors involved in the extrinsic and final common pathways of coagulation were not altered by incubation with Ni-Sepharose. In contrast, aPTT clot times were significantly prolonged in Ni-Sepharose-adsorbed PNP (Fig. 4*A*), indicating that one or more of the contact proteins was depleted by exposure to immobilized Ni^{2+}. When Ni-Sepharose-adsorbed PNP was mixed 1:1 with factor-deficient plasmas and analyzed in aPTT assays, significantly prolonged clot times were observed using FXI-, FXII-, or HK-deficient plasmas, but nearly normal clot times were obtained using FVIII-, FIX-, FX- or PK-deficient plasmas (Fig. 4*B*). Thus, Ni-Sepharose depleted the plasma of FXI, FXII, and HK, suggesting that these three proteins directly interact with immobilized Ni^{2+}.

Direct binding of contact pathway proteins to immobilized transition metal ions was investigated using surface plasmon resonance. NTA sensor chips were preloaded with Ni^{2+} or Cu^{2+} and purified FXI, FXII, PK or HK was flowed over the bound metal ions. Binding data were analyzed by plotting the maximal (steady-state) RU signal versus protein concentration, to which the single site ligand binding equation was fitted to derive K_d values [22]. Control experiments were performed in which proteins were flowed over an NTA surface in the absence of Ni^{2+} or Cu^{2+}, and the values in Table 1 represent the difference in this binding. FXI, FXII and HK bound to Ni^{2+} and Cu^{2+} with high affinity (Table 1). PK was unable to bind to Cu^{2+} and showed a reduced capacity to bind to Ni^{2+}, with a K_d value orders of magnitude higher than the other contact proteins. A positive control of soluble tissue factor with hexahistidine tag [21] also bound to immobilized Ni^{2+}, but intriguingly the affinity was approximately 10-fold weaker than the K_d values observed for binding of FXI, FXII or HK. These results indicate that immobilized Ni^{2+} and Cu^{2+} stimulate coagulation by directly interacting with several of the proteins important in the initiation and propagation of the contact pathway.

Immobilized Ni^{2+} supports activation and autoactivation of FXII and PK

The ability of Ni-NTA-liposomes to promote activation of FXII and PK was quantified. Autoactivation of FXII in the presence of Ni-NTA-liposomes was comparable to reported literature values in the presence of dextran sulfate [6] (Table 2). NTA-liposomes were unable to support FXII autoactivation, with rates similar to the no-surface control (Table 2). Activation of FXII by kallikrein in the absence or presence of HK was also investigated with Ni-NTA-liposomes or dextran sulfate as the activating surface. In the presence of HK, FXII activation by kallikrein was strongly promoted by Ni-NTA-liposomes, but not by NTA-liposomes (Table 2). In the absence of HK, NTA-liposomes alone weakly stimulated FXII activation by kallikrein, but this was dramatically accelerated upon incorporation of Ni^{+} (Table 2).

Ni-NTA-liposomes also strongly supported PK autoactivation in the presence of HK, with apparent rate constants comparable to those of dextran sulfate (Table 3). No autoactivation of PK was observed with Ni-NTA-liposomes or dextran sulfate in the absence of HK, consistent with its role in facilitating binding of PK to a surface (Table 3). NTA-liposomes alone also stimulated autoactivation of PK, but at a 10-fold lower rate. This is potentially explained by the anionic nature of the NTA-DOGS lipid in the absence of bound transition metal ions. Ni-NTA-liposomes and dextran sulfate showed comparable ability to stimulate activation of PK by FXIIa both in the absence and presence of HK (Table 3). However, significant kallikrein activity was detected with NTA-liposomes alone and in the no-surface control.

Discussion

Here we show that immobilization of transition metals (particularly Ni^{2+} and Cu^{2+}) on metal-chelating lipids can initiate activation of the contact pathway. Metal-chelating lipids such as DOGS-NTA-Ni have primarily been used to create two-dimensional crystals of oligohistidine-tagged recombinant proteins for electron crystallography [23]. We previously showed that attaching a C-terminal oligohistidine tag to the isolated ectodomain of tissue factor (normally an integral membrane protein) restores full biological activity to this recombinant fragment when it is bound to Ni-NTA-liposomes [21]. During the course of those studies we discovered that even in the absence of tissue factor, liposomes containing DOGS-NTA-Ni elicit substantial procoagulant activity. We now show that Ni-NTA-liposomes are novel and potent activators of the contact pathway, enhancing the four initial reactions: autoactivation of FXII and PK, and reciprocal activation of FXII and PK by kallikrein and FXIIa, respectively. Activation of this pathway drives coagulation via cleavage of FXI, ultimately resulting in thrombin generation and fibrin formation. It could be especially advantageous to incorporate a potent activator of the contact pathway onto the same liposome surface that also supports the downstream (membrane-dependent) clotting reactions.

The degree of PK autoactivation by Ni-NTA-liposomes was comparable to that with dextran sulfate in the presence of its cofactor, HK. No autoactivation of PK was observed in the absence of HK, consistent with the role of this cofactor in anchoring PK to a surface. Likewise, the K_d derived for the interaction of PK with immobilized Ni^{2+} was several orders of magnitude higher than that observed for FXI and FXII and no binding of PK was observed to immobilized Cu^{2+}. In contrast to the results obtained in the FXII activation studies, the NTA moiety itself was capable of stimulating PK autoactivation and FXIIa-mediated PK activation, with immobilized Ni^{2+} only slightly increasing these rates. This suggests that the NTA group, which is anionic in nature, is sufficiently charged to support binding of PK via HK, thereby resulting in autocatalysis or cleavage by FXIIa. Alternatively, zinc ions that are already complexed to HK may interact with the NTA moiety on the lipid surface to support binding and activation of PK. Despite these observations with PK in the activity assays, the NTA group alone was not sufficient to induce clotting in plasma. The enhanced rate of PK activation observed with Ni-NTA-liposomes in the presence of HK most likely reflects binding of this cofactor, as plasma depletion studies and BIAcore experiments indicate that HK binds strongly to immobilized Ni^{2+}.

Bacterial infection is often accompanied by activation of the contact pathway in the host organism. Bacterial strains from *E. coli* and *S. typhimurium* display fibrous proteins, commonly referred to as curli, on their surfaces [24-26]. These strains bind FXII, PK and HK, and support activation of FXII and HK under certain conditions [25]. Addition of these curlieated strains to plasma, *in vitro*, stimulates release of bradykinin [25]. It is feasible that metal ions on the surface of pathogenic organisms could be involved in activation of the contact pathway. Indeed, there are many examples of pathogens using immobilized metal ions in their virulent attacks. Many strains of pathogenic bacteria utilize Cu, Zn superoxide dismutase to protect themselves from oxidative damage by the host [27,28]. These proteins are expressed either in the periplasmic space or the outer membrane of these pathogenic organisms [29,30] and have unique N-terminal extensions that are rich in histidine residues and bind divalent metal ions, including Ni^{2+}, with high affinity. Efficient colonization of the gram-negative pathogenic bacterium *Helicobacter pylori* requires urease and hydrogenase enzymes, both of which utilize Ni^{2+} as a cofactor [31]. Canatoxin, a highly toxic variant form of urease from jack bean, specifically binds both Ni^{2+} and Zn^{2+} [32]. Studies indicate that this toxin possesses activities distinct from the enzyme's capacity to hydrolyze urea, including the ability to induce platelet activation and aggregation [33,34]. The question of

whether Ni^{2+} bound on these proteins is accessible on their surface and capable of activating the contact pathway remains to be determined.

Exposure to particulate matter from air pollution is linked to arterial and venous thrombosis and is a risk factor for cardiovascular disease and stroke [35,36]. A recent report demonstrated that particulate matter can support activation of FXII *in vitro* and that traffic-related ultra-fine particles induce contact pathway-mediated thrombin generation *in vivo* [37]. The mechanisms underlying these effects require definition, but it is interesting to speculate that the immobilization of transition metals on particulate matter, in a manner similar to that defined here for lipids, may stimulate FXIIa-mediated coagulation. Indeed, transition metals such as Ni^{2+} can be found at high concentrations in particulate matter [38-40], and its presence has been found to correlate positively with cardiovascular and respiratory mortality [41,42]. Water-soluble metals such as Zn^{2+} and Ni^{2+} are capable of translocating to extrapulmonary organs, thereby providing a basis for them to elicit biological effects throughout the systemic circulation [43].

We have shown that immobilization of metal ions on liposomes induces potent activation of the contact pathway and drives FXII-mediated coagulation. These results indicate that when blood contacts immobilized transition metals such as Ni^{2+} and Cu^{2+}, perhaps during pathogenic infection or when individuals are exposed to a high degree of pollution, a procoagulant response may occur as a result of activation of the contact pathway. Several studies have now documented a positive association between Ni^{2+} content of particulate matter and onset of thromboembolic disease (reviewed by [42]). The addition of Ni^{2+} to unleaded fuel and its presence as a catalyst in catalytic converters means it is abundant in areas of increased traffic pollution. This highlights the need to study the effects of immobilization of this metal on particulate matter in relation to activation of the contact system. Further work is necessary to define the exact nature of this interaction in terms of the concentrations of Ni^{2+} that are achieved in the circulation and the threshold at which it can induce contact activation and drive thrombin formation.

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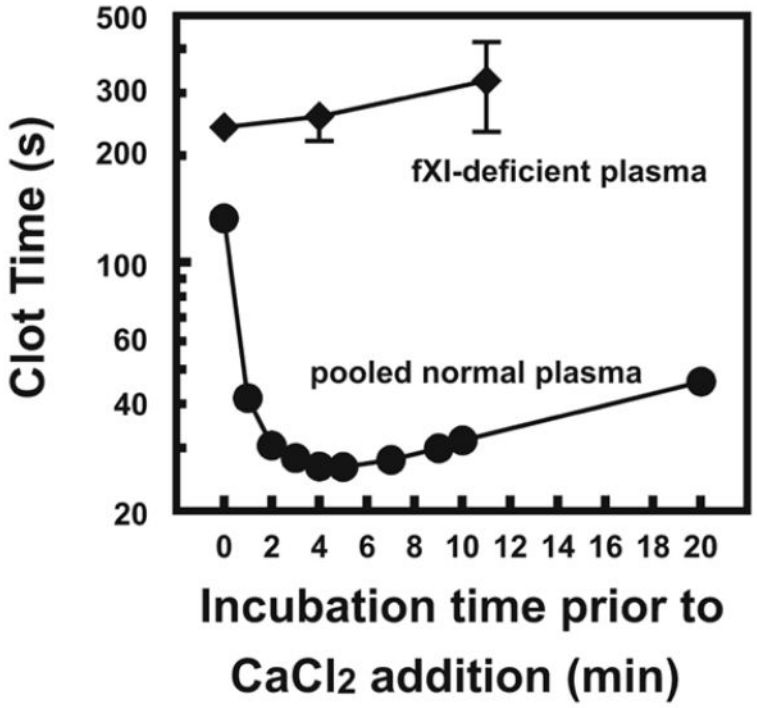

FIGURE 1. Ni-NTA-liposomes are strongly procoagulant

100 μM Ni-NTA-liposomes were incubated with PNP (circles) or FXI-deficient plasma (diamonds) for various times at 37°C before initiation of clotting with 25 mM $CaCl_2$. Clot times of > 500 s were obtained with FXII-deficient plasma and are not shown. Data are mean ± SEM (n = 3).

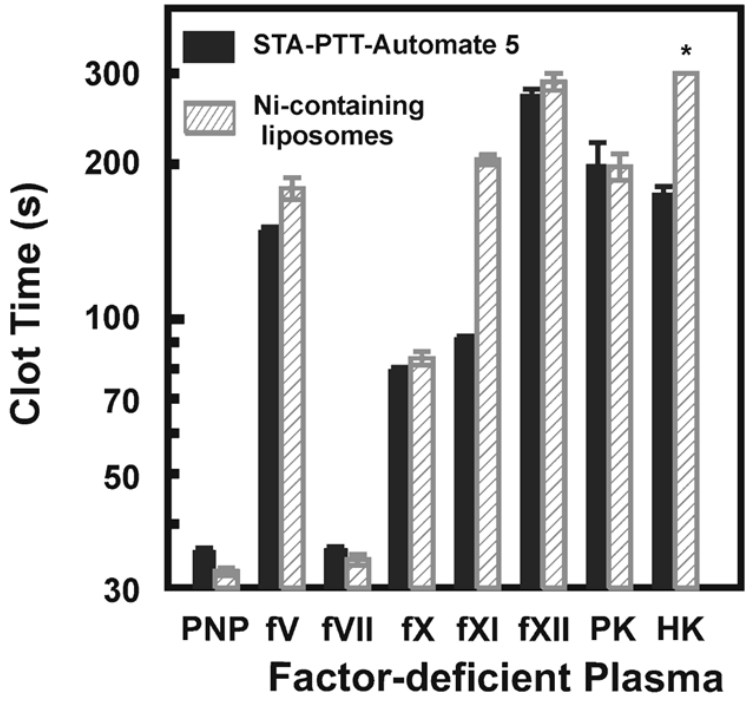

FIGURE 2. Clotting initiated by Ni-NTA-liposomes proceeds via the contact pathway

Ni-NTA-liposomes (50 μM; hatched bars) or the commercial aPTT reagent, STA-PTT-Automate 5, (solid bars) were incubated with PNP or plasma deficient in the indicated clotting factors at 37°C for 180 s, after which clotting was triggered by addition of 25 mM $CaCl_2$. Clot times > 300 s are indicated with an asterisk. Data are mean ± SEM ($n = 3$).

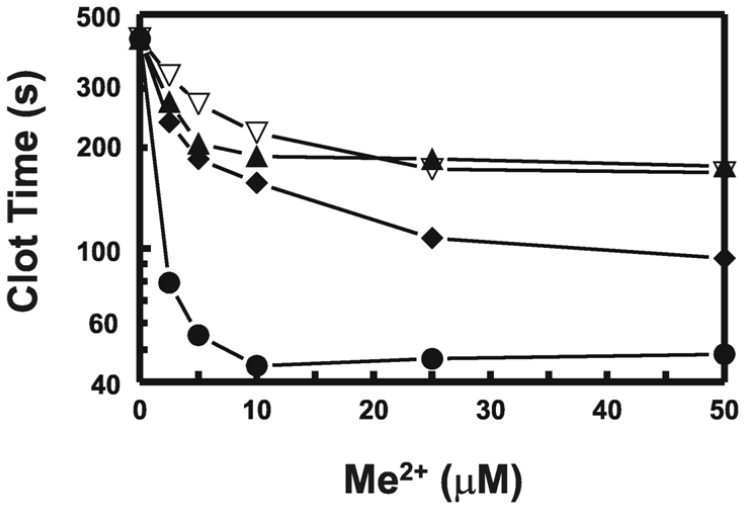

FIGURE 3. Ni^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+} are procoagulant when bound to NTA-liposomes

The indicated concentrations of $NiSO_4$ (closed circles), $CuSO_4$ (closed diamonds), $CoCl_2$ (closed triangles), or $ZnCl_2$ (open, inverted triangles) were incubated with 100 μM NTA-liposomes. aPTT clot times with PNP were then determined as described in Fig. 2. The final DOGS-NTA concentration in these preparations is 15 μM. Assuming half the lipids are exposed to the outer leaflet of the bilayers, then roughly 7.5 μM DOGS-NTA would be available to chelate transition metal ions. Data are mean ± SEM (n = 3).

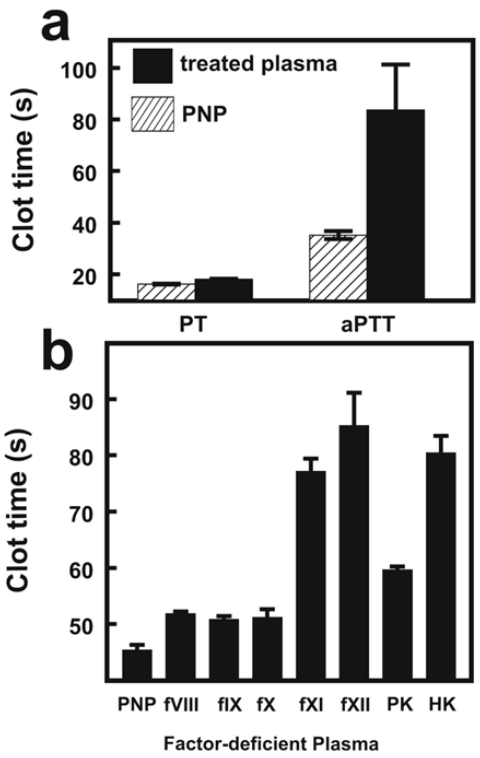

FIGURE 4. Ni-Sepharose beads deplete plasma of specific contact pathway proteins

PNP pre-adsorbed with Ni-Sepharose beads was used in PT and aPTT assays (the latter with the commercial aPTT reagent, STA-PTT-Automate 5). (A) Comparison of clotting times in Ni-Sepharose-treated plasma (solid bars) and untreated plasma (PNP; hatched bars). (B) aPTT assays were performed with 1:1 mixtures of Ni-Sepharose-treated plasma and either PNP or plasma deficient in the indicated clotting factor. Data are mean ± SEM (n = 3).

Table 1

Binding of contact factors to immobilized Ni^{2+} and Cu^{2+}

	Ni^{2+}	Cu^{2+}
	K_d, nM	K_d, nM
FXI	8.03 ± 0.43	1.22 ± 0.13
FXII	38.75 ± 4.36	4.92 ± 0.92
PK	1091.5 ± 344.7	NB[a]
HK	29.82 ± 3.99	5.73 ± 1.28
sTF	198.23 ± 58.4	ND[b]

Ni^{2+} or Cu^{2+} was immobilized on NTA sensor chips and binding affinities were determined using surface plasmon resonance. Data are mean ± SEM (n = 3).

[a] NB, negligible binding.

[b] ND, not determined.

Table 2

Kinetics of FXII activation

	FXII autoactivation (−HK)			FXII activation by kallikrein	
Activating surface	K_m	k_{cat}	k_{cat}/K_m	**initial rate (+HK)**	**initial rate (−HK)**
	μM	min^{-1}	$\times 10^3\ M^{-1}\ s^{-1}$	$nM{\cdot}min^{-1} nM^{-1}$	$nM{\cdot}min^{-1} nM^{-1}$
dextran sulfate	7.5[a]	2.0[a]	4.0[a]	114 ± 14	257 ± 20
Ni-NTA-liposomes	2.6 ± 0.3	1.4 ± 0.2	8.7 ± 1.1	48 ± 5	471 ± 20
NTA-liposomes	NA[b]	NA	NA	NA	119 ± 20
No surface	NA	NA	NA	NA	NA

Data are mean ± SEM (n = 3).

[a]Literature values from Tankersley and Finlayson.

[b]NA, negligible activity.

Table 3

Kinetics of PK activation

	PK autoactivation		PK activation by FXIIa	
Activating surface	**$k_{2,app}$ (+HK)**	**$k_{2,app}$ (−HK)**	**rate (+HK)**	**rate (−HK)**
	$\times 10^5\ M^{-1}s^{-1}$	$\times 10^5\ M^{-1}s^{-1}$	$nM{\cdot}min^{-1}nM^{-1}$	$nM{\cdot}min^{-1}nM^{-1}$
dextran sulfate	2.9 ± 0.25	NA[a]	445 ± 87	374 ± 58
Ni-NTA-liposomes	2.8 ± 0.04	NA	545 ± 43	366 ± 67
NTA-liposomes	1.0 ± 0.10	NA	310 ± 20	348 ± 45
No surface	NA	NA	29 ± 9	126 ± 65

Data are mean ± SEM (n = 3).

[a]NA, negligible activity.