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SCIENCE DIRECT
Biomaterials 27 (2006) 4325–4332
Biomaterials
www.elsevier.com/locate/biomaterials

Autoactivation of blood factor XII at hydrophilic and hydrophobic surfaces ☆

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Received 17 February 2006; accepted 1 April 2006
Available online 27 April 2006

Abstract

Contact activation of blood factor XII (FXII, Hageman factor) in neat-buffer solution is shown not to be specific for anionic hydrophilic procoagulants as proposed by the accepted biochemistry of surface activation. Rather, FXII activation in the presence of plasma proteins leads to *an apparent specificity* for hydrophilic surfaces that is actually due to a relative diminution of the FXII→FXIIa reaction at hydrophobic surfaces. FXII activation in neat-buffer solution was effectively instantaneous upon contact with either hydrophilic (fully water-wettable clean glass) or hydrophobic (poorly water-wettable silanized glass) procoagulant particles, with greater FXIIa yield obtained by activation with hydrophobic procoagulants. In sharp contrast, both activation rate and yield was found to be significantly attenuated at hydrophobic surfaces in the presence of plasma proteins. Putative FXIIa produced by surface activation with both hydrophilic and hydrophobic procoagulants was shown to hydrolyze blood factor XI (FXI) to the activated form FXIa ($FXI \xrightarrow{FXIIa} FXIa$) that causes FXI-deficient plasma to rapidly coagulate.

Keywords: Blood coagulation; FXII; Hageman factor; Contact activation; Autoactivation

1. Introduction

Activation of the blood zymogen factor XII (FXII, Hageman factor) into an active enzyme form, FXIIa, by contact with material surfaces is an important issue in the development of cardiovascular biomaterials. FXIIa is the central member of a self-amplifying activation complex that potentiates the intrinsic pathway of blood plasma coagulation. In turn, activation of the coagulation cascade can lead to formation of thrombus on the surface of biomaterials during the acute phase of blood contact [1]. Hence, this surface-catalyzed FXII→FXIIa reaction (also termed autoactivation in the hematology literature [2]) has been identified as an important cause of poor biomaterial hemocompatibility [3–8]. Indeed, thrombosis remains the significant barrier to development and implementation of advanced in-dwelling blood pumps and ventricular assist devices [9,10].

Contact activation of FXII occurs through a poorly understood interaction of FXII with material surfaces (a.k.a. procoagulants) [2,8] that is a matter of continued investigation in our laboratories [11–16]. It has been long held that FXII activation occurs most efficiently in contact with "anionic" [17–19] or "hydrophilic" (water wettable) procoagulants [12,14–16], presumably due to a chemically specific binding event between FXII and surface-resident negative charges. Technical reasons supporting this contention are many, perhaps originating in the routine hematology-laboratory observation that plasma clots relatively quickly in glass (hydrophilic) tubes but slowly in plastic (relatively hydrophobic) tubes [20]. Specificity for anionic hydrophilic surfaces thus explains that little-or-no FXII activation is observed in plasma brought in contact with hydrophobic procoagulants because these surfaces

☆A Contribution from the Hematology at Biomaterial Interfaces Research Group, The Pennsylvania State University.

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doi:10.1016/j.biomaterials.2006.04.001

bear no anionic functional groups. Indeed, our previous work studying coagulation time of animal [14] and human [12] plasma in contact with procoagulants bearing different oxidized surface chemistries concluded that procoagulants exhibit a surface-energy-dependent "catalytic potential" to induce FXII→FXIIa [11,12,14], in basic accord with the traditional view of FXII activation.

However, results of continued investigation into the details of FXII activation disclosed herein show that FXII is activated *in neat-buffer solution* with nearly equal efficiency by contact with either hydrophobic or hydrophilic surfaces, discounting the specific-binding-event explanation for FXII activation. Moreover, we find that both FXII activation rate and FXIIa yield *in whole plasma* brought in contact with hydrophobic procoagulants is significantly lower than at an equal surface area of hydrophilic procoagulant. Thus, FXII activation is neither specific to anionic hydrophilic procoagulants nor do these surfaces exhibit enhanced catalytic potential. Rather, FXII activation by hydrophobic surfaces immersed in whole plasma is significantly slower than at hydrophilic procoagulants, giving rise to the *appearance of a surface-energy-dependent catalytic potential*.

2. Methods and materials

2.1. Plasma and coagulation proteins

Human platelet poor plasma (PPP) was prepared from outdated (within 2 days of expiration) lots obtained from the M.S. Hershey Medical Center Blood Bank. This work was performed with a single lot of pooled plasma aliquoted into 15 mL polypropylene tubes (Falcon, Becton Dickinson) and frozen at −20 °C until use. We observe consistent results with plasma prepared and stored in this manner over about 1 yr of experimentation. Factor XII depleted plasma (12 dPPP) with a dysfunctional contact activation system was used as received from George King Biomedical, Inc., Overland Park, KS (a negative control). 12dPPP was optionally reconstituted with variable FXII concentrations (R12dPPP) with the same FXII used to prepare neat-buffer solutions (below). Experience has shown that different lots of plasma yield quantitatively different but qualitatively similar results. Coagulation time of recalcified 12RdPPP supplemented with 30 μg/mL FXII (physiologic concentration [21,22]) exceeds 40 min, demonstrating that FXII was not contaminated with measurable quantities of FXIIa. FXI-deficient plasma (11dPPP) was used as received from Haematologic Technologies, Inc. (Essex Junction, VT). Coagulation time of recalcified 11dPPP supplemented with 5 μg/mL FXI (physiologic concentration [23]; R11dPPP) exceeds 40 min, demonstrating that FXI was not contaminated with measurable quantities of FXIa.

Human FXII and FXIIa were used as received from Haematologic Technologies, Inc. (Essex Junction, VT) and Enzyme Research Laboratories (South Bend, IN), respectively. FXIIa activity was specified by the vendor in traditional units of plasma equivalent units per mL (PEU/mL) [24]. FXI was obtained from Enzyme Research Laboratories and FXIa from Haematologic Technologies. FXIa was specified by the vendor in units of μg/mL but the absolute activity of the preparation was unknown. Neat FXII, FXIIa and FXI solutions were prepared in phosphate buffer (Sigma).

2.2. Plasma coagulation-time assay for FXIIa and FXIa

The basic protocol for coagulation-time assay applied in this work has been described in detail elsewhere [12,14–16]. Plasma coagulation time (CT) was used as the traditional hematology method [24] to quantify FXIIa and FXIa in solution through "FXIIa titration" and "FXIa titration" calibration curves, respectively, that relate CT to [FXIIa] and [FXIa]; where square brackets denote concentrations expressed in PEU/mL or μg/mL, respectively. FXIIa and FXIa titrations were carried out by equilibrating 0.5 mL of thawed plasma in 15 × 75 mm polystyrene tubes (VWR) and mixing with increasing volumes of FXIIa or FXIa solution in PBS and diluting with sufficient additional PBS to bring total volume to 900 μL. Coagulation was induced by recalcification with 100 μL of 0.1 M $CaCl_2$ and contents were mixed on a slowly turning hematology mixer (Roto-shake Genie, Scientific Industries, Inc.). Coagulation time CT after recalcification was noted by a distinct change in fluid-like rheology to gel formation, allowing determination of the end point of the coagulation process to within 10 s or so [14]. CT was observed to be exquisitely sensitive to FXIIa and FXIa (see Figs. 1 and 2 Panel C).

2.3. Procoagulant surfaces

Test procoagulants applied in this work were 425–600 μm diameter glass beads (Sigma–Aldrich) in either cleaned or silanized form. Each contact-activation experiment used 100 mg glass beads corresponding to approximately 500 mm^2 surface area based on a nominal diameter of 500 μm. Clean-glass procoagulants were prepared by 3× serial rinses in 18 MΩ water (obtained from a Millipore Simplicity unit), 2-propanol, and chloroform (reagent grade, VWR) followed by air-plasma treatment of a single layer of washed-glass beads held in a 15 mm Pyrex glass petri dish (10 min at 100 W plasma; Herrick, Whippany NY). Clean-glass beads were silanized by 1.5 h. reaction with 5% v/v octadecyltrichlorosilane (OTS; United Chemical Technologies, Inc.) in chloroform. Silanized beads were 3× rinsed in chloroform and dried in a vacuum oven at 110 °C for 24 h. Contact angles of glass-slide witness samples measured by Wilhelmy balance tensiometry (CDCA-100, Camtel Ltd.) typically yielded advancing/receding contact angles 0°/0° and 110°/90° for clean and OTS-treated glass, respectively, with no more than about 10° variation among batches. Contact angles cannot be read directly on glass beads but optical microscopy of the shape of the liquid meniscus of beads partly immersed in water on a microscope slide qualitatively confirmed that the treated beads were not different from the witness samples.

Test procoagulants prepared as above are referred to herein as either "hydrophilic" (clean glass) or "hydrophobic" (silanized glass) for simplicity, in full recognition of the vagaries associated with this terminology [25] for most biomedical applications [26]; as well as dispute in the literature regarding existence of hydrophilic/hydrophobic contrast in the biological response to materials [20,25]. However, in the case of plasma coagulation in vitro, contact activation has been shown to scale very sharply with procoagulant surface energy [11,12,14], leading to a crisp discrimination between surface types that further justifies our choice of nomenclature.

2.4. Surface activation of activation of FXII in neat-buffer solution and plasma

The basic experimental strategy to compare FXII activation in neat-buffer solution (no proteins other than FXII and activation products therefrom) and human-blood plasma is illustrated in Fig. 1. Test solutions were either purified FXII in PBS buffer at physiological concentration 30 μg/mL [21,22] or citrated platelet-poor plasma (Panel A). Two sources of plasma were used; one prepared from blood collected from normal donors (PPP) and the other was a commercial Factor XII-deficient plasma (12dPPP) with a dysfunctional contact-activation system that served as a negative control. In the latter instance, 12dPPP was optionally reconstituted to normal physiological FXII concentration (R12dPPP) with the same FXII used to prepare neat-buffer solutions. Putative FXIIa produced by 30 min contact with hydrophilic or hydrophobic procoagulant surfaces was either released into solution (free) or remained associated with activating surfaces (bound). Supernate containing free FXIIa was separated from surface-bound FXIIa by decantation (Fig. 1, Panel B).