Biomaterials 31 (2010) 1068–1079

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Surface-energy dependent contact activation of blood factor XII☆

Avantika Golas [b], Purnendu Parhi [a], Ziad O. Dimachkie [b], Christopher A. Siedlecki [b,c], Erwin A. Vogler [a,b,*]

[a] Department of Materials Science and Engineering, The Pennsylvania State University, University Park, PA 16802, USA
[b] Department of Bioengineering, The Pennsylvania State University, University Park, PA 16802, USA
[c] Department of Surgery, Pennsylvania State University College of Medicine, Biomedical Engineering Institute Hershey, PA 17033, USA

ARTICLE INFO

Article history:
Received 30 September 2009
Accepted 15 October 2009
Available online 4 November 2009

Keywords:
Autoactivation
FXII
Coagulation

ABSTRACT

Contact activation of blood factor XII (FXII, Hageman factor) in neat-buffer solution exhibits a parabolic profile when scaled as a function of silanized-glass-particle activator surface energy (measured as advancing water adhesion tension $\tau_a^o = \gamma_{lv}^o \cos\theta$ in dyne/cm, where γ_{lv}^o is water interfacial tension in dyne/cm and θ is the advancing contact angle). Nearly equal activation is observed at the extremes of activator water-wetting properties $-36 < \tau_a^o < 72$ dyne/cm ($0^\circ \le \theta < 120^\circ$), falling sharply through a broad minimum within the $20 < \tau_a^o < 40$ dyne/cm ($55^\circ < \theta < 75^\circ$) range over which activation yield (putatively FXIIa) rises just above detection limits. Activation is very rapid upon contact with all activators tested and did not significantly vary over 30 min of continuous FXII-procoagulant contact. Results suggest that materials falling within the $20 < \tau_a^o < 40$ dyne/cm surface-energy range should exhibit minimal activation of blood-plasma coagulation through the intrinsic pathway. Surface chemistries falling within this range are, however, a perplexingly difficult target for surface engineering because of the critical balance that must be struck between hydrophobicity and hydrophilicity. Results are interpreted within the context of blood plasma coagulation and the role of water and proteins at procoagulant surfaces.

1. Introduction

Contact activation of blood-plasma coagulation through the intrinsic pathway has been implicated as an important cause of the poor hemocompatibility of cardiovascular biomaterials (see ref. [1] for a recent review and citations therein that generally support this section). The initiating step of the intrinsic pathway is widely thought to be surface contact activation of the blood zymogen FXII (Hageman factor) into an active-enzyme form FXIIa; a reaction that is sometimes referred to as autoactivation ($\text{FXII} \xrightarrow{\text{surface}} \text{FXIIa}$) in the hematology literature. Surface biophysics of autoactivation is poorly understood, as is the biochemistry that leads to enzyme-like specificity upon surface contact. It is proposed that autoactivation involves cleavage of the Arg_{353}-Val_{354} bond in FXII, generating a two-chain molecule (αFXIIa) comprised of a heavy chain (353 residues) and a light chain (243 residues) held together by a disulfide bond [2]. Other FXII fragments with procoagulant activity such as FXIIf have also been identified [3,4] (see further Section 4.4). As a consequence, amidolytic activity arising from contact activation of FXII may, in fact, be due to one-or-more enzymes.

Experimental evidence clearly shows that plasma coagulation is most efficiently activated by contact with anionic hydrophilic materials such as kaolin clay, glass, or generally any material with an oxidized surface chemistry. In fact, studies carried out with this latter group of activator materials (a.k.a. procoagulants) aimed at elucidating quantitative relationships among surface chemistry, energy, and the propensity to activate the *intact* plasma coagulation cascade show that contact activation is all-but-specific for fully-water-wettable surfaces (water contact angle $\theta = 0^\circ$). Activation by less-water-wettable surfaces ($\theta > 0^\circ$) is sharply reduced, leading to one of the most profound examples of hydrophilic/hydrophobic contrast in the biological response to materials. These observations, together with an evolving understanding of autoactivation biochemistry, naturally led to the conclusion that contact activation of FXII was also specific for (or at least caused most efficiently by contact with) hydrophilic surfaces.

However, recent work demonstrates that autoactivation is not specific for anionic-hydrophilic activators. Instead, it is found that hydrophobic and hydrophilic activators have nearly equal autoactivation properties in neat-buffer solutions of FXII, as measured by the solution yield of enzymes with coagulation activity (putatively FXIIa). The observed contact-activation specificity for hydrophilic procoagulants *in plasma* actually arises from an "adsorption-dilution" effect that causes hydrophobic procoagulants only to *appear* nearly inert. Adsorption of a plethora of blood proteins to hydrophobic surfaces

☆ A Contribution from the Hematology at Biomaterial Interfaces Research Group.

* Corresponding author. Departments of Materials Science and Engineering, The Pennsylvania State University, University Park, PA 16802, USA.
E-mail address: eav3@psu.edu (E.A. Vogler).

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doi:10.1016/j.biomaterials.2009.10.039

significantly blocks adsorption of FXII, leading to a sharp reduction in FXII/surface contacts with commensurate reduction in autoactivation rate and yield. Nevertheless, hydrophobic procoagulants retain small-but-measurable plasma-activation properties, explaining the sluggish coagulation of blood in contact with hydrophobic materials such as plastic tubes [5].

Herein we report measurement of FXII autoactivation in neat-buffer solution with procoagulants sampling the full range of observable water-wettability. Results corroborate our previous findings that FXII activation is not specific to anionic-hydrophilic procoagulants and resolves a surface-energy dependence that leads to a distinct minimum in FXII activation. These findings suggest a route to the surface engineering of cardiovascular biomaterials with improved hemocompatibility.

2. Methods and materials

2.1. Preparation and characterization of particulate activators (procoagulants)

Test procoagulants applied in this work were 425–600 μm diameter glass particles (Sigma Aldrich) in either cleaned or silanized form. The nominal specific area used in this work was 5×10^{-3} m^2/g (based on 512.5 μm mean diameter and 168 μg/particle). The actual surface area measured by the Brunauer–Emmett–Teller (BET) method was 0.25 ± 0.09 m^2/g (Micromeritics ASAP 2000 using liquid nitrogen as the probe gas) was 50× larger than the nominal value, reflecting the large dispersity in particle size and porosity. Nominal surface area was used herein for the sake of consistency with earlier work [6–9]. Choice of surface area had no impact on conclusions drawn herein because qualitative and quantitative comparisons were made among silanized procoagulants prepared from the same lot of glass particles with the same specific area.

Silanes applied in this work (used as received from Gelest Inc., Morrisville, PA) were octadecyltricholorosilane (OTS), 3-aminopropyltriethoxysilane (APTES), *n*-propyltriethoxysilane (PTES), and vinyltriethoxysilane (VTES). OTS-treated glass particles were optionally coated in a 0.2% solution of 1,1-pentadecafluorooctylmethacrylate in tricholorotrifloroethane (“Nyebar”, Nye Lubricants, Fairhaven, MA) by immersion followed by air drying. Glass coverslips (Fisher $22 \times 30 \times 0.1$ mm) were carried through all surface treatments with particles, providing a substrate suitable for measuring buffer contact angles. Glass particles and coverslips were first cleaned and activated by 30 min immersion in heated piranha solution (30% H_2O_2 in concentrated H_2SO_4 at approximately 80 °C) followed by 3× sequential washes in each of 18 MΩ de-ionized water and ethanol. Piranha-solution-oxidized glass was air dried and subsequently oxidized by air–plasma treatment of a single layer of particles (or coverslips) held in a 15 mm Pyrex glass petri dish (10 min at 100 W plasma; Herrick, Whippany, NY) directly before use in silanization procedures or adsorption measurements. Glass surfaces treated in this manner were found to be fully-water-wettable and designated “clean glass”. Clean-glass particles and coverslip samples were silanized by 1.5 h reaction with 5% v/v OTS in chloroform. Silanized samples were 3× rinsed with chloroform before curing in a vacuum oven at 110 °C for 12 h. Cured OTS samples were optionally immersed in Nyebar solution for 10 min and air dried to produce surface slightly more hydrophobic than rendered by OTS treatment alone (see Table 1). APTES silanizations were carried out in 95:5 v/v ethanol–water solutions by 20 min reaction of clean glass with 5% APTES solution that had been hydrolyzed overnight in the ethanol–water mixture before use. APTES-treated glass was washed with ethanol and cured overnight in a vacuum oven at 110 °C. Silanization with PTES and VTES followed the APTES procedure except that 90:10 ethanol–water containing 0.5% glacial acetic acid was used.

Buffer (PBS, see below) contact angles on glass cover-slip witness samples were measured using an automated contact-angle goniometer (First Ten Angstroms Inc., Portsmouth, VA) that employed the captive-drop method of measuring advancing/receding contact angles (see refs. [10,11] for a comparison of goniometric techniques and discussion of experimental errors). Contact angles could not be read directly on glass particles but optical microscopy of the shape of the liquid meniscus of particles partly immersed in water on a microscope slide qualitatively confirmed that treated particles were not different from coverslip witness samples. Water wettability of the *n*th surface type was expressed as (advancing) water adhesion tension τ_n^o (where $\tau_n^o \equiv \gamma_{lv}^o \cos\theta_n$ is the product of pure-buffer interfacial tension $\gamma_{lv}^o = 71.97$ dyne/cm at 25 °C and cosine of the advancing contact angle θ_n observed on the *n*th sample). Results obtained for six surface treatments are collected in column 3 of Table 1. Surface chemistry of glass-particle surfaces was assayed using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) collected on a Bruker IFS-66/S spectrometer using a diffuse-reflectance accessory (Collector II, Thermo Spectra-Tech). The instrument detector constantly purged with CO_2-free dry air while 400 scans at 6 cm^{-1} resolution were acquired for both samples and references. Silica particles were loaded into a macro sampling cup without dilution and leveled with a spatula prior to analysis. Spectra were normalized to arbitrary reflection units relative to an Infragold (Labsphere) reference sample.

Physical mixtures of hydrophilic ($n = 6$) and hydrophobic ($n = 1$) procoagulant surfaces were prepared by gravimetry incrementally sampling 0.0–1.0 weight fractions. Particles were weighed directly into test tubes mixed in the dry state by shaking. Particles were further mixed in suspension with PBS before use in activation experiments described below. A hypothetical “equivalent wetting” parameter was calculated using a linear combination combining rule as described in Appendix B.

Table 1
Water wettability of treated glass-particle procoagulants.

Procoagulant designation # (n)	Procoagulant surface chemistry	Advancing contact angle range (degrees)
1	Nyebar	109–118
2	OTS	106–109
3	PTES	93–97
4	VTES	85–90
5	APTES	42–58
6	Clean glass	0

Notes: 425–600 μm diameter glass particles in either cleaned or silanized/coated form. See Section 2.1 for details.

2.2. Plasma and coagulation proteins

Citrated 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 two different lots (I and II) of pooled plasma aliquoted into 15 ml polypropylene tubes (Falcon, Becton Dickinson) and frozen at −20 °C until use. We have observed consistent results with plasma prepared and stored in this manner over about one year of experimentation. Experience has shown that different lots of plasma yield quantitatively different but qualitatively similar results. Correction for differences between plasma lots I and II was made by measuring a FXIIa-titration calibration curve for each lot (see below).

Two lots of human FXII were used; Lot 1 from Haematologic Technologies, Inc (Essex Junction, VT) and Lot 2 from Enzyme Research Laboratories (South Bend, IN) respectively. The single source of FXIIa was Enzyme Research Laboratories (South Bend, IN). All zymogens and enzymes were used as received. Activity of both FXII and FXIIa was specified by the vendor in traditional units of plasma-equivalent-units-per-mL (PEU/mL) [12]. FXIIa concentrations in mg/mL were converted from PEU/mL for activation yield calculations (Appendix A) using the vendor-supplied conversion factor of 73.5 PEU/mg. Neat-buffer solutions of FXII and FXIIa solutions were prepared in phosphate buffer saline (PBS; Sigma; 0.14 M NaCl, 3 mM KCL prepared from powder in 18 MΩ de-ionized water at pH = 7.2).

2.3. FXIIa assay

Plasma coagulation time (CT) was used as the traditional hematology method to quantify FXIIa by appealing to a “FXIIa titration” calibration curve that related CT to FXIIa concentration (expressed either in PEU/mL or mg/mL) [12,13]. Protocol for the FXIIa assay applied in this work has been described in detail elsewhere [7–9,14,15]. Briefly, FXIIa titrations were carried out by equilibrating 500 μL of thawed PPP in 15 × 75 mm polystyrene tubes (VWR), mixing with increasing volumes of FXIIa 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 tube contents were mixed on a slowly-turning hematology mixer (Roto-shake Genie, Scientific Industries, Inc.). CT after recalcification was noted by a distinct change in fluid-like rheology to gel formation, allowing determination of a coagulation endpoint to within 10 s or so [14–16]. So-measured CT was observed to be exquisitely sensitive to FXIIa with a minimum quantifiable concentration of $\sim 5 \times 10^{-4}$ PEU/mL. FXIIa titration curves in PPP were linear when scaled on a logarithmic concentration axis. Calibration curves were fit to $y = m\log_{10} x + c$ by linear-least-squares regression; where x is FXIIa concentration in PEU/mL and both m and c are adjustable parameters ($m = -10.96 \pm 0.44$, $c = 6.76 \pm 0.80$ with $R^2 = 94.6\%$ from triplicate determinations using plasma Lot I and $m = -8.68 \pm 0.25$, $c = 5.31 \pm 0.48$ with $R^2 = 96.9\%$ from triplicate determinations using plasma Lot II). Strictly speaking, this FXIIa assay measured net plasma-coagulation-inducing activity of the product(s) resulting from contact activation of FXII in buffer solution, reported herein in terms of FXIIa activity deduced from the calibration curve. As such, the coagulation assay did not discriminate among various activated fragments of FXII that might be produced by contact activation of FXII in neat-buffer solution (see further Sections 1 and 4.4). Possible presence of activated forms other than FXIIa did not alter the basic conclusion of this work that FXII activation in neat-buffer solution was a strong function of procoagulant surface energy.

2.4. Autoactivation of FXII in neat-buffer solution

FXII activation in PBS solution was carried out as previously described [8,9]. Briefly, test solutions of FXII in PBS were prepared at nominal physiological concentration