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Surface modification with polyethylene glycol–corn trypsin inhibitor conjugate to inhibit the contact factor pathway on blood-contacting surfaces

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ABSTRACT

Blood contacting surfaces bind plasma proteins and trigger coagulation by activating factor XII (FXII). The objective of this work was to develop blood contacting surfaces having the dual properties of protein resistance and inhibition of coagulation. Gold was used as a model substrate because it is amenable to facile modification using gold–thiol chemistry and to detailed surface characterization. The gold was modified with both polyethylene glycol (PEG) and corn trypsin inhibitor (CTI), a potent and specific inhibitor of activated FXII (FXIIa). Two methods of surface modification were developed; sequential and direct. In the sequential method PEG was first chemisorbed on gold; CTI was then attached to the PEG. In the direct method a conjugate of PEG and CTI was first prepared; the conjugate was then immobilized on gold. The surfaces were characterized by water contact angle and XPS. Biointeractions with the modified surfaces were assessed by measuring fibrinogen adsorption from buffer and plasma and by immunoblot analysis of eluted proteins after plasma exposure. Inhibition of FXIIa, autoactivation of FXII, and clotting times of plasma in contact with the surfaces were also measured. Both the sequential and direct surfaces showed reduced protein adsorption, increased FXIIa inhibition and longer clotting times compared with controls. Although the CTI density was lower on surfaces prepared using the sequential method, surfaces so prepared exhibited greater CTI activity than those generated by the direct method. It is concluded that the activity of immobilized PEG–CTI depends on the method of attachment and that immobilized CTI may be useful in rendering biomaterials more blood compatible.

1. Introduction

Current blood contacting devices have limitations because they trigger thrombus formation [1], resulting in device failure. Protein adsorption is the first event when blood contacts the material in such devices; this leads to platelet adhesion and thrombin generation [1–3]. Approaches to this problem have focused on reducing protein adsorption by modifying surfaces with hydrophilic polymers such as polyethylene glycol [4–7]. Attachment of bioactive molecules has also been investigated as a means of promoting the specific adsorption of proteins that may endow the surface with anticoagulant or antithrombotic properties [8,9]. Heparin, for example, can attenuate clot formation by binding antithrombin and enhancing its capacity to inhibit thrombin, factor Xa, and other clotting enzymes [10]. In more recent studies combinations of PEG and bioactive molecules have been used to modify surfaces [11,12].

Most of the bioactive molecules used for surface modification to prevent clot formation target the later stages of the coagulation cascade [9,12–14]. In this work we focus on the first step of the coagulation cascade, i.e. activation of FXII to FXIIa. Corn trypsin inhibitor (CTI) was used as the surface modifier to bind and inhibit FXIIa. CTI, an ~12,500 Da protein derived from corn kernels, is a known specific and potent inhibitor of FXIIa [15,16]. In other work in our laboratories commercial catheter surfaces were modified with CTI [17] and showed improved blood compatibility compared with unmodified catheters. In the work reported here two different methods of surface modification using both PEG to inhibit nonspecific protein adsorption and CTI to inhibit coagulation were developed. Gold was used as a model substrate because it is amenable to facile modification via gold–thiol chemistry and to detailed surface characterization.

It should be recognized that CTI inhibits FXIIa by formation of a 1:1 complex, and thus on a surface, inhibition will be limited by the amount of CTI present. Heparin, which inhibits activation of later steps in the coagulation pathway, does so by a catalytic mechanism and is thus in principle "renewable". It is important to point out, however, that by blocking upstream at the root cause of

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surface-induced activation of clotting, as anticipated for CTI, a stoichiometric inhibitor may be effective.

2. Materials and methods

2.1. Materials

(2,2′-Dithiobisethylhepa(ethylene glycolic) acid)-N-succinimidyl ester (PEG-NHS ester disulfide), MW = 1109 (Fig. 1a, A), was from Polypure AS (Oslo, Norway). Maleimide PEG amine (MW = 2000, Fig. 1a, B) was from JenKem Technology USA Inc. (Allen, TX). α-Hydroxy-ω-thio-polyethylene glycol (MW = 1100, Fig. 1a, D) was from Polymer Source Inc. (Montreal, Canada). Hydrogen peroxide, ammonium hydroxide, ethanol, 2-iminothiolane-HCl (Traut's reagent) and ethylene diamine tetraacetic acid (EDTA) were from Sigma–Aldrich (Oakville, Canada). CTI was from Haematologic Technologies Inc. (Essex Junction, VT). FXII and FXIIa were from Enzyme Research Laboratories (South Bend, IN). Pefachrome XIIa, a FXIIa-directed chromogenic substrate, was from Pentapharm Ltd. (Basel, Switzerland). PD-10 desalting columns were from GE Healthcare (Baie d'Urfe, Quebec, Canada). Silicon wafers sputter-coated with titanium and then gold (1000 Å), from Silicon Valley Microelectronics Inc. (Santa Clara, CA), were cut into 0.5 × 0.5 cm squares. The plasma used in the experiments was from blood collected from a minimum of 10 healthy volunteers.

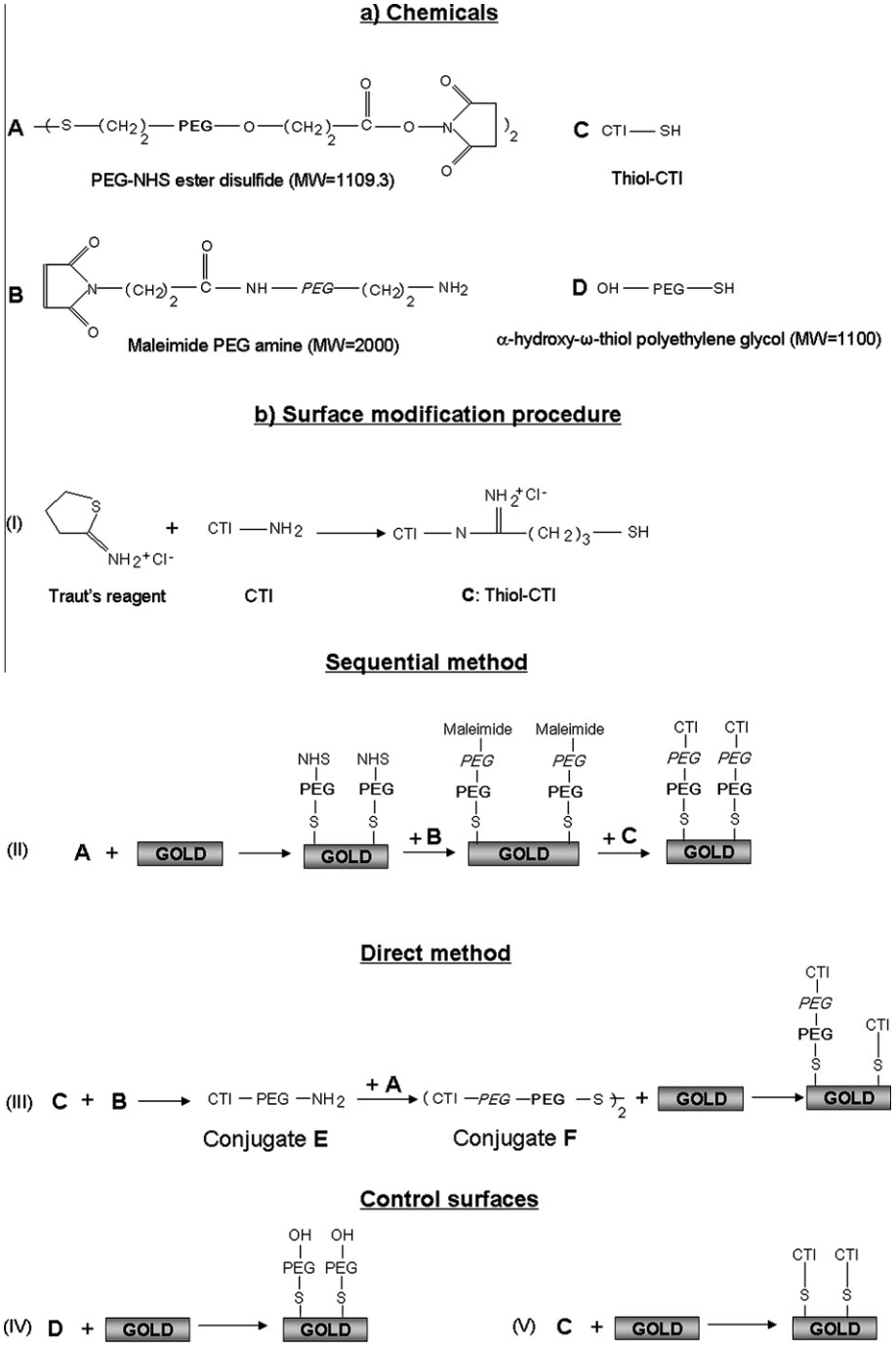

Fig. 1. (a) Reagents used in the surface modification reactions and (b) surface modification reactions. (I) Introducing thiol groups to CTI; (II) sequential method; (III) direct method; (IV) Au–PEG–OH control surface; (V) Au–CTI control surface.