Available online at www.sciencedirect.com
ScienceDirect
Analytical Biochemistry 362 (2007) 98–107
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Probing the interaction of coagulation factors with phospholipid vesicle surfaces by surface plasma resonance

Angelica Wikström [a], Johanna Deinum [b,*]

[a] Department of Applied Physics, Chalmers University of Technology, S-431 90 Göteborg, Sweden
[b] Department of Molecular Pharmacology, AstraZeneca R&D, S-431 83 Mölndal, Sweden

Received 3 October 2006
Available online 29 December 2006

Abstract

The dynamics of the binding of human coagulation factor Xa (FXa) and prothrombin to small unilamellar vesicles (25% phosphatidylserine, 75% phosphatidylcholine) were compared and quantified by Biacore, using two immobilization techniques. The vesicles were either tagged with different molar ratios of cholesterol–DNA and attached on Au chips or fused directly on L1 chips. The diameter in solution was 145 nm, but the more DNA tags/vesicle the more compressed the immobilized vesicles became; with 30 DNA tags the calculated thickness was 88 nm and with 1 DNA tag it was 138 nm. In both models the affinity for the vesicles was higher for the activated coagulation factors than for the corresponding zymogens. FXa and prothrombin had the highest affinities. The affinity was dependent on the vesicle preparation since overall K_D values were up to 10 times lower for N_2-dried than for vacuum-dried phospholipids, although with apparently fewer binding sites. However, compression of the vesicles had no effect on the K_D. In contrast, the rate constants were dependent on the number of DNA tags; thus deformation of the vesicles was observed. The k_a and k_d for FXa were similar for vesicles attached with 30 DNA tags or fused on the L1 chip but higher with fewer tags and approximately 10 times higher if attached with 1 tag. Thus for controlled kinetic studies immobilized DNA-tagged vesicles should be used.

Keywords: Surface plasma resonance; Protein–lipid interaction; Lipid vesicle; Kinetics; Coagulation factors; Gla domain

In a calcium-dependent interaction critical for blood coagulation, vitamin-K-dependent blood coagulation proteins bind to cell membranes containing phosphatidylserine via γ-carboxyglutamic-acid-rich (Gla) [1] domains [1] including prothrombin, factors X, IX, and VII, and proteins C, S, and Z. Gla-domain-mediated protein–membrane interaction in the presence of Ca^{2+} is required for generation of thrombin, the terminal enzyme in the coagulation cascade, on a physiologic time scale [2,3]. Binding of coagulation factors containing a Gla domain is greatly enhanced after platelet activation due to the exposure of negatively charged phosphatidylserine on the cell surface [4]. The Gla domains of all these proteins contain homologous amino-terminal sequences of about 45 amino acids that include 9–13 Gla residues [2]. Specificity for binding to anionic phospholipids in vivo may aid in regulation of coagulation and localization of the blood clotting response to sites of cell damage.

Calcium binding to the Gla domain is known to be crucial for the induction of a conformation change in the protein domain that mediates membrane binding [5]. Despite much study, structural features of the protein membrane contact remain speculative and somewhat contradictory. While the requirement for calcium provides a popular concept of calcium-bridging between protein carboxyl groups and anionic phospholipids, no direct evidence exists to support this model. The X-ray crystal structure shows seven calcium ions

* Corresponding author. Fax: +46 31 7762737.
E-mail address: Johanna.Deinum@astrazeneca.com (J. Deinum).

[1] *Abbreviations used:* BSA, bovine serum albumin; Chaps, 3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonate;EDTA, ethylenediaminetetraacetic acid; Gla, γ-carboxyglutamic acid; PC, 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine; PS, 1,2-dioleoyl-sn-glycero-3-[phospho-L-serine]; PSPC, mixture of the two phospholipids PS and PC; QCM-D, quartz crystal microbalance with dissipation monitoring; RU, resonance units; SPR, surface plasmon resonance; FX, factor X; FV, factor V; LPS, lipopolysaccharide.

0003-2697/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2006.12.009

bound to prothrombin fragment 1 in a relatively planar configuration with a cluster of hydrophobic amino acid side chains projecting outward [6]. Protein–membrane association has been suggested to occur via penetration of these hydrophobic side chains into the hydrocarbon region of the membrane to a depth that allows simultaneous calcium chelation by the protein and the membrane phospholipids. In some cases, prothrombin association with the phospholipid monolayer occurred without increase in monolayer surface pressure [7]. These properties suggest interaction with only the phospholipid head group.

The reported K_D values for the binding of coagulation factors to negatively charged membranes under similar conditions by different methods vary by as much as several orders of magnitude. Membrane binding of vitamin-K-dependent coagulation factors has previously been studied by ellipsometry, light scattering, and fluorescence polarization [1,8–19]. It has been found in a variety of studies that, to support the generation of thrombin in vitro from prothrombin by the complex of factor Xa (FXa) with factor Va (FVa) on a phospholipid surface [14,20,21] or in the thrombin generation assay in plasma [22], small unilamellar vesicles made from a mixture of 20–25% phosphatidylserine (PS) and 80–75% phosphatidylcholine (PC) can be used as a membrane substitute. We therefore decided to investigate membrane binding by surface plasmon resonance (SPR) using well-defined vesicles without the need for labeling the proteins, allowing determination of the kinetics of membrane interaction in real time. SPR has been used to study protein/membrane interactions for all sorts of proteins with different types of liposomal surfaces [23]. However, SPR has seldom been used to characterize membrane interaction with the blood coagulation factors, except for bovine coagulation factor X. Erb et al. [8] studied the calcium dependency of the interaction of FX with unilamellar liposomes on a Biacore L1 sensor chip. Recently, it has been shown that the activity of the complex of tissue factor with coagulation factor VIIa (FVIIa) fused in vesicles depends on the degree of incorporation [24].

To be able to study membrane interaction with SPR the lipid vesicles need to be captured on a sensor chip. Moreover, a stable surface is needed, thereby allowing multiple regenerations. The commercial L1 chip from Biacore contains a dextran matrix modified with hydrophobic residues to capture lipid vesicles through hydrophobic interaction or insertion of the residues in the membrane [25]. Intact vesicles have also been immobilized to SPR chips using small amounts of biotinylated lipids that were bound to avidin covalently attached to the surface [26–28], vesicles containing lipopolysaccharide (LPS) via LPS-specific antibodies [29], and DNA-modified vesicles hybridizing to complementary DNA strands attached to the sensor surface. We used a bivalent cholesterol-tagged DNA coupling, first studied by Pfeiffer and Höök [30], giving the possibility to control the number of molecules attaching the vesicle to the surface. The bivalent cholesterol-based coupling was accomplished by hybridization between a 15-mer and a 30-mer DNA strand, modified with cholesterol in the 3′ and 5′ end, respectively (Fig. 1A).

In this model study we have used PSPC vesicles (25:75), id est consisting of 25% phosphatidylserine and 75% phosphatidylcholine, extruded through a 100-nm filter. We have furthermore characterized the properties of the vesicles on the L1 chip and DNA-tagged vesicles on the Au chip and compared and quantified the binding of the human coagulation factors, prothrombin and thrombin and the factors VII, VIIa, IX, IXa, X, and Xa in a calcium-containing buffer with 0.1% BSA. It was found that the preparation method of the lipids was crucial for the affinity of the factors for the vesicles. Affinity constants for vesicles prepared from lipids dried with N_2 differed by a factor of at least 10 from those dried under vacuum. Furthermore, to obtain reproducible surface properties it was important to carefully follow the cleaning procedure of the Au chips. Moreover, although the degree of deformation of the immobilized vesicles was found to have no effect on the equilibrium constants, the rate constants were strongly dependent on the curvature. Since the vesicles bound with many DNA tags and the vesicles on the L1 chip were apparently compressed, the best model for vesicles in solution was obtained when only one or two DNA tags per vesicle was used.

Materials and methods

Materials

Phospholipid vesicles. The phospholipids were 1,2-dioleoyl-*sn*-glycero-3-[phospho-L-serine] (PS) from Avanti

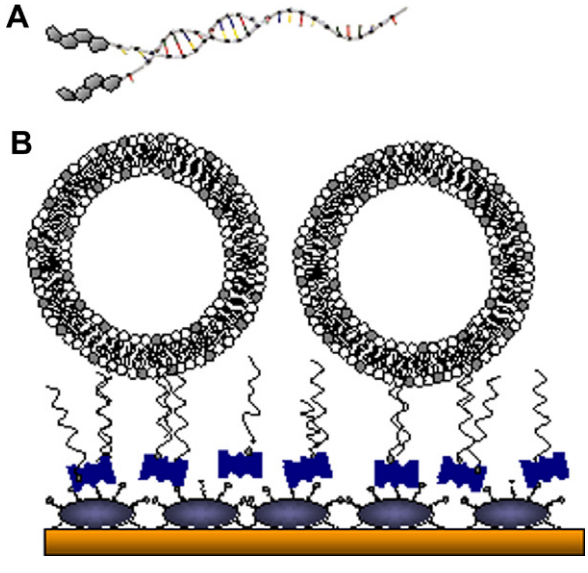

Fig. 1. Schematic illustration of the DNA-tagged vesicle immobilization. (A) DNA tag: Bivalent cholesterol-modified DNA strand accomplished by hybridization between a 15-mer and a 30-mer, modified with cholesterol in the 3′ and 5′end, respectively. (B) Vesicle adsorption: A gold surface prepared by (i) adsorption of biotinylated BSA, (ii) binding of neutravidin, (iii) binding of biotinylated DNA, and (iv) anchoring of vesicles preincubated with defined number of DNA tags.