26 Antiprothrombin Antibodies

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Introduction

In clinical practice, anticardiolipin antibody (aCL) detected by ELISA and lupus anticoagulant (LA) detected by clotting assays have been standardized for the diagnosis of the antiphospholipid syndrome (APS). However, it is now established that antiphospholipid antibodies (aPL) are a large and heterogeneous family of immunoglobulins, which, despite their name, do not seem to bind phospholipids, but are directed to plasma proteins with affinity for anionic surfaces (i.e., phospholipids).

Amongst these phospholipid binding proteins, the best studied is β_2-glycoprotein I (β_2-GPI), which bears the cryptic epitope(s) for aCL binding. These epitopes are exposed when β_2-GPI binds to negatively charged phospholipids such as cardiolipin, or irradiated plastic plates [1]. Several studies have highlighted the significance of anti–β_2-GPI antibodies (anti–β_2-GPI) as an alternative ELISA with higher specificity than the conventional aCL ELISA [2–4].

Prothrombin, another phospholipid binding protein, was first proposed as a possible co-factor for LA by Loeliger in 1959 [5]. In subsequent years, the interest regarding this protein has increased and several groups have investigated the significance of antiprothrombin antibodies.

Prothrombin

Prothrombin is a single chain glycoprotein, synthetized in the liver, recognized very early as the prime contributor to the blood coagulation process. This protein is found in plasma at a concentration of around 2.5 μmol/L. Its gene spans 21 kilobase pairs [6] on chromosome 11. Mature prothrombin contains 579 amino acid residues with a molecular mass of 72 kD, including 3 carbohydrate chains and 10 γ-carboxyglutamic acid residues [7].

The tenase complex, entailing factor Xa and factor V, calcium and phospholipids as co-factors, physiologically activates prothrombin. Once negatively charged, phospholipids bind prothrombin and tenase converts prothrombin to thrombin, which triggers fibrinogen polymerization into fibrin [8]. In addition, thrombin binds thrombomodulin on the surface of endothelial cells and activates protein C, which then exerts its anticoagulant activity by digesting factor V and depriving in this way the tenase complex from its most important co-factor. Due

to this negative feedback pathway prothrombin/thrombin behaves as an "indirect" anticoagulant.

The prothrombin molecule consists of 3 functional domains: Gla, kringle, and catalytic. During its liver byosinthesis, prothrombin undergoes γ carboxylation (10 glutamic acid residues in proximity of its amino terminus). These γ carboxyglutamic residues, known as Gla domains and located on fragment 1 of the prothrombin molecule, are essential for the calcium dependency of phospholipid binding to prothrombin, necessary for the conversion of prothrombin to biologically active α-thrombin. Two kringle domains follow this region and are involved in pro(thrombin) binding to fibrin [6]. Tenase selectively hydrolyses 2 peptide bonds on the catalytic domain of the prothrombin molecule. Cleavage at Arg273–Thr274 results in the liberation of prothrombin fragment 1+2 (residues 1–273) and prothrombin 2 (residues 274–581); further cleavage at Arg322–Ile323 results in the formation of α-thrombin. The latter, one of the most potent enzymes known, not only converts fibrinogen to fibrin but acts upon factors V, VIII, XIII, protein C, platelets, and endothelial cells [9]. The schematic representation of the prothrombin molecule is shown in Figure 26.1.

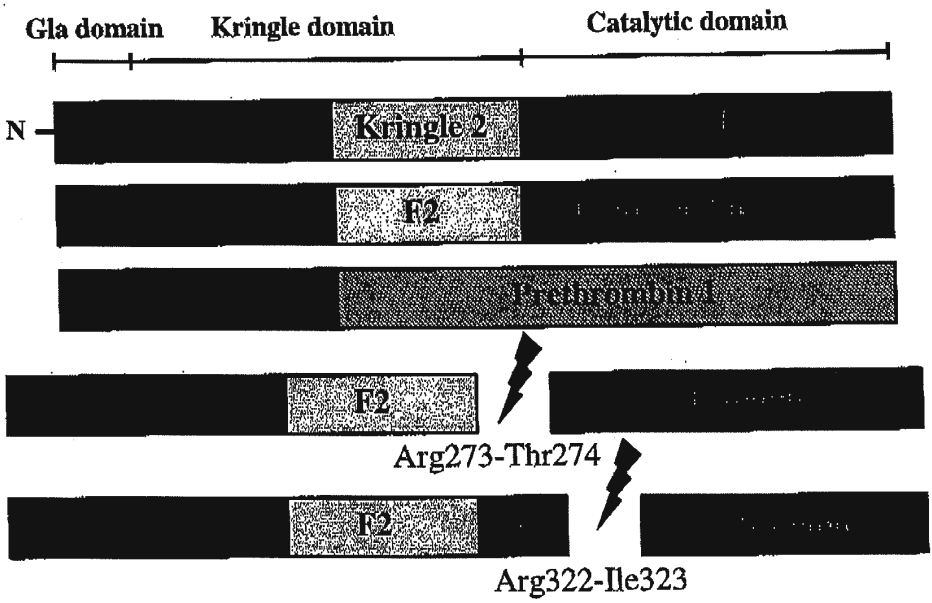

Figure 26.1. Schematic representation of the prothrombin molecule. Cleavage at Arg273–Thr274 results in the liberation of prothrombin fragment 1+2 (residues 1–273) and prethrombin 2 (residues 274–581); further cleavage at Arg322–Ile323 results in the formation of α-thrombin.