FEBS Letters 587 (2013) 2606–2611

journal homepage: www.FEBSLetters.org

Immobilized amyloid Aβ peptides support platelet adhesion and activation

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ARTICLE INFO

Article history:
Received 30 April 2013
Revised 10 June 2013
Accepted 24 June 2013
Available online 4 July 2013

Edited by Jesus Avila

Keywords:
Amyloid Aβ peptide
Human platelet
Adhesion
Cerebrovascular inflammation

ABSTRACT

Accumulation of amyloidogenic Aβ peptides in the brain contributes to the onset of Alzheimer disease. Aβ peptide deposits are also present in blood vessel walls, mainly deriving from circulating platelets. However, their effect on platelet function is unclear. We demonstrate that immobilized Aβ peptides induce platelet adhesion and spreading through metalloproteinase-sensitive surface receptors. Aβ peptides also fasten platelet spreading on collagen, and support the time- and ADP-dependent activation of adherent platelets, leading to stimulation of several signalling proteins. Our results indicate a potential role for peripheral Aβ peptides in promoting platelet adhesion and activation in the initiation of thrombus formation.

1. Introduction

Amyloidogenic Aβ are heterogeneous peptides deriving from the amyloidogenic proteolysis of the amyloid precursor protein APP, and accumulate in the senile plaques in the brain during the onset of Alzheimer disease (AD) [1]. Aβ peptides are also present in small arteries and capillaries of leptomeninges and cerebral cortex, causing cerebral amyloid angiopathy [2], and are involved in the inflammatory pathology of atherosclerotic vascular disease [3]. Amyloid Aβ peptides present in the bloodstream mainly derive from circulating platelets. Platelets express amyloid precursor protein APP_{751} and APP_{770}, as well as all the enzymes for APP proteolysis through amyloidogenic and non-amyloidogenic pathways [4]. In addition, a considerable amount of amyloidogenic $Aβ_{1–40}$ and $Aβ_{1–42}$ peptides is stored in platelet α-granules and is secreted upon platelet stimulation [3,5,6]. Once released in the plasma, Aβ peptides are able to induce platelet activation, establishing a positive feedback to potentiate cell activation. The mechanism by which Aβ peptides trigger platelet activation is unknown. A previous study has demonstrated that aggregated Aβ peptides, as other misfolded proteins, induce platelet activation through two different pathways initiated by CD36 or GPIb, respectively [7]. Shen and collaborators have demonstrated that the ability of $Aβ_{1–40}$ to promote platelet activation and to potentiate the effect of weak agonists is completely conserved by the smaller $Aβ_{25–35}$ peptide, a fragment of 11 amino acids corresponding to the intermembrane segment of the entire peptide [8]. It is now clear that $Aβ_{25–35}$ reproduces all the biological functions of Aβ peptides, as it also retains the toxicity of the entire peptides and is able to aggregate innately in fibrils with β-structure [9]. $Aβ_{25–35}$ is also physiologically present in elderly people, suggesting its importance in the pathogenesis of AD [10,11].

In the present study we have investigated the ability of different heterogeneous amyloidogenic peptides, $Aβ_{1–40}$, $Aβ_{1–42}$ and the short $Aβ_{25–35}$ immobilized on coated dishes to promote specific platelet adhesion, spreading and activation, in order to clarify their potential role in the early phases of thrombus formation.

2. Materials and methods

2.1. Materials

$Aβ_{1–40}$, $Aβ_{1–42}$ and $Aβ_{25–35}$, bovine serum albumin (BSA), dimethylsulfoxide (DMSO), prostaglandin E_1, apyrase, indometacin,

Abbreviations: AD, Alzheimer disease; PLC, phospholipase C; PI3K, phosphatidylinositol 3 kinase; APP, amyloid precursor protein; PKC, protein kinase C

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.06.041

TRITC-conjugated phalloidin, leupeptin and aprotinin were purchased from Sigma–Aldrich. W7 was from Alexis. Monomeric type I collagen was provided by Prof. M.E. Tira (University of Pavia, Pavia, Italy). The rabbit polyclonal Abs against Rap1(121), as well as the mAb anti-tubulin (DM1A) were from Santa Cruz Biotechnology. Anti phospho-Akt(S473), anti phospho-p38MAPK(T180/Y182), anti phospho-ERK1/2(T202/Y204), anti phospho-MLC(S19) and anti PKC phospho-substrates were from Cell Signaling Technology. Appropriate peroxidase-conjugated anti-IgG Abs were from Bio-Rad. The bicinchoninic acid assay was from Pierce and the enhanced chemiluminescence substrate was from Millipore.

2.2. Preparation of washed human platelets

Human platelets were obtained from healthy volunteers (<60 years) enrolled by the local hospital. Whole blood in citric acid/citrate/dextrose (152 mM sodium citrate, 130 mM citric acid, 112 mM glucose) was centrifuged at 120×*g* for 10 min at room temperature. Apyrase (0.2 units/ml), prostaglandin E_1 (1 μM), and indometacin (10 μM) were then added to the platelet-rich plasma. Platelets were recovered by centrifugation at 720×*g* for 15 min, washed with 10 ml of PIPES buffer (20 mM PIPES, 136 mM NaCl, pH 6.5), and finally gently resuspended in HEPES buffer (10 mM HEPES, 137 mM NaCl, 2.9 mM KCl, 12 mM $NaHCO_3$, pH 7.4) containing 1 mM $CaCl_2$. The cell count was typically adjusted to 3×10^8 platelets/ml unless otherwise stated.

2.3. Adhesion assay

Polystyrene dishes (60 mm) were coated overnight at room temperature with 25–50 μg/ml of collagen type I, or 5–10 μM $Aβ_{1-40}$, $Aβ_{1-42}$ and $Aβ_{25-35}$, (as indicated) diluted in PBS, and then blocked with 1% BSA for 2 h at room temperature. Washed human platelets (0.5 ml) were added to Aβ- or collagen-coated dishes in the presence of 1 mM $CaCl_2$ or of 1 mM $MgCl_2$ and 1 mg/ml of BSA, respectively. After incubation at room temperature for 10–60 min, non-adherent cells were discharged, and adherent cells were directly solubilized with 0.3 ml of 2% SDS, for whole cell lysates preparation. For analysis of Rap1 activation, adherent platelets were lysed with 0.3 ml of ice-cold RIPA buffer (50 mM Tris/HCl, pH 7.4, 200 mM NaCl, 2.5 mM $MgCl_2$, 1% Nonidet P-40, 10% glycerol, 1 mM PMSF, 5 μg/ml leupeptin, 5 μg/ml aprotinin, and 2 mM Na_3VO_4) and lysates were centrifuged at 18000×*g* for 10 min. Aliquots of each sample containing the same amount of proteins were used for immunoblotting analysis or for Rap1b activation assay, as previously described [12].

Evaluation of platelet adhesion and spreading was performed using a fluorescence microscopy-based method after incubation

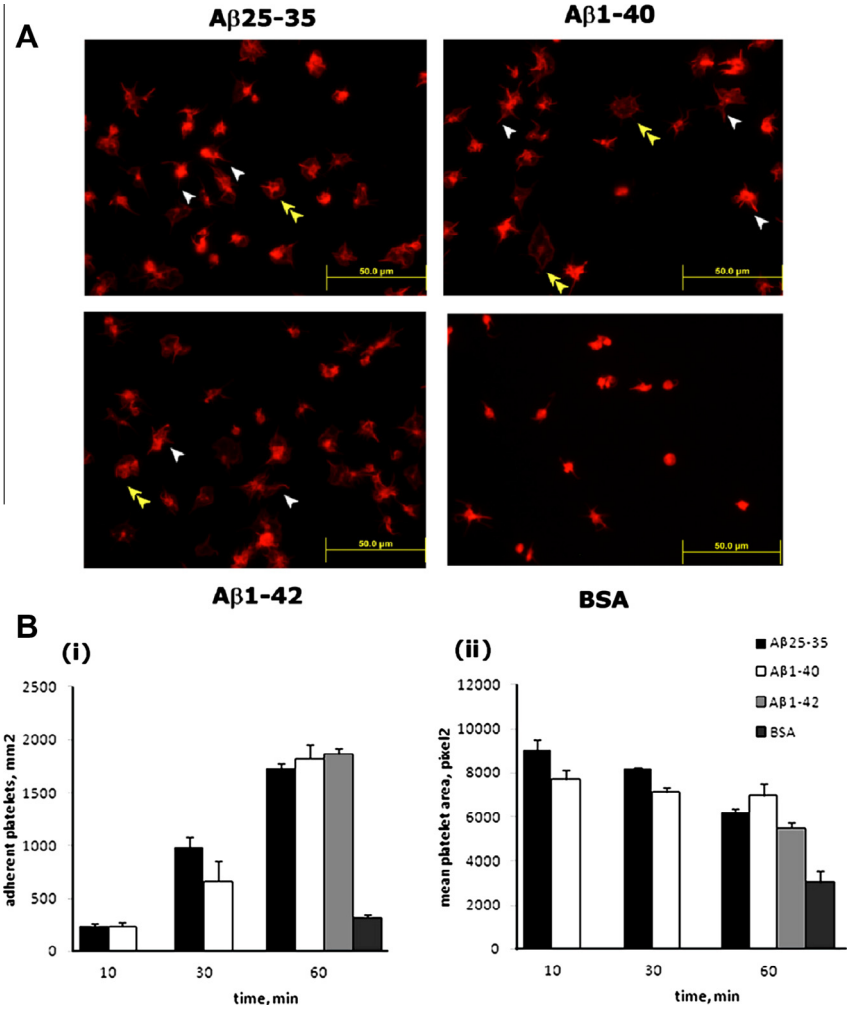

Fig. 1. Immobilized amyloid peptides $Aβ_{25-35}$, $Aβ_{1-40}$ and $Aβ_{1-42}$ support platelet adhesion. (A) Washed human platelets were plated on glass coverlips coated with 10 μM $Aβ_{25-35}$, $Aβ_{1-40}$, $Aβ_{1-42}$ or 0.5% BSA, as indicated, for different time. Adherent platelets were fixed, permeabilized and stained with TRITC conjugated phalloidin. Representative images at 1000× magnification of adherent platelets to the indicated substrates after 60 min are reported. Filopodia and lamellipodia formation are indicated by single and double white arrows, respectively. (B) Quantification of platelets adhesion, evaluated as number of adherent platelets, and platelets spreading, evaluated as mean platelet area is reported in panels (i) and (ii), respectively.