



The integrin α L leg region controls the Mg/EGTA mediated activation of LFA-1



Siyu Guan, Ming Cheng¹, S.K. Alex Law^{*}

School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore

ARTICLE INFO

Article history:

Received 13 January 2015

Available online 30 January 2015

Keywords:

Divalent cation
Integrin activation
Domain swapping
Cell adhesion

ABSTRACT

We have shown that Mg/EGTA (5 mM Mg²⁺ and 1.5 mM EGTA) could effectively promote the adhesion of integrin α L β ₂ to its ligand ICAM-1 but could not promote that of the α M β ₂ to denatured BSA. In order to determine the structural differences between α L and α M that specifically contribute to Mg/EGTA sensitivity, a series of α L/ α M chimeras were constructed. Our results showed that α L β ₂ with α M calf-1 domain completely lost the response to Mg/EGTA activation. In the reverse experiment, α M β ₂ would require the presence of both the α L calf-1 and calf-2 domain to initiate the Mg/EGTA sensitivity.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Integrins are a family of membrane glycoproteins that serve as bi-directional signaling molecules between the interior of the cell and its extracellular environment. Integrins are heterodimers, each member is consisted of one α subunit and one β subunit, which associate with each other non-covalently [1]. In human, there are 24 integrin heterodimers formed by selective combinations from 18 different α subunits and 8 β subunits. Of specific interest to us are the members of the β ₂ integrin family. Four α subunits, namely α L, α M, α X, and α D, combine with the common β ₂ subunit to form a closed set of α L β ₂, α M β ₂, α X β ₂, and α D β ₂ integrins. They are expressed exclusively on leukocytes. Specifically, α L β ₂, α M β ₂, and α X β ₂ are expressed on peripheral blood leukocytes and the regulation of their adhesion properties has been studied extensively [2–5]. Generally, these integrin molecules are at a resting state, i.e. incapable to mediate cell adhesion to their ligands.

Integrin activation is regulated bi-directionally. The inside-out signal can be from another receptor on the cell, such as the CD14 on monocytes and CD40 on B cells [4]. The signal is transmitted to the cytoplasmic tail of the β ₂ subunit by intracellular proteins such

as talin and kindlin [6]. Upon such interaction, the ectodomain of the integrin changes from a folded compact conformation to an extended and open conformation. Functionally, the open integrin can adhere to ligand with high affinity. Activation can also be induced from outside via ligand binding. The signal can be manifested to cellular activities such as cell spreading and cell migration. The α subunits of the β ₂ integrin family belong to the subset that has an inserted I-domain, which we shall refer to as the α I domain. For these integrins, the α I domain is the major ligand binding site [7].

In the laboratory, the transition of integrin activity states can be studied by the manipulation of extracellular conditions: either by antibodies that promote the conformational change associated with activation, or by the alteration of the concentrations of divalent cations. Ca²⁺, Mg²⁺ and Mn²⁺ are capable in regulating the adhesion between leukocyte integrins and ligands, among which Ca²⁺ generally inhibits the ligand-receptor binding, whereas Mg²⁺ and Mn²⁺ generally promote the adhesion [8–10]. Divalent cation binding sites have been identified in the headpiece of the integrin heterodimer, i.e. in the β -propeller of the α subunits, the β I domain of the β subunits, and also on the α I domain for those α subunits with this domain. As of today, the structure of the ectodomain of three integrin heterodimers had been solved by X-ray crystallography; they are the α V β ₃ [11], α IIb β ₃ [12] and α X β ₂ [13] integrins. For α IIb and α V, a Ca²⁺ binding site has also been found in the genu region between the thigh and the calf-1 domain. However, this Ca²⁺ binding site was not identified in the α X subunit.

It was discovered in 1992 that the high concentration of Mg²⁺ coupled with the depletion of Ca²⁺ by the inclusion of EGTA in the

Abbreviations: EGTA, Ethylene glycol tetra-acetic acid; LFA-1, Leukocyte function associated antigen-1 (α L β ₂); Mac-1, Macrophage antigen-1 (α M β ₂); ICAM, Inter-cellular adhesion molecule; BSA, Bovine serum albumin; WT, Wild-type.

^{*} Corresponding author. Fax: +65 6515 1632.

E-mail address: alaw@ntu.edu.sg (S.K.A. Law).

¹ Current address: Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

medium would activate LFA-1 from a resting to a ligand binding state [9]. In contrast, it appears that both Ca^{2+} and Mg^{2+} are required to activate Mac-1 to adhere to iC3b [8]. In this study, we focus to discern the difference of Mg/EGTA mediated activation between LFA-1 and Mac-1 and to identify the domain responsible for this difference.

2. Material and methods

2.1. Antibodies and reagents

Hybridomas of mAbs were obtained from different sources: MHM24 (anti- αL blocking mAb) and MHM23 (β_2 heterodimer specific mAb) [14] were provided by Professor AJ McMichael (John Radcliff Hospital, Oxford, UK). KIM185 (anti- β_2 activating mAb) [15] was obtained from Dr. MK Robinson (UCB, CellTech, UK). Hybridoma of LPM19c (anti- αM blocking mAb) [16] was purchased from ATCC. All mAbs were purified from hybridoma supernatant using Hi-Trap protein G or A columns (Amersham Pharmacia Biotech). Preparation of recombinant human ICAM-1-Fc was described previously [17]. Bovine serum albumin (fraction V, BSA) was bought from Sigma–Aldrich.

2.2. Plasmids

The pcDNA3 expression plasmids containing αL , αM and β_2 cDNA were described previously [18]. Each domain swapping plasmid was constructed by a two-step PCR. In the 1st step, a pair of 64–70-mer primers containing donor sequences at 5' tails that were ~30 bases in length and receptor sequences at 3' tails that were ~35 bases in length was designed. A mega primer containing the domain of interest from the donor in the middle, and flanked by the receptor sequences were synthesized by PCR using Advantage[®]-GC cDNA Polymerase Mix kit (ClonTech), followed the manufacturer's instruction. The mega primer was obtained by gel purification. In the 2nd step, the purified mega primer was used to clone the chimera using Pfu Turbo polymerase (Stratagene). Setting of thermo cycling parameters was followed the manufacturer's instruction with the exception of using a gradually decreased annealing temperature which dropped 1 °C every 30 s from 76 °C to 45 °C. Subsequent procedures after PCR were followed by the instruction from the manufacturer. All constructs were verified by sequencing (Axil Scientific, Singapore).

2.3. Cell culture and transfection

Human embryonic kidney 293T (HEK293T) cells were purchased from ATCC, cells were cultured in DMEM (Hyclone) supplemented with 10% (v/v) heat-inactivated FBS (Hyclone), 100 IU/ml penicillin and 100 µg/ml streptomycin (Hyclone) at 37 °C in 5% CO_2 humidified incubator. HEK293T cells were transfected with expression plasmids using polyethylenimine (PEI) according to a previously described method [19].

2.4. Flow cytometry

Surface expression detection was described previously [17] using a β_2 heterodimer specific mAb MHM23 followed by FITC-conjugated sheep anti-mouse IgG [20]. Background histograms were obtained using the LPM19c mAb (αM specific) for LFA-1, and MHM24 mAb (αL specific) for Mac-1. Stained cells were collected on a FACS Calibur (BD Biosciences). Data were analyzed using Flowjo (Tree Star).

2.5. Cell adhesion assay

For ICAM-1 adhesion, 100 µl of 5 µg/ml goat anti-human IgG (Fc specific) (Sigma–Aldrich) in bicarbonate buffer (50 mM, pH 9.2) was introduced into each well in 96-well microtiter plates (Polysorb, Nunc Immno-Plate) and left overnight at 4 °C. Non-specific sites were blocked with 130 µl of 0.5% (w/v) BSA in PBS for each well. 50 µl of ICAM-1-Fc in PBS with 0.1% BSA (w/v) at 1 µg/ml was added into each well and left at RT for 2 h. For BSA adhesion assay, 100 µl of 100 µg/ml BSA in bicarbonate buffer was coated on each well and the plate was left overnight at 4 °C. Non-specific sites were blocked with 130 µl of 0.2% (w/v) polyvinylpyrrolidone (PVP) in PBS for each well. In both cases the wells were washed twice with wash buffer (RPMI1640 with 10 mM HEPES and 5% v/v FBS) before adding cells. Transfected cells were harvested, washed and incubated with 1 µg/ml BCECF (Invitrogen) in wash buffer at 37 °C for 20 min. Labeled cells were transferred to each wells with or without activation reagents (Mg/EGTA: 5 mM MgCl_2 and 1.5 mM EGTA; Mn^{2+} : 0.5 mM MnCl_2 ; mAb KIM185: 10 µg/ml) and incubated at 37 °C for 30 min. To establish specificity, appropriate antibodies were added to block adhesion. Unbound cells were removed by washing twice with wash buffer. Fluorescence signal, which correlates with the number of cells adhering to the ligand-coated well, was measured using a FL600 fluorescence plate reader (Bio-Tek instruments, Winooski, VT).

3. Results

3.1. The effect of Mg/EGTA on $\alpha\text{L}\beta_2$ and $\alpha\text{M}\beta_2$ mediated adhesion

Manipulation of divalent cation concentrations, generally Ca^{2+} , Mg^{2+} and Mn^{2+} , in the cell medium had been found to affect their integrin mediated adhesion to ligands. Mn^{2+} , at sub mM level, has been shown to promote $\alpha\text{L}\beta_2$ and $\alpha\text{M}\beta_2$ mediated adhesion to their ligands. However, the requirements for Mg^{2+} and Ca^{2+} are different for the two integrins. It was shown that both Mg^{2+} and Ca^{2+} are required for $\alpha\text{M}\beta_2$ mediated adhesion [8,21] whereas Ca^{2+} is inhibitory to $\alpha\text{L}\beta_2$ mediated adhesion [9]. This observation is confirmed in an experiment shown in Fig. 1. $\alpha\text{L}\beta_2$ and $\alpha\text{M}\beta_2$ transfectants were assayed for their adhesion to their respective ligands ICAM-1 and denatured BSA. Adhesion can be promoted for both transfectants with the β_2 -specific activating mAb KIM185, $\alpha\text{L}\beta_2$ transfectants, but not the $\alpha\text{M}\beta_2$ can be activated by Mg/EGTA. Since the two integrins share the common β_2 subunit, it is logical to argue that this difference is due to certain structural features that are different in αL and αM subunits.

3.2. αL calf-1 domain is required for Mg/EGTA mediated activation

A series of $\alpha\text{L}/\alpha\text{M}$ chimera were constructed such that the domains of the leg region of the αM subunit were introduced into the αL background. These chimeras, together with the β_2 subunit, were co-transfected into HEK293T cells. Expression of the chimeric integrins was monitored by flow cytometry using the mAb MHM23, and adhesion to ICAM-1 was performed. The results are shown in Fig. 2. Chimeras with the calf-1 domain of αM would render the integrin $\alpha\text{M}\beta_2$ -like, in that Mg/EGTA cannot stimulate the adhesion of the transfectants to ICAM-1.

Significantly, the integrin with only the αM calf-1 domain on an $\alpha\text{L}\beta_2$ background is not responsive to Mg/EGTA stimulation, suggesting that Mg/EGTA response element is located in the calf-1 domain of αL subunit.

3.3. The restoration of Mg/EGTA mediated activation on $\alpha\text{M}\beta_2$

The converse series of chimera were constructed where domains of the leg region of αL were introduced into the αM

Download English Version:

<https://daneshyari.com/en/article/10753271>

Download Persian Version:

<https://daneshyari.com/article/10753271>

[Daneshyari.com](https://daneshyari.com)