VIROLOGICA SINICA, February 2008, 23 (1):1-7
DOI 10.1007/s12250-008-2886-2

CLC number: R511 Document code: A Article ID: 1674-0769 (2008) 01-0001-07

Integrin Activation and Viral Infection*

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Abstract: Integrins are members of a ubiquitous membrane receptor family which includes 18 different α subunits and 8 β subunits forming more than 20 α/β heterodimers. Integrins play key functions in vascular endothelial cell and tumour cell adhesion, lymphocyte trafficking, tumor growth and viral infection. Current understanding of the molecular basis of integrins as viral receptors has been achieved through many decades of study into the biology of transmembrane glycoproteins and their interactions with several viruses. This review provides a summary of the current knowledge on the molecular bases of interactions between viruses and integrins, which are of potential practical significance. Inhibition of virus-integrin interactions at the points of virus attachment or entry will provide a novel approach for the therapeutic treatment of viral diseases.

Key words: Integrins; Cellular receptor; Viral infection

Integrins are a family of ubiquitous α/β heterodimeric glycoproteins that mediate cell migration and adhesion. The major ligands for integrins are coagulation and fibrinolytic factors, complement proteins and cellular counter-receptors, epithelial and vascular matrix components in addition to viral proteins. Integrins and their ligands are currently an intensely investigated topic in fields as hematology, neurobiology, thrombosis, cancer biology, developmental biology, inflammation, gene therapy and viral We have splited the long sentence to short sentences. (2, 4, 18, 19, 20, 22, 30). Increasing evidences have demonstrated that many viruses such as human coxsackievirus A9, human papillomavirus, adeno-associated virus 2 (AAV2), hantaan virus (HT-NV), foot-and-mouth disease virus (FMDV), human parechovirus 1 and human echovirus (1, 8, 9, 22) can initiate infection by attaching to activated integrins. This review focuses on recent advances relating to factors contributing to intergrin activation and the roles of integrins in viral infection.

STRUCTURE OF INTEGRIN

Integrin is about 280 Å long and consists of one α (150 to 180 kDa) and one β (about 90 kDa) subunit, both of which are type I membrane proteins. To date,

Received: 2007-06-13, Accepted: 2007-09-12

* Foundation item: The National key Basic Research (973) Program (2005CB523201) and National Key Technology R&D Program (2006BAD06A14)

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there are at least 18 α（α1~α11, αD, αE, αL, αM, αv, αX, αIIb）and 8 β（β1~β8）subunits which combine to form 25 different non-covalently bounded α/β heterodimers. The heterodimers can then be grouped into subfamilies according to their α and β subunits or the ligand specificity (8, 15). Each integrin subunit is composed of a large extracellular domain, a transmembrane region, and a short cytoplasmic domain that in most cases consists of 20-70 amino acid residues (except for β4 which contains a very large cytoplasmic domain of some 1000 amino acids). The structure amongst the α subunits is very similar, as for the β subunits. According to the identity of the α subunits, integrins can be subdivided into 2 groups; one group with α subunits（α1, α2, α10, α11, αL, αM and αX）that contain an I ("inserted") domain (about 180 amino acids) which is a member of a family of von Willebrand A domains (VWA); the other group (α3, α5, α6, α7, α8, αIIb, and αV) share a post-translational cleavage of their precursors into a heavy and a light chain. The light chain is composed of the cytoplasmic domain, the transmembrane region and a part of the extracellular domain (about 25 kD). The remainder of the heavy chain is contained on the extracellular domain (about 120 kD) (34). Nevertheless, all α subunits contain a β-propeller consisting of 7 homologous repeats of 30-40 amino acids in extracellular domain, spaced by stretches of 20-30 amino acids (Fig.1). The extracellular three or four repeats contain sequences Asp-X-Asp-X-Asp-Gly-X-X-Asp with cation-binding properties. Moreover, all the α subunits share the 5 amino acid motif GFFKR, directly under the transmembrane region. The leg of the α-subunit consists of a thigh domain and two calf domains. The β subunit is a four-fold repeat of cysteine-rich segments believed to be internally disulfide bonded, and the N-terminal 40-50 KD is tightly folded with internal disulfide loops and contributes to the ligand-binding domain. The head of all β-subunits contains an I-like domain, which shares a common structure with α I domains (Fig. 1). The β I-like domain interacts with an α-subunit, forming an interface for ligand binding. The leg of the β-subunit has a hybrid domain, a plexin-semaphorin-integrin (PSI) domain, four cysteine-rich repeats (epidermal growth-factor-like domains, EGF-like) and a novel cystatin-like fold (β-tail domain). A metal-ion binding site (MIDAS), essential for ligand binding, is present in both the αI domain and β I-like domain. As for the α/β heterodimers, the N-terminal domains of α and β subunits combine to form a ligand-binding head which is connected by two stalks; each makes up of one of the membrane-spanning segments and connects the two cytoplasmic domains (Fig.1). These cytoplasmic domains are believed to interact with cytoskeletal proteins and perhaps with other cytoplasmic components (5).

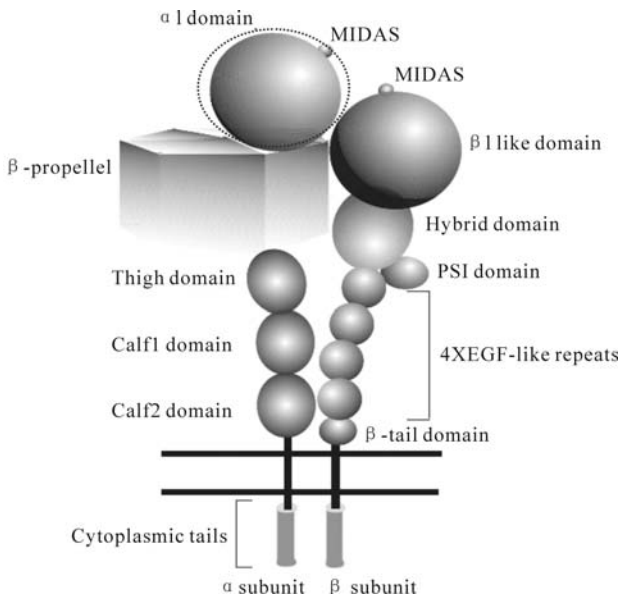

Fig. 1. The structure of integrin. (Modified from Humphries, 2000)

INTEGRIN ACTIVATION

Integrin activation initially refers to the changes required to enhance ligand-binding activity (the primary effector function of adhesion receptors), whereas activation of signalling receptors generally refers to the changes induced by ligand binding that enhance signal transduction (the primary effector function of signaling receptors). The finding that integrins also play an important role as signalling receptors emphasizes the importance of providing a clear definition of this term (31). A general property of integrins is that they have at least two conformations: active (able to bind ligands) and inactive (unable to bind ligands). Conversion from an inactive status to an active one (which is called integrin activation) is postulated to occur through two different mechanisms collectively referred to as "inside-out signaling". The first one is called affinity modulation, which is mediated through conformational changes in the integrin ectodomain, whereas the second, avidity modulation, is mediated by clustering of heterodimers at the cell surface (32).

Integrin affinity

One important mechanism by which cell regulates integrin function is through tight spatial and temporal control of integrin affinity for extracellular ligands. This is achieved by rapid, reversible conformational changes of the extracellular domains of the integrin heterodimer (3). Transition between these conformations has been shown to be regulated by both divalent cation occupancy and ligand binding (Fig. 2). Since integrins are conformationally flexible and contain a number of key hinge regions, exquisite changes in cationic environment regulate a complex pivoting of both α and β subunits about these hinges. The subsequent conformational alterations have a direct effect on ligand-binding capacity. In general, Mn^{2+} and Mg^{2+} usually promote ligand binding and Ca^{2+} usually has an inhibitory effect (23, 25).

Integrin clustering

Integrins differ from other cell-surface receptors in that they bind their ligands with a low affinity (10^6-10^9 liters/mole) and they usually present at 10-100 fold higher concentration on the cell surface. So only when stimulated by a certain kinase (11) do these integrins cluster in focal contacts, then their combined weak affinities give rise to a spot on the cell

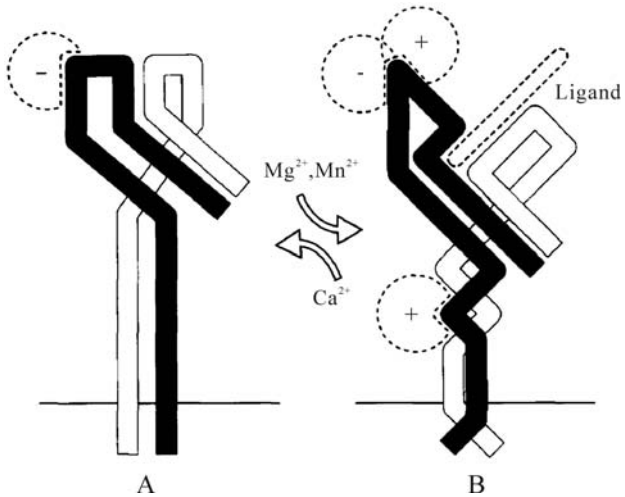

Fig. 2. A model of the major conformational states of integrin extracellular domains, showing the effects of divalent cation occupancy and ligand binding. A: Ca^{2+}-occupied inactive integrin can not bind ligand. B: Mg^{2+} or Mn^{2+} binding elicits a major conformational change and generates an active conformer with the potentiality to bind ligand.

surface which has enough adhesive capacity to bind ligands. Clustering is considered to increase avidity, but not affinity of molecular interactions, thereby increasing receptor occupancy by increasing the on-rate of binding (6). This occurs inside (for signalling and cytoskeletal proteins) and outside the cell (for ligands), and can be controlled bidirectionally. Thus binding of integrins to multivalent ligands in the extracellular matrix or on other cell surfaces causes accumulation of signalling complexes on the cytoplasmic face of the plasma membrane, and a wide selection of intracellular factors can induce formation of cytoskeletal and signalling complexes, which, in turn, recruit integrins via linker proteins.

INTEGRIN AND VIRAL INFECTION

General principles of virus-integrin interactions

Many viruses exploit the endocytic machinery of the host for invasion in which viruses must attach to the specific receptor(s) on the cell surface. These receptors usually play important roles in cell adhesion, cell-cell interactions, signalling and defence mechanisms. The binding of a virus to a receptor can elicit changes in receptor conformation. These alterations may bring about signalling events which regulate both the viral entry and the cellular response to the infection (13, 21). In addition, conformational changes in viral particles triggered by receptor binding can also facilitate viral entry and uncoating.

Integrins seem to be the "doors" for some viruses to enter the cell (Table 1). The interaction between virus and integrin is important in the viral replication cycle. This interaction brings about membrane permeabilization, fusion, and endocytosis. There are different complex strategies of integrin-dependent viral infection. Integrins can be used either as primary attachment receptors or as co-receptors in the entry process. When virus-integrin interaction occurs, viruses bind to integrin using pattern recognition sequences such as RGD, GRRP, LDV and QAGDV, which are important for natural ligands (16), or interact with unique regions of integrins without necessarily having a recognition sequence.

Viruses utilize integrins as receptors

The RGD-binding integrins are among the most promiscuous in the integrin family. Several viruses

Table 1. Integrins in viruses entry

Virus	Integrin receptor	Route	Kinase/ GTPase
Adenovirus	αvβ1, αvβ3, αvβ5	Clathrin, microtubules	PI3K, CAS, PKA, p38/ Rab5, dynamin, Rac1, Cdc42
CoxackievirusA9	αvβ3, αvβ6		
Echovirus	αvβ3, α2β1		/ dynamin
Foot and mouth disease virus	αvβ1, αvβ3, αvβ6, αvβ8	Clathrin	AKT deactivation, GSK3 dephosphorylation
Hantaan virus	αvβ3, αⅡbβ3		
Human parechovirus 1	αvβ3		
Human immunodeficiency virus 1	αvβ3		
Rotaviruses	α2β1, αXβ2, αvβ3, α4β1	Non-clathrin, non-caveolin	/ dynamin
KSHV/HHV-8	α3β1, α2β1	Clathrin microtubules	FAK, Src, PI3K, ERK, PKCζ- MEK-ERK/ RhoA, Cdc42
Papillomavirus	α6β4, α6β1		
AAV-2	αvβ5(α5β1)		PI3K
Parechovirus 1	αvβ1, αvβ3		

have been reported to utilize RGD-dependent integrins to initiate infection. Kaposi's Sarcoma-Associated Herpesvirus (KSHV/HHV-8) uses β1 integrin for infection (8). Field strains of FMDV use at least four integrins αvβ1, αvβ3, αvβ6, αvβ8 as receptors to initiate infection on cultured cells, and integrins are believed to be the receptors used to target epithelial cells in animals (17, 35). Adenovirus has been shown to interact with αvβ3, αvβ5, and αvβ1 integrins via a high-affinity arginine-glycineaspartate (RGD) domain present in the penton bases of the viral capsids (14). Both human parechovirus type 1 and coxsackievirus A9 can use αvβ3 and αvβ1 (33). In addition, αvβ3 and α5β1 have been shown to be receptors of the Barty strain of echovirus type 9 and adenovirus, respectively. Integrins have also been demonstrated as receptors for rotaviruses and papillomaviruses.

Some virus-integrin interactions, however, are independent of RGD motif. Cytomegalovirus can interact with integrin via a highly conserved disintegrin-like domain (10). Hantaan virus, which causes severe respiratory disease in human, has also been reported to use integrin α3β3 for infection, but this interaction seems to be independent of the RGD motif (7). AAV2 lacks the RGD motif, but uses integrin αvβ5 as a coreceptor for cell entry. A recent study demonstrated that a highly conserved domain that contains an asparagine-glycinearginine (NGR) motif on the VP3 domain of the AAV2 could bind integrin α5β1 with moderate affinity, thus mediating AAV2 infection in human embryonic kidney 293 cells (1). Increasing evidences also support the hypothesis that bacteriophages (bacterial viruses) use a cellular receptor β3 integrin for their attachment to eukaryotic cells (12). For example, in T4 phages, there are 55 copies of the KGD motif in the head corner protein; therefore, phages could bind cells that express β3 subunit (platelets, monocytes, some lymphocytes and some neoplastic cells) and downregulate activities of those cells by inhibiting integrin functions (12).

ROLES OF ADAPTOR PROTEINS AND KINASES

The cytoplasmic domains of integrins, particularly β-subunits, interact with adaptor proteins (e.g., vinculin, talin, and α-actinin) which are critical for transmitting mechanical force between the extracellular matrix (ECM) and the actin cytoskeleton. In addition, integrins and their associated adaptor proteins recruit signaling molecules such as focal adhesion kinase, proline-rich tyrosine kinase 2, Src, c-Src kinase, integrin-linked kinase (ILK), protein kinases GSK-3, protein kinase C (PKC), and p21-activated kinase (PAK) to the membrane. Kinases have been known for some time to regulate endocytosis, and recently an unexpectedly large number of kinases were shown to regulate clathrin and caveolin-mediated endocytosis (27). Increasing evidence suggests that small GTPases such as Arf6 and members of the Rab family control integrin internalization and recycling back to the plasma membrane along microtubules. Several kinases, which have well-established roles in integrin signaling, can move integrins from the back to the front of migrating cells, thus facilitate virus adsorption (24, 26). For instance, echovirus 1 and rotavirus enter cells via α2β1 integrin-mediated endocytosis, regulated by dynamin-dependent mechanisms (28). Adenoviral entry via αV integrin depends on GTPase Rab5 (29). The rotaviral spike protein VP4 is able to bind both to the extracellular domain of an α2 subunit and to Rab5 in the

cytosol (9). More targets downstream of these kinases will hopefully be identified, and will provide new insight as to how different endosomal pathways integrate extracellular signals and coordinate their activities in viral infection.

CONCLUSION

The growing evidence for viruses using integrins for cell entry suggests that these receptors are not only convenient portals for cell entry, but also have more significant functions. Integrin ligation increases the kinetics of pathogenic virus internalization into cells, a situation that would protect viral particles from being rapidly inactivated by antibodies and/or complement. Integrin signaling could also provide a more stable environment for the microbe once it has established the infection by down-regulating the host immune response. Microbes that use β1 integrins on cells of the immune system also provide an opportunity for establishing a latent infection in these cells, as well as facilitating pathogen dissemination within the host. Microbial pathogen spread could also be enhanced through integrin-mediated cell migration. However, pathogen entry is a complicated process that is also regulated by enzymes and signaling molecules secreted by the pathogen; further studies with viruses that are related to use of integrins and kinases should help in better understanding the precise function of integrin in cell entry of microbial pathogenesis.

Acknowledgments

We thank Martin J. Humphries for permission to use his schematic model of integrin structure and Zhidong Zhang, Yanmin Li for their constructive criticism of the manuscript. The work is supported by National key Basic Research (973) Program (2005CB-523201) and National key Technology R&D Program (2006BAD06A14). Apologies to those authors whose work we could not cite due to space restrictions.

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