Integrin-mediated signal transduction

C. M. Longhurst* and L. K. Jenningsa,b,*

aUniversity of Tennessee, Memphis, Department of Medicine, 956 Court Avenue, Rm. H335, Memphis (Tennessee 38163, USA), Fax +1 901 448 7181, e-mail: ljennings@utmem1.utmem.edu
bUniversity of Tennessee, Memphis, Department of Biochemistry, Memphis (Tennessee 38163, USA)

Abstract. Integrins, expressed on virtually every cell type, are proteins that mediate cellular interactions with components of the extracellular matrix (ECM) and cell surface integral plasma membrane proteins. In addition, integrins interact with the cytoskeleton and through this process participate in cell migration, tissue organization, cell growth, haemostasis, inflammation, target recognition of lymphocytes and the differentiation of many cell types. Signals generated from ligand-integrin interactions are propagated via the integrin cytoplasmic tails to signal transduction pathways within the cell (outside-in signalling). Information from within the cell can also be transmitted to the outside via integrin affinity modulation (inside-out signalling). Protein tyrosine phosphorylation has a central role in integrin-initiated cell signalling, leading to cytoskeletal organization and focal adhesion formation. This review will examine the current understanding of integrin function, focusing on the intracellular consequences of integrin-ligand interaction.

Key words. Integrin; cell adhesion; extracellular matrix; signalling; focal adhesion kinase.

Introduction

Integrins bind to a variety of ligands including extracellular matrix (ECM) proteins [1], cell surface immunoglobulin (Ig) superfamily receptors [2] and certain plasma proteins [3]. Integrin-mediated signal transduction contributes to the coordination of the actin cytoskeleton organization and cellular responses to ECM proteins and growth factors. The adaptability and rapid modulation of integrin-ligand interactions make integrins ideal coordinators of dynamic cellular processes. As integrin binding to extracellular ligands precedes diverse cell functions, the accompanying signalling pathways must be tightly regulated and coordinated. Inactivated integrins usually have a low affinity for ligand; however, on activation via appropriate signals, integrins can participate in high-affinity binding. This unique function allows modulation of cell adhesive properties without changes in integrin gene expression.

The understanding of signal transduction pathways involved in integrin-mediated cellular events has been the focus of intense interest for several years. The manipulation of the integrin αIIbβ3 expressed on human platelets by mimetic agents has already demonstrated that integrins provide potential sites for therapeutic intervention in thrombotic states [4]. In this review, we shall discuss the ongoing dissection of integrin-signalling pathways and their potential role in cell function.

Structure of integrins

Sixteen α and eight β integrin subunits have been cloned. These structures assemble to form the 22 described integrin heterodimers. These subunits are the products of two different genes, and the expression of both subunits is required for cell surface expression [5]. The α subunits comprise a short cytoplasmic tail, a transmembrane domain and a large extracellular domain of 1000 amino acids (aa) approximately. The α extracellular domain consists of seven tandem repeats

* Corresponding author.
of 60 aa containing three or four EF-like divalent cation-binding sites. The EF-hand domains have homology with a helical-loop Ca\(^{2+}\)-binding structure found in proteins such as calmodulin, trophin C and parvalbumin [6]. The non-I domain \(\alpha\) subunits \((\alpha 3, \alpha 5, \alpha 6, \alpha 7, \alpha IIb, \alpha v)\) contain four EF-like motifs and a disulphide cleavage site near the transmembrane region. \(\alpha IIb\beta 3\) was the first integrin to be purified to homogeneity and demonstrated to be a calcium dependent heterodimeric complex [7].

Electron microscopy studies [8] have shown that integrins are asymmetric structures consisting of a mushroom-like extracellular region and two flexible tails. The N-terminal regions of the \(\alpha\) and \(\beta\) subunits form the extracellular domains which span the membrane ending in cytoplasmic C-terminals of both subunits. The eight described \(\beta\) subunits of approximately 750 aa have an overall homology of approximately 40\%. Each \(\beta\) subunit has a short cytoplasmic tail with the exception of \(\beta 4\), which has an extended cytoplasmic region containing four fibronectin (FN) type III repeats and two alternatively spliced sites. The \(\beta\) extracellular domain has a highly conserved region of approximately 200 aa which has similarities to the I domain (A-like domain) found in some \(\alpha\) subunits and may contain a divalent cation-binding site [9]. The \(\beta\) subunit contains four cysteine-rich motifs within the C-terminal proximal to the transmembrane region. Additional variability is provided by alternative splicing of the cytoplasmic tails of the \(\beta 1\) and \(\beta 3\) subunits [10]. Disulphide bond arrangements and intersubunit contacts of proteolytic fragments of \(\alpha IIb\beta 3\) have been proposed [11]. These models relate to biophysical and electron microscopy data [12]. Integrins are subject to disulphide bond rearrangements and alternative splicing [10].

**Binding of integrins to their ligands**

Integrins generally recognize ligand amino acid sequences which contain a key acidic residue that is essential for receptor binding. An example is the Arg-Gly-Asp (RGD) peptide motif which is present in a number of integrin ligands. Studies of the three-dimensional structure of several unrelated RGD-containing ligands showed structural and topological similarities in the RGD motif [13]. The RGD-binding motif of the type III FN repeat is presented in a flexible structure between two \(\beta\) loops. Additional discontinuous regions of this protein may provide secondary binding sites [14, 15].

Divalent cations play a role in regulating integrin binding to the majority of ligands [7, 16]. Integrin-mediated cell adhesion as well as ligand binding to purified receptor is dependent on millimolar concentrations of calcium [17–19]. A relationship between cation, ligand and integrin was demonstrated in studies with \(\alpha IIb\beta 3\) utilizing the mAb PMI-I [19, 20]. PMI-I binds minimally to an epitope on the \(\alpha IIb\) subunit in the presence of physiological levels of cations and binds maximally upon chelation of cations with EDTA. Direct evidence for integrin-cation interactions comes from affinity-labeling experiments using cobalt ions and the integrin \(\alpha v\beta 3\) [21]. An irreversible linkage between \(\text{Co}^{3+}\) and \(\alpha v\beta 3\) was demonstrated. The \(\text{Co}^{2+}\) ion also supported ligand binding to \(\alpha v\beta 3\) in a similar manner to other divergent cations. When cobalt ions were irreversibly bound to the receptor by oxidation (\(\text{Co}^{3+}\)), \(\alpha v\beta 3\) was no longer able to interact with vitronectin. A recombinant fragment of \(\alpha IIb\) containing the four putative cation-binding sites described earlier bound fibrinogen optimally when all four sites had bound cation [22].

In general, the divergent cation \(\text{Ca}^{2+}\) plays either a facilitating or inhibitory function on integrin binding depending on the integrin-ligand interaction in question. Ligand-binding domains in the \(\alpha\) subunit have been found in or around cation-binding sites [23]. Ligand mimetic peptides can be cross-linked to the N-terminal of the \(\alpha IIb\) [24] and \(\alpha v\) [25] subunits. A critical site for divergent cation binding at aa 296–306 in the \(\alpha IIb\) subunit has been identified [24], and peptides from this region inhibit fibrinogen binding to \(\alpha IIb\beta 3\) [26]. A highly conserved 72-aa sequence in the \(\beta 3\) chain (D109-E171) has been implicated in \(\beta 3\) chain ligand recognition and can be chemically cross-linked to bound peptide ligand [27]. Point mutations and monoclonal antibody (mAb) binding within D109-E171 can inhibit ligand binding [27–29]. Divalent metal ions are involved in ligand binding mediated by D109-E171. The naturally occurring mutation \(\beta 3\) (D119Y) altered the conformation of \(\alpha IIb\beta 3\) in a way that suggests loss of bound divalent cation [19]. Binding of a luminescent calcium analogue to a synthetic peptide corresponding to \(\beta 3\) 118–131 was reduced by the substitution of D119 by alanine [30]. Alanine substitutions at D119 also reduced macromolecular ligand binding [31]. The cluster of D119, S121 and S123 is highly conserved among the \(\beta 3\) integrin subunits. A peptide representative of \(\beta 3\) (211–222) bound competitively with \(\alpha IIb\beta 3\) to fibrinogen [32]. In addition, \(\beta 3\) (211–222)-specific mAbs inhibited fibrinogen binding to purified \(\alpha IIb\beta 3\) [32], and mutations in this region \((\beta 3\ R214)\) blocked ligand binding [33] and caused instability of the \(\alpha IIb\beta 3\) heterodimer [34].

**Affinity modulation of integrins**

Cells can rapidly change integrin function by altering the binding affinity of integrin for ligand [35, 36]. Inte-