Adhesive Recognition Sequences

Kenneth M. Yamada
From the Laboratory of Developmental Biology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892

Specific cellular adhesion and migration of cells are recurring themes in embryonic development, tumor cell metastasis, and wound healing. Recent advances in our understanding of the molecular basis of cell adhesive and migratory interactions with extracellular matrix molecules have converged on the concept that many cell interactions are dependent on specific adhesive recognition sequences (reviewed in Refs. 1–8). As will be described in detail below, certain short peptide sequences in adhesion proteins are thought to serve as sites for recognition and binding by specific plasma membrane receptors (Table I).

A number of specific cell surface receptors, particularly integrins, mediate the adhesion of cells to fibronectin, laminin, or collagen by recognizing different, specific peptide sequences in each (Fig. 1). Moreover, some receptors can recognize the same specific sequence in several different proteins (1–5, 8). Conversely, a single adhesion protein can contain several different sequences that are recognized by distinct receptors (1–8). The existence of so many potential combinations of binding activities provides considerable complexity to the repertoire of interactions possible for an individual cell.

Fibronectin, a Prototype Cell Adhesion System

The adhesive glycoprotein fibronectin is involved in a variety of biological processes, particularly in mediating cell attachment and cell migration (reviewed in Refs. 1–5). Fibronectin is bound by several cell surface receptors, including the “classical” fibronectin receptor α5β1 and several other integrin receptors (2–5, 8). As reviewed below, short peptide sequences appear to be key determinants in the recognition of fibronectin by these receptors, although contributions from other polypeptide sequences are also quite important (Fig. 2). Most concepts in this review were established initially for fibronectin, as were most of the current tests of biological relevance summarized in Table II. Consequently, this review will focus in detail on fibronectin and then will compare adhesive recognition sequences in other molecules.

Adhesion and Competition Assays—The first peptide sequence in fibronectin to be identified as an adhesive recognition sequence was Arg-Gly-Asp (RGD) (9–11). Subsequently other cell adhesion sites of fibronectin have been identified, including a Leu-Asp-Val (LDV)-containing sequence that displays cell-type specificity and alternative splicing (12–15). Synthetic peptides based on the RGD or LDV motifs can mimic activity of the intact protein at least partially. They can mediate cell attachment when adsorbed to a substrate or when conjugated to a carrier, e.g. to albumin, IgG, beads, or a substrate (9, 14, 16). Conjugation to a carrier may avoid substrate adsorption artifacts (cf. Refs. 13 and 14).

An equally important property of such peptides is their capacity for competitive inhibition of adhesion or of other processes involving the native protein, e.g. to compete for the ligand in cell attachment and spreading assays or to compete for binding of radiolabeled ligand to a cell surface receptor (9–11, 14, 15). Both adhesion and competitive inhibition assays demonstrate specificity for such peptides when comparing a series of peptide substitutions. For example, RGDS-containing peptides are active in the binding of the central cell-binding domain of fibronectin by α5β1, α5β3, and α6β1, whereas RGES-containing peptides are much less active (10). In contrast, LDV-containing peptides are active for the α5β1 receptor, whereas LEV-containing peptides have minimal activity (15).

Analysis by Mutagenesis or Natural Mutation—The most direct test for function of a putative peptide recognition sequence is mutation. Deletion or mutation of RGD to RGE in fibronectin cDNA expressed as a fusion protein in bacteria leads to a loss of activity (17). Interspecies comparisons of evolutionarily mutated sequences can also be enlightening; although the LDV sequence is present in fibronectins from a variety of species, the REDV adhesion sequence is missing from chicken fibronectin (15, 18). Surprisingly, even though the RGD sequence is present and potentially functional in chicken tenascin and mouse laminin, it is missing from mouse tenascin and moved in position in human laminin (19–22); the RGD sequences in tenascin and laminin may thus be fortuitous or used variably. The CS1 alternatively spliced sequence appears to contribute 40% or much less of the activity of intact fibronectin depending on the cell type and the system for analyzing its activity (23–26).

Loss of Avidity and Specificity in Synthetic Peptides—Although peptide sequences such as RGD and LDV appear to be critical minimal sequences, they do not function alone. Competitive inhibition assays and avidity determinations show that these sequences by themselves are substantially less active than either the native protein or fully active fragments (9, 11, 27). The RGD sequence has about 100-fold less activity than intact fibronectin according to binding assays (27), and the LDV sequence is up to 25-fold less active than the complete CS1 sequence of 25 residues (15). Since the RGD sequence can also inhibit the function of receptors that do not normally bind to fibronectin (1–4), this minimal adhesion sequence alone also appears to lack information determining receptor specificity.

Synergistic or Helper Sequences—Recent studies indicate considerable complexity in the effects of other regions of the molecule on the function of minimal adhesion sequences (15, 17, 24–33). The simplest case so far appears to be that of the LDV sequence in the alternatively spliced CS1 region of fibronectin. The full-length CS1 peptide displays an impressive 40% of the full activity of fibronectin (14). Truncation of the 25-mer CS1 sequence leads to a gradual loss of activity, until reaching the LDV sequence which shows a 20–25-fold loss of activity (15); structural determinations and amino acid substitution studies should help elucidate why activity is lost during truncation.

The well studied RGD sequence is dependent on additional polypeptide information for full function, but studies to date can be interpreted as supporting roles for such sequences in either conformational stabilization or function as a second binding region (1–5). Cyclizing the RGD sequence can dramatically increase its effectiveness for interacting in vitro with vitronectin receptors, but not with fibronectin receptors (34). Moreover, antibodies can recognize different conformations or environments of the RGD sequence in different proteins (35, 36). These studies support the importance of conformation of the RGD sequence.

On the other hand, mutagenesis studies have located key functional polypeptide regions considerable distances away from the RGD site, as far as 14,000 and 28,000 daltons toward the amino terminus (17, 29–32). Deletions show 100–200-fold less activity than the native protein, consistent with a synergistic function (Refs. 17 and 32, but compare Ref. 30). Weak synergism can even be found between such sequences and the RGD sequence when each is located in separate polypeptides (17). Moreover, a monoclonal antibody binding to an epitope mapping about 15,000 daltons away from RGD blocks cell adhesive functions, whereas another monoclonal binding closer to the RGD site does not inhibit, ruling out steric inhibition (31).

Interestingly, the absolute distance between at least one of these sequences and the RGD sequence may also be important,
since moving a putative synergistic sequence away from the RGD sequence causes a major loss of biological activity (30). Taken together, these and other studies suggest that both RGD conformation and external sequence information contribute to interactions of the RGD sequence with the \( \alpha \beta_1 \) fibronectin receptor.

Function of such "synergistic" sequences is also important for cell migration mediated by a fibronectin substrate (24, 31) and for assembly of fibronectin into extracellular fibrils (31). It is obvious that the three-dimensional structure of fibronectin needs to be determined to complement these functional studies.

Other Recognition Sequences in Fibronectin—Besides the CS1 site, another alternatively spliced region that contains cell adhesion activity is the REDV sequence (13). This sequence, like LDV, is recognized by the \( \alpha_5 \beta_1 \) integrin receptor (8). It has been suggested that the presence of a common D (Asp) residue in RGD, LDV, and REDV may reflect its use as part of hypothesized cation-binding structures found in a number of integrins (37).

The high affinity heparin-binding domain of fibronectin also contains sequences that show adhesive activity, either when adsorbed to substrates or when used as competitive inhibitors of cell attachment (38, 39) (Table I). These sequences may function independently of the CS1 sequence, and they bind to heparin.

Laminin: A Surprising Number of Active Peptides

The glycoprotein laminin is a prominent constituent of basement membranes and can serve as an adhesion protein for a variety of cell types, especially epithelial and neuronal cells (reviewed in Refs. 6 and 7). A number of peptides with attachment activity has been derived from this protein (Table I, Fig. 3). The first sequence reported from this protein was the YIGSR sequence (43).

FIG. 1. Schematic model of several cell interactions with extracellular glycoproteins via specific recognition sequences. Cells can use integrin receptors (as depicted here) or other types of receptors to interact with specific peptide regions of ligands. Integrin receptors consist of heterodimers with one \( \alpha \) and one \( \beta \) subunit, each of which can determine ligand specificity. For example, \( \alpha_5 \beta_1 \) can bind collagen, while \( \alpha_5 \beta_3 \) binds fibronectin; at least 17 different vertebrate integrins have been described so far. Some of their cell adhesion ligands such as fibronectin can contain multiple sites involved in recognition by several different integrin receptors. These receptors can participate in a number of biological processes, including adhesion, migration, and assembly of a fibronectin matrix. Matrix assembly may involve other molecules (\( M \)) and is accompanied by clustering of receptors and assembly of intracellular microfilament bundles (thin lines).
The same plasma membrane receptor is used for the intact protein as for similar activities are present in the intact protein and are retained in progressively truncated proteolytic fragments or recombinant proteins. Synthetic peptides competitively inhibit function, and biological activity is lost after site-directed mutagenesis. The rather large number of proposed recognition sequences reported to date in laminin is puzzling (at least 8 have been reported so far, Table I). It will be more reassuring when more of the criteria listed in Table II are applied to each of these sites in laminin (and fibronectin) to establish the biological relevance of each. The activities of different sites for different cell types also need further evaluation. Finally, it will also be important to learn which receptor(s) recognize each sequence and the biological sequelae of binding.

Other Adhesive Extracellular Matrix Proteins

A number of other proteins can mediate cell adhesion or serve as cell migration substrates. Many of them contain the RGD sequence, e.g. vitronectin, fibronogen, von Willebrand factor, entactin, thrombospondin, and collagen (Table I). Since these proteins are bound by specific integrin receptors, the roles of other sequences in determining specificity requires elucidation. A variety of novel adhesive recognition sequences are also present in these and other proteins (Table I); the relative importance of each in vivo remains to be established.

Sequences in Receptors and Cell-Cell Adhesion Molecules

Surprisingly, a short peptide from the αIIβ5 integrin retains the capacity to bind directly to fibronogen, while retaining RGD specificity; this Thr-Asp-Val-Asn-Gly-Asp-Gly-Arg-His-Asp-Leu peptide is highly conserved among integrins (61). Besides their involvement in cell-substrate interactions, adhesive recognition sequences may also be involved in recognition events involving certain cell-cell adhesion molecules. The octapeptide Tyr-Lys-Leu-Aas-Val-Asn-Asp-Ser inhibits aggregation of Dictyostelium mediated by the glycoprotein 80 adhesion molecule and can mimic its homophilic binding in vitro (62). The tripeptide sequence His-Ala-Val present in a variety of cadherin molecules inhibits mouse embryo compaction (63). These promising findings suggest that cell-cell adhesion mechanisms may also have a component involving recognition of a short peptide sequence.

Synthetic Peptides as Probes of Biological Functions

Since synthetic peptides can specifically inhibit the function of adhesive recognition sites, they can be used to test the roles of these sites (and of the protein as a whole) in living animals. Roles for RGD-dependent processes have been identified for gastrulation, neural crest cell migration, and experimental metastasis (Ref. 64, reviewed in Refs. 1-6). Similarly, the YIGSR peptide has been reported to inhibit experimental metastasis and migration of neural crest cells (reviewed in Refs. 1 and 7). Platelet functions such as attachment to extracellular matrix proteins and aggregation in suspension are inhibited by RGD peptides.

Table II

| Synthetic peptides containing the sequence display activity after conjugation to a carrier, even if inactive when adsorbed directly on substrates. | Synthetic peptides competitively inhibit function of the intact protein. Biological activity is lost after site-directed mutagenesis. | Anti-peptide antibodies inhibit function of the native protein. The same plasma membrane receptor is used for the intact protein as for synthetic peptides. |
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which block interactions with the α5β1 (glycoprotein IIb-IIIa) receptor (reviewed in Refs. 1–4 and 8).

**Signaling Activities of Synthetic Peptides**

Besides serving as competitive inhibitors of cell adhesion and migration, synthetic peptides containing adhesive recognition sequences appear to signal certain metabolic responses directly. RGD-containing peptides can induce cell-cell adhesion of segmental plate cells in vitro and promote somite formation in vivo (65), perhaps by increasing expression of cell-cell adhesion molecules. RGD-containing peptides can also dramatically signal the secretion of certain proteases such as collagenase and stromelysin from cultured cells (66).

Certain peptides from laminin containing the IKVAV sequence can display strong biological activities, some of which may be distinct from those of the intact molecule. The PA-22-2 peptide and promote the metastatic process via increased cell invasion (68). It is conceivable that this sequence may function in some cases only after proteolytic degradation of laminin, e.g. in promoting angiogenesis after tissue destruction.

**Summary and Perspective**

The importance of short peptide recognition sequences in binding to cell surface receptors such as the integrins during cell adhesion has been well established. Less clear, however, is the manner in which additional polypeptide sequences in some proteins such as fibronectin function to enhance or synergize with such sequences and to provide receptor specificity. Such contributions are important to elucidate, since they can account for 100- to 200-fold differences in biological activity. Although a number of short adhesive recognition sequences has been proposed, their overall patterns of cell-type specificity remain to be determined. Some of these sites may be cryptic or otherwise inactive in the native protein and may therefore require proteolysis of the molecule for function. Some putative recognition sequences may ultimately fail the functional tests listed in Table II.

The receptors for most of these sequences still remain to be determined, and the relationship of receptor expression to cell-type specificity also remains to be clarified. The use of these peptides as probes of biological functions in living animals has established the general importance of certain sequences in the recognition and adhesion of cells. Certain peptides from laminin containing the IKVAV sequence can display strong biological activities, some of which may be distinct from those of the intact molecule. The PA-22-2 peptide and promote the metastatic process via increased cell invasion (68). It is conceivable that this sequence may function in some cases only after proteolytic degradation of laminin, e.g. in promoting angiogenesis after tissue destruction.

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