Journal of Cellular Biochemistry 61:562–568 (1996)

Carbohydrate–Carbohydrate Interactions in Adhesion

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Abstract Cell–cell interactions play an important role in the development, maintenance, and pathogenesis of tissues. They are highly dynamic processes which include migration, recognition, signaling, adhesion, and finally attachment. Cells on their pathway to a final location have to pass and interact with their substratum formed of matrix and cell layers. Testing and recognition are important keys for the proper result of tissue formation. They can, however, also lead to diseases when they are misused in pathological situations, by microorganisms or malignant cells, for instance.

Carbohydrates, which are the most prominent surface-exposed structures, must play an important role as recognition molecules in such processes. The rich variability of carbohydrate sequences which cell surfaces can present to lectins, adhesion molecules, and other ligands creates a refined pattern of potential attachment sites. The subtle control of the surface presentation density can provide variations in attachment strength. Not only the carbohydrate sequences but also the fact that carbohydrates can be branched while proteins cannot and that the oligosaccharide chains can be attached to the protein backbone in different densities and patterns will create yet more interaction possibilities.

Maximal use of the combinatorial richness of carbohydrate molecules would be made when carbohydrate sequences could interact with other carbohydrate sequences. Such interactions have only very rarely been considered for biochemically and biologically relevant situations since they are difficult to measure. A few are known and will be summarized here with the hope that this wealth of possible chemical interactions may be considered more and more by surface cell biochemists when analyzing fine tuning in cellular interactions.

Key words: cell adhesion, cell recognition, proteoglycan, carbohydrate, polyvalence, sponge

Cell adhesion as an instrument for the formation and maintenance of a functional tissue is a process which can be modulated. Intermediate strength of attachment and release must be granted to migrating cells in order to allow them to test their pathway. Cells of the lymphoid system, for instance, find their homing centers after a rolling process based on intermediate adhesion to the vessel wall. During embryogenesis adhesive connections are built up and destroyed again. Filopodia of migratory cells test the substratum of their surroundings and the result of this testing is converted into signals to direct the migration of the moving cell [Burger, 1979]. In contrast to firm adhesions between stationary cells formed by stable protein complexes in the form of tight junctions, such short-term adhesions must be of reversible, but nevertheless specific, nature. What are the molecular mechanisms underlying such phenomena?

Carbohydrates are versatile structures and a multitude of different sequences can be created by a limited number of enzymes only. Furthermore, carbohydrate–protein interactions are characterized by fairly weak forces which, however, can easily be potentiated by orders of magnitudes when multimerized [Bundle and Young, 1992]. Glycans are therefore predestined to serve as crucial molecules at the moment of the encounter between two cells [Burger, 1979]. Given the presence of carbohydrates on cell surfaces of all multicellular organisms as well as on pathogens from viruses to bacteria, the question should not be their involvement as such but their precise role in such encounters.

Carbohydrates have been recognized as interaction sites in cell adhesion, whether this be lymphoid cell homing [Springer, 1990], tissue maintenance, or host–pathogen interaction [Karlsson, 1989]. Most of these interactions are

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Received April 24, 1995; accepted December 7, 1995.

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attributed to lectin or lectin-like molecules and their specific carbohydrate ligands. The strategy by which the disadvantage of the low affinity of many of such single-cell recognition sites is overcome consists of arranging multiple lectin molecules in clusters and concentrating the carbohydrate motives in patches [Sharon and Lis, 1989; Feizi et al., 1994; Varki, 1994].

In this review we focus on direct interactions between carbohydrate sequences. Such carbohydrate–carbohydrate interactions can easily be modulated by enzymes and divalent cations, and they represent a highly versatile form of recognition and adhesion-promoting complexes [Spillmann, 1994], which have barely been considered by either cell biologists or carbohydrate biochemists.

CARBOHYDRATE INTERACTIONS IN STRUCTURAL COMPONENTS

Classically, carbohydrates are believed to be space fillers and structural components. In microbes and plants carbohydrate structures are widely used to create stable cell walls. A very simple form of carbohydrate–carbohydrate interaction can be found in plants already, namely the formation of cellulose microfibrils where hydrogen bonds crosslink and stabilize individual polyglucose chains with one another. Other components of plant cell walls, namely the more heterogeneous hemicellulose and pectin chains, are similarly interacting with one another to participate in the plant cell wall architecture. Regular sequences within the carbohydrate chains have the capacity to form helical bundles. These structures are interrupted by irregular sequences that lack such properties. In this way, a single sugar chain can form bundles with more than one other chain to provide a three-dimensional network [Bryce et al., 1974]. Agar is another example of such a network created from regular and random stretches of alginate chains which form a hydrated, elastic gel and which represent a classical example for functional carbohydrate–carbohydrate interactions.

In all of these examples rather simple, homogeneous chains of carbohydrates interact directly with neighboring chains of carbohydrates. The presence of repetitive, interactive sequences provides enough avidity to create stable structures. In many of these simpler structures, cations—often Ca^{2+}—contribute to the formation of the associating superstructures.

The extracellular matrix of higher eukaryotic tissues consists of an assembly of highly glycosylated molecules [Wight et al., 1992]. Isolated glycosaminoglycan chains have for instance been found to form helical structures with other glycosaminoglycans *in vitro* [Fransson and Cöster, 1979; Fransson et al., 1983]. When glycosaminoglycan chains were immobilized on beads, thereby reconstituting their natural appearance on a protein core, different types of glycosaminoglycan chains showed specific interactions [Turley and Roth, 1980]. Whether such specific glycosaminoglycan–glycosaminoglycan interactions *in vitro* do reflect a physiologically relevant structural or a recognition-mediating mechanism in the extracellular matrix remains to be shown. The presence of a large number of glycan chains and the functions of the extracellular matrix to provide both a structural support as well as a communication mediator for cells should have raised some considerations about carbohydrate–carbohydrate interactions a long time ago. Whether they reach the degree and the relevance already now assigned to the countless protein–carbohydrate interactions in the extracellular space remains to be seen.

CARBOHYDRATES IN SPONGE CELL ADHESION

The first time cell–cell recognition has been demonstrated was on marine sponge cells. Since the beginning of the century marine sponges have been used as models to elucidate the secrets of recognition and adhesion in multicellular organisms [Wilson, 1907; Galtsoff, 1925]. The limited number of cell types and extracellular components has been a considerable advantage for the analysis of the recognition and adhesion processes in the sponge organism. The extracellular matrix of the sponge contains proteoglycan-like complexes, collagen, and other glycoproteins just as higher eukaryotes do. The question of which were the adhesion-mediating molecules for reaggregation of dissociated sponge cells could be resolved when a glycosaminoglycan complex was isolated from *Microciona prolifera* [Humphreys, 1963]. A large, EDTA-sensitive molecular complex containing at least 50% carbohydrate by weight [Humphreys, 1967; Henkart et al., 1973; Cauldwell et al., 1973] turned out to mediate species-specific reaggregation of dissociated sponge cells in the presence of calcium ions [Humphreys, 1963; Jumblatt et al., 1980]. The complex appears as a sunburst in

electron microscopy [Humphreys et al., 1977], consisting of a ring of ≈200 nm diameter to which up to 20 arms are attached [Dammer et al., 1995]. This aggregation factor or adhesion proteoglycan [Misevic and Burger, 1993] contains several hundred Ca^{2+} binding sites [Cauldwell et al., 1973] which are important for the structural integrity of the complex and are functional in mediating factor self-interaction [Jumblatt et al., 1980].

The aggregation factor contains two distinct types of binding sites [Jumblatt et al., 1980], one binding to a cell surface receptor [Varner et al., 1988] which is independent of Ca^{2+} and one for self-association which is Ca^{2+} dependent and which provides the intercellular adhesive force [Jumblatt et al., 1980]. A participation of the carbohydrate part in the adhesion process was anticipated after it was found that functional activity was lost after glycosidase treatment [Turner and Burger, 1973; Misevic and Burger, 1990b] or periodate oxidation [Jumblatt et al., 1980] of the adhesion proteoglycan. Direct evidence for the involvement of the glycan chains came from reconstitution experiments with isolated, protein-free glycan chains, provided they were repolymerized into a multimeric complex [Misevic et al., 1987].

MULTIVALENCY OF INTERACTIVE CARBOHYDRATES AND CARBOHYDRATE–CARBOHYDRATE INTERACTIONS IN SPONGES

Ca^{2+} is essential for aggregation of the glycans and cannot be replaced by other divalent cations [Rice and Humphreys, 1983]. However, polycations as polybrene or polylysine are able to replace Ca^{2+} at much lower concentrations, indicating a cooperative effect of the polycations with the polyanionic carbohydrate chains. The quantitative differences in interactive strength of various polycations most likely reflect the variations around an optimal fit of the complementary polycations to the polyanionic glycan chains of the aggregation proteoglycan, as demonstrated by phase-partition assays [Burkart and Burger, 1981]. The necessity for polyvalent interactions was corroborated by the demonstration that single glycan chains could not interfere with aggregation or with factor binding, but that for species-specific binding the glycopeptides had to be polymerized again [Misevic et al., 1982; Misevic and Burger, 1986].

To mediate cell or bead aggregation there must be either a functionally intact aggregation factor present or the fragmented glycans have to be repolymerized to create *de novo* polyvalence through crosslinking of isolated glycan chains with diepoxybutane/glutaraldehyde or by attachment to beads [Misevic and Burger, 1986; Misevic et al., 1987]. Whereas many different polycations are able to precipitate the intact factor glycans, the self-interaction of glycans under physiological conditions, i.e., in the presence of Ca^{2+} ions, is restricted to glycan chains of the same species. These findings indicate that the self-interaction of the glycan chains is not due to unspecific charge interaction of polyionic chains. Rather, the glycans contain a species-specific arrangement of residues in a proper spacing of interactive sites, whether these are single charged residues in a defined distance [Burkart and Burger, 1981] or sequences of several residues [Spillmann, 1994]. Monoclonal antibodies which inhibit the glycan-mediated self-aggregation of factor molecules [Misevic et al., 1987; Misevic and Burger, 1993] are directed against distinct carbohydrate motives [Spillmann et al., 1993, 1995], favoring the sequence model. Observation of crossreactivity of some of the monoclonal antibodies with glycans from different species [Misevic et al., 1987] and the observation that different sponge-type cells may first aggregate randomly before sorting out [Burger, 1979] could easily be explained by the different arrangement of otherwise identical or practically identical motives.

Molecules involved in cell–matrix and cell–cell adhesion have mostly association constants above $10^5\ M^{-1}$ since they are mono- to oligovalent. Potential adhesion-mediating molecules with poor association constants have so far been mostly neglected. At first, we have also questioned the biological relevance of a glycan of $M_r = 6.3 \times 10^3$ from the sponge adhesion proteoglycan which bound only with a $K_a \leq 10^3\ M^{-1}$ to its own cell. When we reconstituted approximately the proteoglycan size ($M_r = 2 \times 10^7$) by polymerizing the glycans, binding could be raised, however, by more than six orders of magnitude ($1.6 \times 10^9\ M^{-1}$), restituting thereby essentially the full biologically relevant binding strength and specificity [Misevic and Burger, 1990a].

These conclusions were reached for interactions between an oligosaccharide epitope of the aggregation proteoglycan and the cell surface receptor protein (Fig. 1).

When searching for the chemical nature of the factor–factor interaction we decided to profit

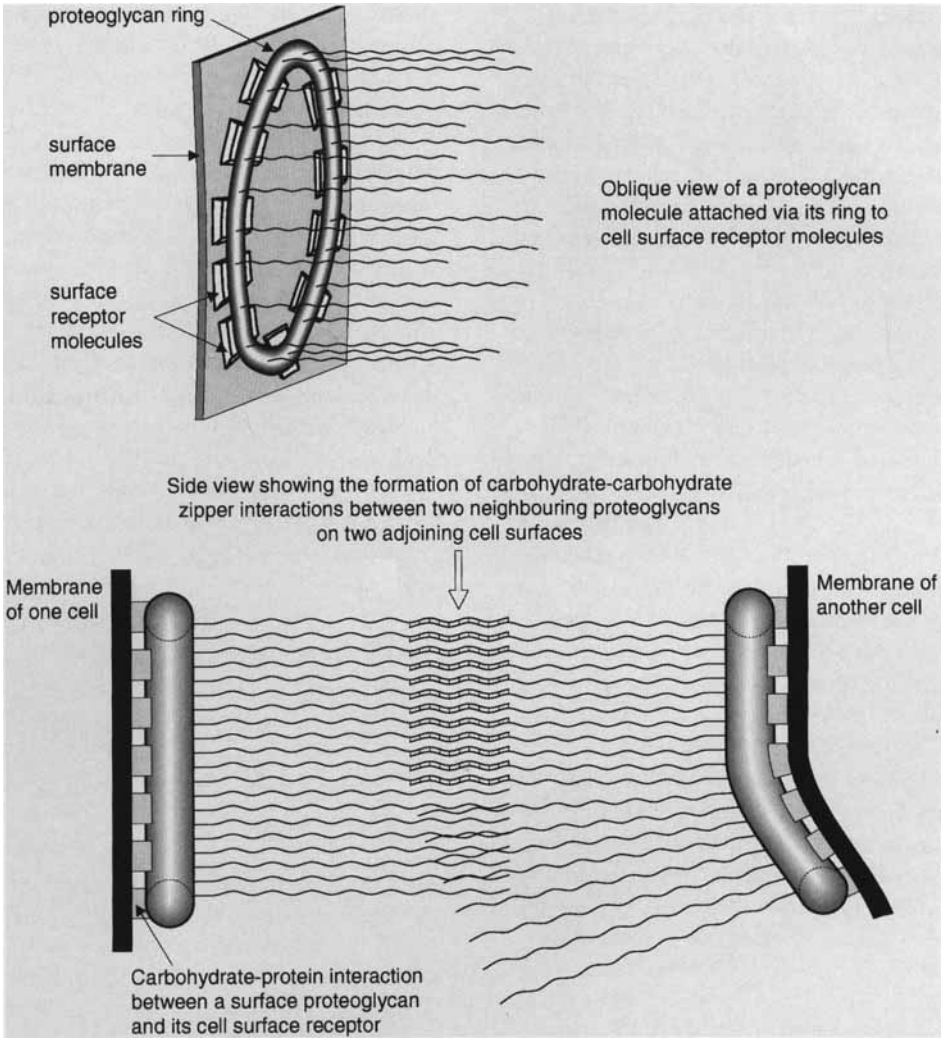

Fig. 1. Model of polyvalent zipper interactions between surface–bound adhesion molecules in sponges. A proteoglycan molecule ($M_r = 2 \times 10^7$) interacts in a Ca^{2+}-independent mode with cell surface receptor molecules ($M_r = 6.8 \times 10^4$) via short glycan chains located on a ring structure of 200 nm diameter. The long arms (≈ 180 nm) of the proteoglycan extending from the ring backbone adhere in a Ca^{2+}-dependent carbohydrate–carbohydrate interaction mode to the symmetric structures of a proteoglycan molecule attached to a neighboring cell. Closure of the double zipper (between a pair of glycan arms as well as between the 20 successive pairs of a proteoglycan molecule) provides through its cooperativity by far enough strength for sponge cell aggregation, even though the individual carbohydrate epitope interaction is fairly weak and was so far neglected as a potentially powerful cell–cell interaction force.

from this experience since no glycans could be found which inhibited the Ca^{2+}-mediated interaction. Despite the fact that the glycan involved in factor–factor interaction was 33 times larger than the glycan interacting with the cell surface and thus should have been quite polyvalent, only very poor binding to the aggregation factor could be detected. However, when the glycan was polymerized with diepoxybutane/glutaraldehyde to approximately the size of the native aggregation factor, not only interaction with the aggregation proteoglycan could be shown but homophilic carbohydrate–carbohydrate interaction in solution as well as when the pure glycan was attached to beads [Misevic and Burger, 1993], confirming earlier proposals and data [Burkart and Burger, 1981].

In the carbohydrate–carbohydrate model of cell interaction (Fig. 1), advantage is taken of single low-affinity sites. Control of the polyvalence by various means (surface density of presented structures, ionic strength to modulate attractive *vs.* repulsive forces, subtle changes in biosynthesis of the carbohydrate sequences, etc.) to change the affinity of the interactive molecules provides a highly adaptable system be-

yond a structural scaffold. Recognition systems are thereby created which allow different cells to test surrounding surfaces and release or reinforce interactions [Burger, 1979]. Species-specific sorting out is one example of the value for moderate interaction strengths which achieve biological relevance through polyvalence. Moderate strength is also required within one species, i.e., within an individual sponge, otherwise the cells could not migrate past each other. In an experiment where factor-mediated aggregation was induced by polybrene instead of Ca^{2+}, secondary migration of cells was inhibited. This observation demonstrates the drastic effect of "locking" the molecules in a permanent tight *interaction mode* [Burkart and Burger, 1981].

The zipper can be used as a simple model to highlight the value of such carbohydrate–carbohydrate interactions and to demonstrate the simplicity by which nature may create specificity, determining differences from compositionally rather similar structures [Spillmann, 1994]. The creation of repetitive, interacting glycan sequences as such is feasible in different modes. First, oligosaccharide motives can be repeated along the primary glycan sequence; second, glycans can be arranged in a repetitive pattern along a protein backbone structure not directly participating in binding; and finally, the proteoglycans, glycoproteins, or glycolipids can be presented in clusters or surface superstructures partly due to mobile anchors. Only in this last version is advantage taken of the fluidity of the biomembrane for control of the surface density of these glycans and therefore their avidity [Kojima, 1992; Spillmann, 1994; Varki, 1994].

CARBOHYDRATE–CARBOHYDRATE INTERACTIONS IN HIGHER EUKARYOTES

In higher eukaryotes glycolipid recognition seems so far to be involved in a specific instance during mouse embryogenesis. The compaction of early morula stage mouse embryos can be disrupted by multivalent Lewis x (Le_x) carbohydrate determinants, but not by the monomeric Le_x antigen [Fenderson et al., 1984]. During the same stage of mouse embryogenesis the SSEA-1 antigen, identical to the Le_x antigen, is at its maximal expression level on the cell surfaces [Solter and Knowles, 1978]. Analysis for Le_x ligands in F9 mouse teratocarcinoma cells have revealed the presence of Le_x on glycolipids [Eggens et al., 1989] and protein [Kojima et al., 1994] as receptor molecules. As model studies revealed specific interactions between Le_x glycolipids on liposomes and Le_x glycolipid-coated plates [Eggens et al., 1989], carbohydrate–carbohydrate interaction is suggested to mediate at least the primary recognition and adhesion process between mouse morula cells. Similarly, G_{M3}-ganglioside on B16 mouse melanoma cell surfaces seem to interact with G_{g3} glycosphingolipids on mouse lymphoma cells since interactions could be abolished with sugar-specific antibodies and since the two glycolipids interacted specifically in a liposome-monolayer assay [Kojima and Hakomori, 1991]. The interactive capacity of specific glycolipids is not restricted to a positive effect, i.e., binding, but shows also the opposing, i.e., repelling, effect [Hakomori, 1990] and is dependent on the environmental stress factors as tested in a fluid system with high shear forces copying the situation in vessels [Kojima et al., 1992].

PERSPECTIVES

The combination of the highly variable carbohydrate chains which offer simultaneously flexible, ordered, and easily modulatable motives for recognition and interaction together with the possibility of controlled forces in the form of multiple low-affinity interaction sites is difficult to follow experimentally but probably of considerable value in nature. The number of carbohydrate sequence permutations created by a rather limited number of enzymes is manyfold higher than that in any other biopolymer including proteins. The specific arrangement of such sequences creates interactive patches, whether in linear stretches on glycosamino- or other glycans, on core proteins, or on freely movable lipid anchors, to interact either with other carbohydrate structures [Spillmann, 1994] or, with carbohydrate-recognizing proteins like lectins [Varki, 1994]. Such primary carbohydrate–carbohydrate interactions during migration and homing of cells could be both specific and easy to control by external influences like ionic conditions, shear forces, substrate supply, or gene activation/repression. This concept goes beyond the classical function of glycans as intercellular space fillers and storage molecules only. The molecular mechanisms of these interactions are open to elucidation. Molecular modeling has shown complementarity of different sequences that allow interactions based on a combination of hydrogen bridges and hydrophobic interactions. Ionic interactions on the other hand are suggested by the involvement of divalent cations (primarily Ca^{2+}) not only in the sponge system but also in the mouse embryo and melanoma cell

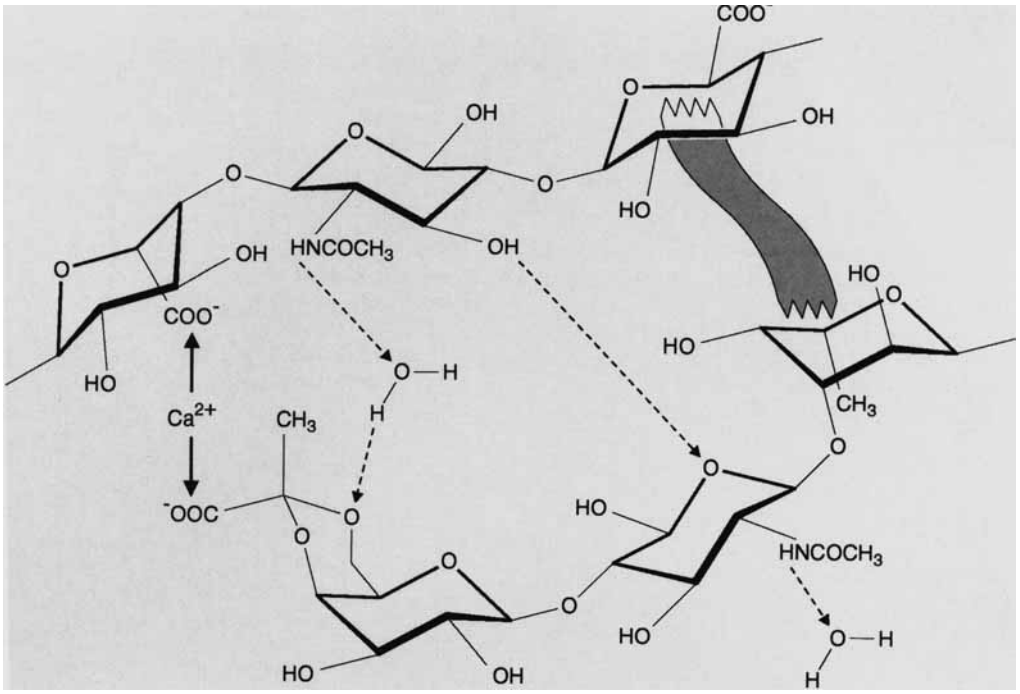

Fig. 2. Schematic drawing of stabilizing forces between two carbohydrate chains in sponge glycans. Possible hydrogen bridges (arrows with dashed lines), hydrophobic surfaces (shaded area), and ionic interaction (Ca^{2+} between arrows) sites are sketched between two carbohydrate chains. Ca^{2+} is thought to stabilize conformations which lead to hydrogen and hydrophobic interactions. Interactions do not have to occur between different oligosaccharide sequences of a glycan as depicted; they can also occur between identical sequences.

system. An example for all three types of interactions is depicted in Figure 2 for sponge glycan sequences.

How these different mechanisms may be used in nature and how relevant low-affinity carbohydrate–carbohydrate *vs.* the classical carbohydrate–protein interactions, for instance, may turn out to be will be interesting to follow and remain to be elucidated. Physicochemical approaches [Dammer et al., 1995] as well as the availability of monoclonal antibodies [Feizi, 1985; Misevic et al., 1987; Hakomori, 1991], the characterization of their carbohydrate epitopes [Spillmann et al., 1993, 1995], and their synthesis [Ziegler, 1994] will help in the future analysis of these phenomena.

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