9 The Biochemistry and Mechanics of Gastropod Adhesive Gels

Andrew M. Smith

9.1 Introduction

A wide variety of organisms attach to surfaces using gels as glues, but the mechanism by which a gel can form a strong attachment has only recently been studied in depth. The adhesive gels used by animals are unusual biomaterials. Their structure and properties are strikingly different from common commercial glues. Commercial glues are generally solids; they may be applied in liquid form and then solidify, or they may be deformable, tacky solids (Wake 1982). In either case, their final form consists entirely of polymers or crosslinked material. In contrast, adhesive gels typically consist of dilute polymer networks that contain more than 95% water. These gels are highly deformable. One would not expect such a dilute hydrogel to be suited for adhesion. In fact, dilute polymer gels are often excellent lubricants. Nevertheless, a wide array of animals use such gels as powerful glues (Smith 2002).

Because they are gels, these glues have a variety of interesting and useful properties. Foremost among these are their great flexibility and their ability to bond to wet, untreated surfaces. Furthermore, despite being dilute and easily deformable, these adhesive gels provide surprisingly strong attachments. Snails such as limpets can be extraordinarily difficult to detach by hand. They use gels to create tenacities (attachment force per unit area) ranging from 100 to 500 kPa (Branch and Marsh 1978; Grenon and Walker 1981; Smith 1992; Smith and Morin 2002). This approaches the adhesive strength of the solid cements of mussels and barnacles, which is typically 500–1000 kPa (Waite 1983; Yule and Walker 1987).

Since the adhesive gels have unusual properties that would not be predicted for such dilute polymer mixtures, the mechanism by which they work should be interesting. What features of these gels make them such strong adhesives instead of lubricants? The goal of this chapter is to describe the structure and mechanics of adhesive gels focusing on those of gastropod molluscs. Gastropods are particularly interesting because of the diversity and impressive performance of their adhesive gels. They are also notable because a key structural feature has been discovered that appears to control the mechanics of the gels; this is the presence of specific glue proteins that are not found in the

Department of Biology, Ithaca College, Ithaca, New York 14850, USA

Biological Adhesives
(ed. by A.M. Smith and J.A. Callow)

non-adhesive forms of the gels. These glue proteins may crosslink other polymers, thus strengthening the gel and possibly contributing to interfacial adhesion.

9.2 Background

A key step in understanding how snails such as limpets can create strong adhesion with a gel was the recognition that the glue was a different form of gel than the normal mucous gel. It is now becoming clear that there are many ways to construct gels, and these differences give rise to substantial functional differences (Smith 2002). As anyone who has handled an invertebrate can appreciate, the normal mucus covering their outer surface is not inherently sticky. This type of mucus appears to be used during suction adhesion of limpets. If suction is eliminated through a leak, or because the animal is not forcibly contracting the musculature that creates suction, the adhesive strength in shear is low to non-existent (Smith 1991b, 2002). Thus, the mucus that the animals normally crawl on provides virtually no adhesive strength on its own. The mucus that is used in adhesion, though, is different. When limpets glue down in an aquarium, they are easily distinguished from limpets that are not glued down (Smith 1992). In addition to having a high shear tenacity, detachment of limpets that are glued down occurs abruptly and usually leaves a thin film of gel stuck firmly to the glass. One can remove this gel with a razor blade to get an elastic mass that is unlike the loose slime that many snails produce across their general body surface. Thus, it is likely that there are substantial structural differences between these gels. Indeed, there are probably a wide variety of invertebrate gels. The use of the term mucus, therefore, is probably misleading in that it implies a unity of structure that does not appear to exist.

9.3 Adhesive Gels Used by Different Animals

There are probably a wide variety of animals that use adhesive gels. Many echinoderms adhere using secretions that are described as containing either mucopolysaccharides or protein (Flammang 1996; Chap. 10, this volume). A wide variety of worm-like invertebrates also adhere using such viscous secretions (Hermans 1983). Any time a polymeric adhesive secretion contains a high percentage of water and is easily deformable but strikingly viscous and even elastic, it is likely to be a gel. Some species of frogs can produce a sticky gel that is markedly different from common mucous secretions (Graham et al., Chap. 11, this volume). Many microscopic organisms also adhere with gels (Callow and Callow. Chap. 4, this volume).

Adhesive gels have been studied in depth for four gastropod molluscs. These represent a range of habitats and functions. Of these, the attachment of limpets has been studied for the greatest length of time. Most limpets live on rocky intertidal coasts. Limpets in the genus *Lottia* use their adhesive gel to glue down when they are exposed and inactive during low tide (Smith 1992). The adhesive strength protects them from dislodgement by predators such as shorebirds (Hahn and Denny 1989). When the tide returns, the limpets typically become active and at this point rely on suction for adhesion (Smith 1992). The adhesive might also be used instead of suction to attach when wave surge is particularly strong. It is likely that other limpets also alternate between attachment mechanisms, though the cues may be different.

The marsh periwinkle, *Littoraria irrorata,* can also produce adhesive and non-adhesive gels. These snails forage along mud flats, but when the tide returns they climb marsh grass stems and glue the lip of their shell down. In this way, they avoid aquatic predators such as crabs and fish (Warren 1985; Vaughn and Fisher 1988). When the tide recedes they break their adhesion and return to the mud flats. The shear tenacity created by their adhesive gel can exceed 100 kPa. This is an order of magnitude greater than the tenacity these snails create using suction and any viscous contributions from the mucus they crawl upon (Smith and Morin 2002). As with limpets, the adhesive gel is surprisingly elastic, and significantly firmer than the mucus the animal crawls upon (Fig. 9.1A,B). One difference from limpets is that the glue forms a thin strip along the edge of the shell, while limpets secrete the glue under the sole of the foot. This means that periwinkle glue, unlike limpet glue, is exposed to the elements. Thus, it may dry into a solid sheet in warm, dry weather. In some species of periwinkle, such as *L. aspera*, the glue may always dry (Denny 1984) while in others it may typically stay gelled. The peak force required to detach marsh periwinkles using the gelled glue, though, is not significantly different from that of the dried film (Smith, unpublished). If anything, the flexibility of the gel may provide better adhesive performance by absorbing energy during detachment rather than failing as a brittle solid. While *L. irrorata* has been studied in depth, many other periwinkles also use glues to attach to rocks or vegetation, often switching between active and inactive states.

Some land snails such as *Helix aspersa* also glue the lip of their shell onto the substrate during periods of inactivity. In this case they stay glued for longer periods, sometimes months, estivating until conditions are sufficiently moist (Wells 1944; Campion 1961; Barnhart 1983). Unlike marsh periwinkles, the glue always seems to dry into a tough film. It also forms a seal around the entire circumference of the opening instead of just the anterior end. Whereas the marsh periwinkle glue functions solely in holding the animal's position above the water, the land snail's glue also plays an important role in desiccation resistance by limiting airflow into the area under the shell (Campion 1961; Barnhart 1983). As with limpets and marsh periwinkles, adhesion is not immediate. Marsh periwinkles take roughly 10 min to form the glue (Bingham 1972). The

limpets and land snails that have been studied seem to take at least that long to form the fully functional adhesive bond (personal observation), though limpet feet can become tacky to the touch within seconds (Smith 1991b).

Finally, many terrestrial slugs, such as *Arion subfuscus*, use an adhesive gel as a defensive secretion (Mair and Port 2002). When disturbed, *A. subfuscus* secretes a markedly sticky, orange gel from its dorsal surface. This typically appears as a liquid on the dorsal surface but soon stiffens into a rubbery mass (Fig. 9.1C–E). Like the glues of limpets and marsh periwinkles, it is a gel. It may be slightly more concentrated, but is still roughly 95% water. When used to glue acrylic disks together, it sets within seconds and creates tenacities up to 100 kPa (Smith, unpubl.). As with the other gastropods, the glue was distinct from the normal mucus used in locomotion and lubrication.

It is interesting to note the substantial functional variation among the adhesive gels. Analysis of the adhesive gels of other animals will likely show further variation. In order to understand this variation, it is first necessary to understand gel mechanics in general.

9.4 Principles of Gel Mechanics

A gel is a dilute polymer network within a liquid (Tanaka 1981). The liquid, which is water in biological systems, keeps the polymer network from collapsing. The polymers trap the liquid so that it cannot easily flow. Thus, a gel has solid properties, despite its high water content. Gels are typically viscoelastic; when a stress is applied to them, they have a viscous and an elastic resistance

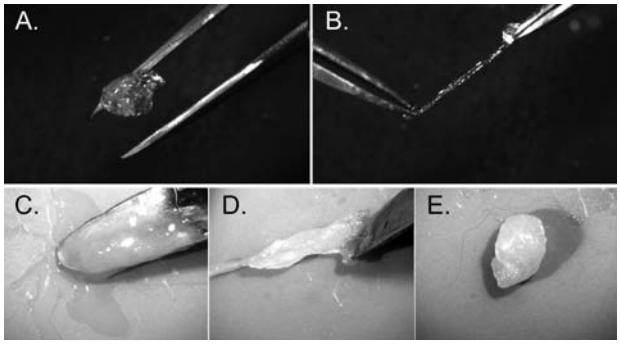

Fig. 9.1. The physical characteristics of two gastropod adhesive gels: **A,B** the glue from the marsh periwinkle *L. irrorata* forms an irregular mass (a) that can be stretched extensively (b). This deformation is reversible. The glue in A and B is held by fine-tip forceps; **C–E** the glue from the slug *A. subfuscus* is often secreted in a form that appears fairly fluid (c), but which sets into an elastic gel that sticks strongly to most surfaces, and can also be stretched extensively (d). This deformation is also reversible (e). In C and D the glue is attached to a metal spatula

to deformation. For a full description of viscosity and elasticity see Wainwright et al. (1976) or Denny and Gosline (1980). In brief, a purely viscous material will flow in response to an applied stress, resulting in a permanent deformation. Higher viscosities give more resistance to flow. A purely elastic material will not flow. Instead, it will deform in proportion to the applied stress and will maintain that stable deformation as long as the force is applied. When the force is removed, the material will spring back to its original configuration. A viscoelastic material may resist deformation elastically at first, but over time the molecules rearrange and flow in response to the stress. Note that these properties depend on the rate of deformation (Wainwright et al. 1976). Gels range from highly viscous secretions that have little elasticity to elastic materials that barely flow (Tanaka 1981).

The ability to gel and the mechanics of the gel depend largely on interactions between polymers (Williams and Phillips 2000). Polymers can entangle or crosslink to form a network that will not dissolve in water. Only certain kinds of polymers are likely to interact in this way, though. If each polymer occupies a relatively small volume because of its size and configuration, it is unlikely to interact with its neighbors, and the solution will not have elasticity (Fig. 9.2A). In contrast, if a polymer occupies a large volume, it may begin to overlap its neighbors at low concentrations (Fig. 9.2B). There will be a critical concentration at which this overlap begins. Once there is overlap, the viscosity of the solution increases markedly and begins to depend on the rate of shear (Williams and Phillips 2000). Very large, extended molecules may begin to overlap near a concentration of 1%, while similar-sized molecules that fold into a compact shape may not overlap until the concentration reaches 20% or higher. Smaller compact molecules may not interact at all even though they become noticeably more viscous (Williams and Phillips 2000). Thus, most gels contain molecules that take on an extended configuration in order to occupy a large volume and achieve overlap. In many cases, these molecules will be unusually large, but that is not always so.

If the only interactions among polymers are through physical entangling due to overlap, the polymer solution will be viscoelastic, but may not solidify to form a classic gel. The behavior is dominated by reptation, which is the untangling process that occurs in response to stress (Doi and Edwards 1988; deGennes and Leger 1982). Initially, the stress deforms the polymers elastically as each polymer is stretched against the resistance of its neighbors. The polymers can flow, though, creeping through the boundaries imposed by their neighbors. The ability of the polymers to move in this way determines the mechanics of the material. To form a more elastic gel, polymers generally form intermolecular crosslinks (Williams and Phillips 2000). When the material is strained, the polymers are deformed, but the crosslinks prevent them from flowing appreciably to alleviate the stress. In this case, the elastic contribution to the mechanics is greater than the viscous contribution, and neither depends much on shear rate (Williams and Phillips 2000). Thus, the material may behave more like a solid, even though it may still consist of over 95% water.

Thus, two major characteristics of polymers that affect the mechanics of the gel are the following; (1) the size and configuration of the polymers, and (2) the ability of the polymers to crosslink. Large, extended molecules entangle to a greater extent and thus have greater difficulty working their way through the twisted path imposed by the network (Fig. 9.2C) (Doi and Edwards 1988). Branching also impedes this flow. Increased concentration also increases the extent of entangling, restricting the movements of the polymers further (Doi and Edwards 1988). If a gel is crosslinked (Fig. 9.2D), the size of the individual polymers does not matter as much. For example, with gelatin, the strength of the gel increases as the gelatin fragments get bigger, up to 100 kD. Increasing the size of the polymers beyond this does not strengthen the gel further (Williams and Phillips 2000). Instead, the elasticity of the gel would depend on the number and strength of the crosslinks (Denny 1983).

The final aspect of gel mechanics to consider is the mode of failure. Because gels are highly deformable, brittle failure through simple crack propagation is less likely than with solids. As cracks form, their leading edges are usually blunted by flow. Thus, a major component of the energy required to break the attachment goes towards deforming the gel (Wainwright et al. 1976). When one of the adhering surfaces is flexible, as is the case with gastropod feet, failure would often occur by peeling (Gay 2002). Even if the surfaces are sufficiently rigid, and pulled directly apart, failure will often not occur uniformly. If cracks do not form and propagate within the glue or along

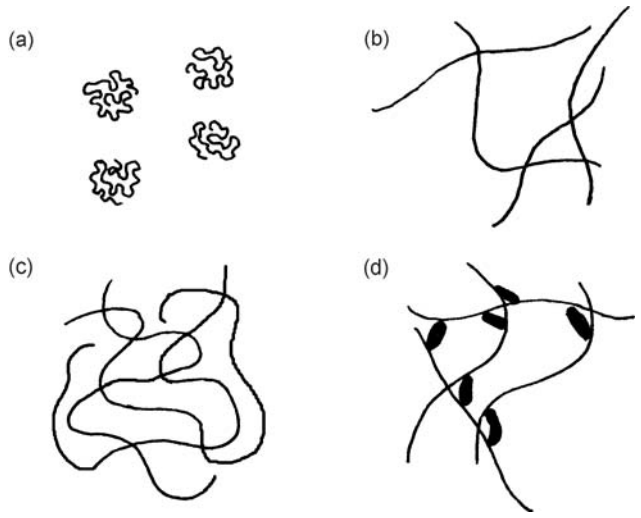

Fig. 9.2. The effect of size, configuration and interactions of polymers on gel mechanics: (a) compact polymers do not interact at low concentrations; (b) if the polymers take on an extended configuration, they are more likely to entangle, increasing viscosity and stiffness; (c) longer polymers entangle to a greater extent, creating a stiffer, more viscous material; (d) crosslinks between polymers can dramatically increase the stiffness of the material

the adhesive interface, failure typically occurs when regions of instability trigger localized flow, resulting in "fingers" of air or water being sucked between the surfaces (Gay 2002). The glue would deform dramatically during this process, dissipating a great deal of energy. In addition, bubble formation by cavitation can occur (Gay 2002). In cavitation, the detaching force creates a reduced pressure in the water that makes up the gel. This may be sufficient to trigger the expansion of microscopic air pockets (Smith 1991a). As with fingering, cavitation would initiate failure, but in doing so would create scattered regions of high deformation and thus energy dissipation. Because of the mechanical strength of the gel, the bubbles would not expand rapidly and form one large bubble, as would happen in pure water (Gay 2002).

An interesting feature of gastropods is that a number of them can use either suction or glue (Smith 1991b, 1992). During suction, the muscles of the foot create a reduced pressure in the water under their foot. Of course, the water is presumably in the mucus layer between the foot and substratum. It is clearly suction, though, as shown by direct pressure measurements and the effect of leaks (Smith 1991b). During suction, the mechanics of the gel do not appear to play a role in adhesion. When the same gastropods glue themselves down, though, the mechanical properties of the gel dominate and failure is more typical of a viscoelastic solid. There will also likely be forms of adhesion that are intermediate between suction and glue, as observed by Smith (1992). For example, when animals use suction, they could easily strengthen the seal with the adhesive gel.

9.5 Adhesive Gel Structure

Unlike most mucus-like secretions, which appear to consist primarily of giant protein-carbohydrate complexes (Denny 1983; Davies and Hawkins 1998), molluscan adhesive gels contain a substantial fraction of smaller proteins. The term "giant polymers" will be used to describe molecules with an apparent size of roughly 1000 kD or more that do not dissociate into subunits under heat and dissociating conditions. Such molecules should tangle easily to form a loose network, and are common in mucus. The importance of smaller proteins, though, has often been overlooked; these proteins appear to play a major role in controlling gel mechanics. The size of these proteins and their amount relative to the giant polymers differs among molluscan adhesive gels (Fig. 9.3). In some cases there are no giant polymers, and only smaller proteins that presumably crosslink. In other cases there is a mixture of giant polymers that may contribute by entangling and smaller proteins that may be involved in crosslinking. In all four species studied, though, the primary characteristic that distinguished the adhesive gel from the non-adhesive mucus was the presence of specific proteins (Fig. 9.4). These were named glue proteins (Pawlicki et al. 2004).

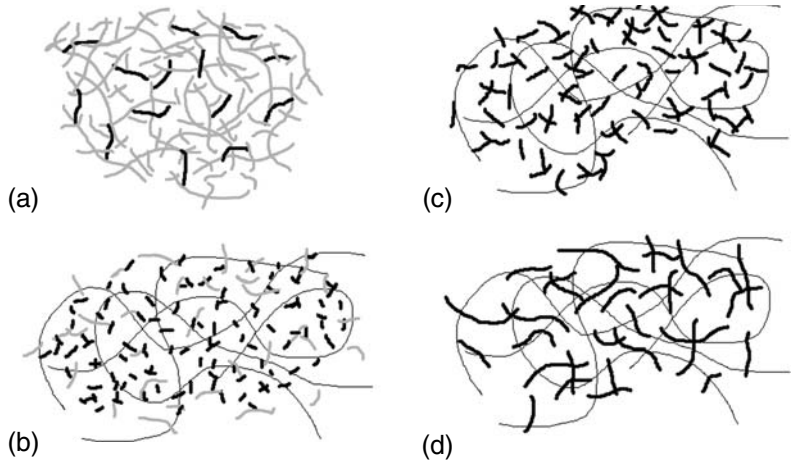

Fig. 9.3. Schematic illustrations of the relative size and abundance of the components of adhesive gels from: (a) the limpet *L. limatula*; (b) the periwinkle *L. irrorata*; (c) the slug *A. subfuscus*; (d) the land snail *H. aspersa*. Polymers of roughly 1000 kD or larger are drawn as *thin black lines*, smaller proteins (10–200 kD) are drawn as *thicker lines*, with glue proteins in *black* and other proteins in *gray*. The size of the polymers and relative amounts of each are depicted to scale using data from Smith et al. (1999), Smith and Morin (2002) and Pawlicki et al. (2004)

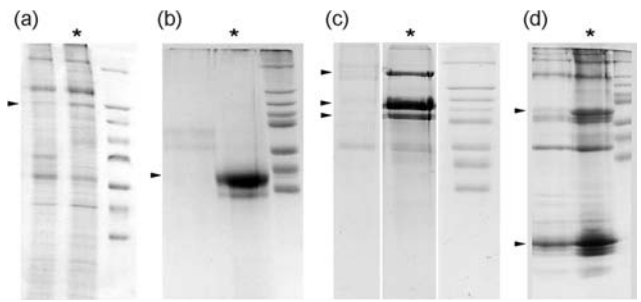

Fig. 9.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis comparison of non-adhesive (*left lanes*) and adhesive mucus (*) from four molluscs: (a) the limpet *L. limatula*; (b) the marsh periwinkle *L. irrorata*; (c) the land snail *H. aspersa*; (d) the slug *A. subfuscus*. Note the difference in specific proteins (*arrowheads*) between the adhesive and non-adhesive lanes. These are identified as glue proteins. MW markers (*right lanes*) are 205, 116, 97, 84, 66, 55, 45 and 36 kD. For each species, the same amount of dried sample was present in the adhesive and non-adhesive lanes. From Smith et al. (1999), Smith and Morin (2002), and Pawlicki et al. (2004)

Limpet glue appears to be constructed primarily of 20–200-kD proteins (Figs. 9.3A and 9.4A) (Grenon and Walker 1980; Smith et al. 1999). In order for proteins of this size to form a gel, it is highly likely that they take on an extended configuration and interact with each other. Compact proteins of this size that did not crosslink would be incapable of gelling. The non-adhesive

mucus from *L. limatula* is structurally similar to the glue, but the glue has a 118-kD glue protein in addition to the other proteins.

Unlike limpets, the glues of the marsh periwinkles, land snails, and terrestrial slugs that have been studied consist of roughly equal parts giant polymers (≥1000 kD) and smaller proteins (Figs. 9.3 and 9.4) (Smith and Morin 2002; Pawlicki et al. 2004). In the marsh periwinkle (*L. irrorata*) and land snail (*H. aspersa*) glues, two or three glue proteins make up most of the protein content. The trail mucus has giant polymers, but almost no small proteins. In the slugs (*A. subfuscus*) there are a significant number of proteins besides the glue proteins. Among the three different gastropods, the giant polymers differ in their carbohydrate content, with marsh periwinkle giant polymers consisting primarily of carbohydrate (Smith and Morin 2002), and land snail and slug giant polymers appearing to consist mostly of protein with much less carbohydrate (Pawlicki et al. 2004).

The glue proteins of all four species may be structurally similar. They typically have acidic isoelectric points and a large proportion of charged and polar amino acids. For the limpet *L. limatula*, the proteins have isoelectric points that are typically between 4.7 and 5.3, and 65% of the amino acids would be polar or charged at neutral pH (Smith et al. 1999). For marsh periwinkles the two glue proteins have an isoelectric point of 4.75 and contain 49 and 52% charged or polar amino acids (Smith and Morin 2002). For land snails (*H. aspersa*) and slugs (*A. subfuscus*), the isoelectric points fall in the same range (Smith, unpubl.). The glue proteins differ in size, which is likely to be functionally significant. The glue proteins range in mass from 14 kD for the primary slug glue protein to 118 kD for the primary limpet glue protein (Smith et al. 1999; Pawlicki et al. 2004). The land snail also has a glue protein with a mass of roughly 175 kD (Pawlicki et al. 2004). Note that these molecular masses are based on gel electrophoresis, and may not be precise.

Little is known at present about the giant polymers. They are defined primarily by their size; in Sephacryl S-400 gel filtration they elute in the void volume, implying a mass greater than 1000 kD (Smith and Morin 2002; Pawlicki et al. 2004). In most invertebrate mucus, such polymers are carbohydrate-rich and anionic. The charge typically comes from sulfated or carboxylated sugars, with the former more common in seawater (Denny 1983). A large amount of negative charge would generally cause the polymers to take on an extended configuration, which would assist gel formation. The fact that the giant polymers from land snail glue do not appear to have much carbohydrate is surprising. The assay used for carbohydrates in the work of Pawlicki et al. (2004) would not have detected amino sugars, and may miss other sugar derivatives. Smith and Morin (2002) addressed this, estimating that the carbohydrate concentrations may be as much as 50% greater than assayed, based on the relative proportions of different types of sugars in other molluscs. Even considering this, the giant polymers of land snails appear to be mostly protein. It is possible that they consist of smaller proteins that are

covalently crosslinked or associated into tight complexes as collagen fibers are. The same may be true of the giant polymers from slug glue.

9.6 The Role of Different Proteins in Adhesion

Because a major difference between the adhesive gels and non-adhesive mucus from the same animal was the presence of glue proteins, these proteins are likely to play a central role in adhesion. This was confirmed by Pawlicki et al. (2004), who purified the glue proteins from different molluscs and showed that they are potent gel-stiffeners. They are able to stiffen non-specifically gels formed from large, negatively charged polymers (Fig. 9.5). Their effect was weak or absent on similar, neutral polymers, which suggested that electrostatic interactions may play a role. Other proteins found in the gel did not have this stiffening effect.

The experiments of Pawlicki et al. (2004) suggest that the glue proteins crosslink the other polymers in the gel. This is the most likely way that they could stiffen gels. Without crosslinking, a dilute gel will lack the stiffness necessary to maintain a strong adhesive bond for an extended period (Eagland 1990). In order to demonstrate crosslinking directly, an important step is to show that the glue proteins bind to other proteins in the secretion. The behavior of the glue proteins in a variety of biochemical techniques suggests a strong tendency to aggregate with themselves and other polymers in the secretion, particularly the giant polymers (Smith et al. 1999; Pawlicki et al. 2004). Most notably, the glue proteins tend to coelute with other polymers in gel filtration chromatography, even under dissociating conditions (Pawlicki et al. 2004).

The relative insolubility of the adhesive gels also suggests that crosslinking occurs. These gels are difficult to dissolve, unlike most mucous secretions (Smith et al. 1999). To dissolve them, a combination of shear and dissociating agents such as urea and non-ionic detergents are typically used (Smith et al. 1999; Smith and Morin 2002; Pawlicki et al. 2004). Milder treatments

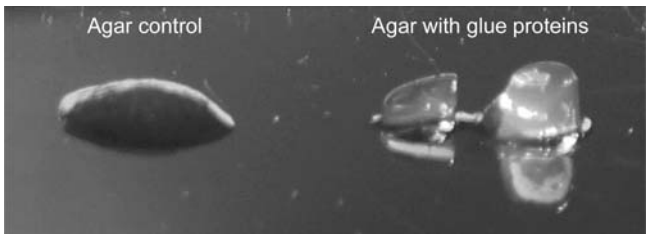

Fig. 9.5. The gel-stiffening effect of gastropod glue proteins. The following shows 0.6% agar with either bovine serum albumen (*left*) or glue proteins from *L. irrorata* (*right*) added. The control is a viscous liquid, while the glue proteins stiffen the agar into a firm gel. From Pawlicki et al. (2004)

extract the same proteins, but at much lower concentrations. These observations suggest that typical covalent bonds are not essential, as they would not be broken under these conditions, but there must be strong non-covalent bonds. The relative insolubility of the gels makes sense for an adhesive that must maintain its integrity underwater.

While an ability to crosslink and stiffen a gel would be a key step in adhesion, that alone is not sufficient. In addition, the gel must adhere to the surfaces to which it is exposed. This aspect has not yet been studied in gastropod adhesive gels. If the glue proteins are capable of non-specifically binding to polymers in the gel, it is likely they can also bind to molecules on the substrate. Surfaces in the ocean are typically covered with an organic film that would be rich in negatively charged molecules (Kamino, Chap.8, this volume). These molecules are probably quite diverse structurally, though, and the mechanism of interaction may not be the same. It is possible that other molecules in the glue are responsible for interfacial adhesion. It is intriguing, though, that Pawlicki et al. (2004) note that the glue proteins increase the ability of solutions to wet surfaces.

In addition to the glue proteins, another difference between non-adhesive and adhesive mucus is the overall concentration (Smith et al. 1999; Smith and Morin 2002). The concentration of the adhesive may be twice the concentration of the non-adhesive mucus, though still less than 3% organic material by weight. An increased concentration would increase tangling interactions, and thus strengthen the glue. This may play a role in addition to the glue proteins, but it is unlikely to be sufficient without crosslinking. Data from Smith (2002) and Ben-Zion and Nussinovitch (1997) for a wide variety of polymers show that increased concentration can improve adhesive strength somewhat, but even a much greater change in concentration would not come close to accounting for the great adhesive strength of molluscan adhesive gels.

The other proteins that are present in the glue of slugs and limpets may play a role in attachment as well. Recent work with barnacles (Kamino, Chap. 8, this volume) and mussels (Sagert et al., Chap. 7, this volume) has made it clear that adhesion in animals often depends on the action of a variety of proteins, each with somewhat different functions. While most of the proteins in limpet glue share similar amino acid compositions, Smith et al. (1999) found other differences between them. One of the two most common proteins in limpet glue, the 140-kD protein, is glycosylated, unlike the other proteins in the glue. It also has substantially more proline than the other proteins. One relatively less common protein, at 53 kD, has a basic isoelectric point (8.6), while the pIs of the other proteins typically fall between 4.7 and 5.3. These differences may relate to their function, but further research would need to be done to elucidate this.

It is also worth noting that there are often several glue proteins in each adhesive gel. Usually there is one that is noticeably more common, but there are often others that are also characteristic of the glue. The evidence suggests that these are related, and in some cases may just be different size variants of

the same protein. The 41- and 36-kD glue proteins from marsh periwinkles, for example, have strongly similar amino acid compositions and the same isoelectric point (Smith and Morin 2002). In limpets, the 118- and 80-kD glue proteins behave similarly in a number of biochemical procedures. For example, they both transfer substantially slower than other proteins in electrophoretic blotting procedures (Smith et al. 1999). It is still possible, though that each protein has slightly different functions.

The mechanism of detachment of these animals may or may not involve biochemical changes. In periwinkles it has been reported that the animal simply eats the glue or tears it down with its radula (Bingham 1972). In other snails, the detachment mechanism has not been tested. In *H. aspersa*, it has been suggested that a protease present in the mucus breaks down the glue (Campion 1961). In limpets, the glue forms a thin layer between the foot and substratum, so one possibility is that they secrete a layer of non-adhesive mucus over the top of the glue. This mucus could include molecules that compete for binding sites and block them, or it could include an enzyme that breaks bonds in the glue. Alternatively, the animal may break the bonds mechanically, by generating sufficient shear. It is worth noting that there is a 68-kD protein that is unique to the non-adhesive mucus of the limpet *L. limatula* (Smith et al. 1999). This may play a role in detachment, though it is found in the pedal mucus used during locomotion, not solely during detachment.

9.7 Mechanisms of Crosslinking

While Pawlicki et al. (2004) suggested that electrostatic crosslinks were important for glue proteins, the actual nature of the crosslinks is likely to be more complex. A key factor is that the glue proteins operate in an aqueous environment. Water has a high dielectric constant; it interacts strongly with charges on the polymers, effectively weakening the electrical field between them (Waite et al. 2005). In essence, water masks the charges. Anything that removes this water would strengthen the interactions between charged groups. Given the usefulness of detergents in solubilizing the adhesive gels, it is possible that hydrophobic interactions assist bonding. They may contribute by excluding water from certain regions, thus allowing the formation of stronger ionic interactions. The charged regions may also be involved in forms of bonding that involve more than simple electrostatic interactions.

The use of a combination of bonding mechanisms is not unusual for gels. A variety of different types of interactions have been demonstrated in commercially used gels (Phillips and Williams 2000). Hydrogen bonding and hydrophobic interactions between helical regions are important in gels such as agar and gelatin. Electrostatic bonding using divalent ions to bridge polymers is common among gels such as pectins. Recently, a number of researchers have synthesized gels with controllable mechanical properties.

These have used a variety of different mechanisms to cross-link the gels. Miyata et al. (1999) used antigen-antibody interactions to crosslink gels. These would presumably depend on several different types of interactions. Several groups have used coiled-coil interactions to link together chains (Petka et al. 1998; Wang et al. 1999, 2001). Coiled-coil interactions depend on hydrophobic interactions between helices that wind around each other. These are sometimes stabilized by electrostatic interactions. Nowak et al. (2002) used polypeptides whose ability to gel depended on having separate hydrophobic and charged regions, both of which presumably contribute to bonding. Alternatively, some researchers have used covalent bonds to crosslink gels. Lee et al. (2002, 2004) incorporated the amino acid dihydroxyphenylalanine (DOPA) into polymer hydrogels in order to form crosslinks. Hu and Messersmith (2003) also created hydrogels with the crosslinking enzyme transglutaminase.

These mechanisms suggest the breadth of ways that gels can be crosslinked. Based on the type of treatments used to solubilize molluscan adhesive gels and keep the proteins from aggregating during chromatography, gastropod glue proteins may depend on similar types of non-covalent interactions. Another possibility is that metal ions could be involved in crosslinking. Multivalent ions typically have large effects on the mechanics of gels (Tanaka 1981). Metals such as iron are also able to form other types of bonds, such as coordinate covalent bonds. Interactions involving metal ions appear to play a key role in mussel adhesion (Sagert et al., Chap. 7, this volume).

9.8 Comparison of Gel Structure Among Gastropods

It is interesting to consider possible explanations for the differences in adhesive gel structure among gastropods. It is possible that the different structures merely reflect evolutionary heritage, where animals evolved glue proteins to work with whatever polymers were present in the trail mucus. In this case, the differences may be unrelated to adhesive performance. Alternatively, they may reflect adaptations for different performance requirements.

One factor that could have a functional impact is the relative sizes of the molecules in the glue. Limpet glue is interesting because it does not appear to contain any giant polymers. Both the non-adhesive and adhesive gels are built primarily of smaller proteins. Given that the limpet is most well-known among gastropods for its adhesive strength, and has produced the highest recorded adhesive strengths for gels, this may suggest that a network of smaller, crosslinked proteins is stronger. It is difficult to compare adhesive strengths among these gastropods, though, as the geometry of detachment varies markedly. Interestingly, Williams and Phillips (2000) note that the strength of crosslinked commercial gels typically reaches a maximum when

the polymer size is near 100 kD. This may also be relevant to the fact that the primary glue proteins of the land snail *H. aspersa* and the limpet *L. limatula* are 97 and 118 kD.

At 14 kD, the glue protein from the slug *A. subfuscus* is markedly smaller than the other glue proteins. The slug glue appears to be defensive, and its most notable characteristic is its ability to set rapidly and generate interfacial adhesion. Perhaps the small size of the glue proteins facilitates this speed, forming interactions more rapidly than the bulkier glue proteins of other species. It is often advantageous to make glues out of smaller polymers that can flow more readily to interact with the adhering surfaces before crosslinking (Bikerman 1958; Wake 1982; Waite 1983).

The land snail and marsh periwinkle glues are both interesting in that they have a substantial amount of giant polymer, and there are no other proteins besides the glue proteins. Thus, the proportion of glue proteins to other components in these two species is also typically higher. A functional difference that may correlate with this is that these glues sometimes (marsh periwinkles) or always (land snails) dry into tough films.

9.9 Conclusion

The adhesive gels produced by molluscs have unusual and potentially useful properties. They are highly flexible, strong and adhere well underwater. Thus, it will be interesting to determine how they function. There are a variety of ways of making a gel, and the mechanics of the resulting gels will vary considerably. Often unusually large molecules (>1000 kD) play a role, but crosslinking by much smaller proteins seems to be a central factor in creating adhesive strength. Specific glue proteins have been identified, and these have a gel-stiffening action that is relatively non-specific. The mechanism by which they do this is probably non-covalent. There are a number of non-covalent interactions that could create stiff gels, but at present it is unclear which mechanism the glue proteins use. In the long run, determining the nature of these interactions and characterizing the functional effects of different gel structures should lead to useful insights into gel design.

Acknowledgments. I would like to thank R. Shadwick for comments on the manuscript, and J.H. Waite and S. Werneke for helpful discussions. This work was supported in part by an Ithaca College summer research grant.

References

Barnhart MC (1983) Gas permeability of the epiphragm of a terrestrial snail, *Otala lactea*. Physiol Zool 56:436–444

Ben-Zion O, Nussinovitch A (1997) Hydrocolloid wet glues. Food Hydrocolloids 11:429–442
Bingham FO (1972) The mucus holdfast of *Littorina irrorata* and its relationship to relative humidity and salinity. Veliger 15:48–50
Bikerman JJ (1958) The rheology of adhesion. In: Eirich FR (ed) Rheology: theory and applications, vol III. Academic Press, New York, pp 479–503
Branch GM, Marsh AC (1978) Tenacity and shell shape in six *Patella* species: adaptive features. J Exp Mar Biol Ecol 34:111–130
Campion M (1961) The structure and function of the cutaneous glands in *Helix aspersa*. Quart J Microscop Sci 102:195–216
Davies MS, Hawkins SJ (1998) Mucus from marine molluscs. Adv Mar Biol 34:1–71
deGennes PG, Leger L (1982) Dynamics of entangled polymer gels. Annu Rev Phys Chem 33:49–61
Denny MW (1983) Molecular biomechanics of molluscan mucous secretions. In: Wilbur K, Simkiss K, Hochachka PW (eds) The mollusca, vol I. Academic Press, New York, pp 431–465
Denny MW (1984) Mechanical properties of pedal mucus and their consequences for gastropod structure and performance. Am Zool 24:23–36
Denny MW, Gosline JM (1980) The physical properties of the pedal mucus of the terrestrial slug *Ariolimax columbianus*. J Exp Biol 88:375–393
Doi M, Edwards SF (1988) The theory of polymer dynamics. Clarendon Press, Oxford
Eagland D (1990) What makes stuff stick? Chemtech (April):248–254
Flammang P (1996) Adhesion in echinoderms. In: Jangoux M, Lawrence JM (eds) Echinoderm studies, vol 5. Balkema, Rotterdam, pp 1–60
Gay C (2002) Stickiness -some fundamentals of adhesion. Integr Comp Biol 42:1123–1126
Grenon JF, Walker G (1980) Biomechanical and rheological properties of the pedal mucus of the limpet, *Patella vulgata* L. Comp Biochem Physiol B 66:451–458
Grenon JF, Walker G (1981) The tenacity of the limpet, *Patella vulgata* L.: an experimental approach. J Exp Mar Biol Ecol 54:277–308
Hahn T, Denny M (1989) Tenacity-mediated selective predation by oystercatchers on intertidal limpets and its role in maintaining habitat partitioning by *'Collisella' scabra* and *Lottia digitalis*. Mar Ecol Prog Ser 53:1–10
Hermans CO (1983) The duo-gland adhesive system. Oceanogr Mar Biol Annu Rev 21:283–339
Hu BH, Messersmith PB (2003) Rational design of transglutaminase substrate peptides for rapid enzymatic formation of hydrogels. J Am Chem Soc 125:14298–14299
Lee BP, Dalsin JL, Messersmith PB (2002) Synthesis and gelation of DOPA-modified poly(ethylene glycol) hydrogels. Biomacromolecules 3:1038–1047
Lee BP, Huang K, Nunalee FN, Shull KR, Messersmith PB (2004) Synthesis of 3,4-dihydroxyphenylalanine (DOPA) containing monomers and their co-polymerization with PEG-diacrylate to form hydrogels. J Biomater Sci Polym Ed 15:449–64
Mair J, Port GR (2002) The influence of mucus production by the slug, *Deroceras reticulatum*, on predation by *Pterostichus madidus* and *Nebria brevicollis* (Coleoptera: Carabidae). Biocontrol Sci Technol 12:325–335
Miyata T, Asami N, Uragami T (1999) A reversibly antigen-responsive hydrogel. Nature 399:766–769
Nowak AP, Breedveld V, Pakstis L, Ozbas B, Pine DJ, Pochan D, Deming TJ (2002) Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. Nature 417:424–428
Pawlicki JM, Pease LB, Pierce CM, Startz TP, Zhang Y, Smith AM (2004) The effect of molluscan glue proteins on gel mechanics. J Exp Biol 207:1127–1135
Petka WA, Harden JL, McGrath KP, Wirtz D, Tirrell DA (1998) Reversible hydrogels from self-assembling artificial proteins. Science 281:389–392
Phillips GO,Williams PA (2000) Handbook of hydrocolloids. Woodhead Publ, Cambridge
Smith AM (1991a) Negative pressure generated by octopus suckers: a study of the tensile strength of water in nature. J Exp Biol 157:257–271
Smith AM (1991b) The role of suction in the adhesion of limpets. J Exp Biol 161:151–169

Smith AM (1992) Alternation between attachment mechanisms by limpets in the field. J Exp Mar Biol Ecol 160:205–220
Smith AM (2002) The structure and function of adhesive gels from invertebrates. Integr Comp Biol 42:1164–1171
Smith AM, Morin MC (2002) Biochemical differences between trail mucus and adhesive mucus from marsh periwinkles. Biol Bull 203:338–346
Smith AM, Quick TJ, St. Peter RL (1999) Differences in the composition of adhesive and non-adhesive mucus from the limpet *Lottia limatula*. Biol Bull 196:34–44
Tanaka T (1981) Gels. Sci Am 244:124–138
Vaughn CC, Fisher FM (1988) Vertical migration as a refuge from predation in intertidal marsh snails: a field test. J Exp Mar Biol Ecol 123:163–176
Wainwright SA, Biggs WD, Currey JD, Gosline JM (1976) Mechanical design in organisms. Princeton Univ Press, Princeton
Waite JH (1983) Adhesion in bysally attached bivalves. Biol Rev Camb Philos Soc 58:209–231
Waite JH, Andersen NH, Jewhurst S, Sun C (2005) Mussel adhesion: finding the tricks worth mimicking. J Adhesion 81:1–21
Wake WC (1982) Adhesion and the formulation of adhesives. Applied Science Publ, London
Wang C, Stewart RJ, Kopeček J (1999) Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. Nature 397:417–420
Wang C, Kopeček J, Stewart RJ (2001) Hybrid hydrogels cross-linked by genetically engineered coiled-coil block proteins. Biomacromolecules 2:912–920
Warren JH (1985) Climbing as an avoidance behaviour in the salt marsh periwinkle, *Littorina irrorata* (Say). J Exp Mar Biol Ecol 89:11–28
Wells GP (1944) The water relations of snails and slugs III. Factors determining activity in *Helix pomatia* L. J Exp Biol 20:79–87
Williams PA, Phillips GO (2000) Introduction to food hydrocolloids. In: Phillips GO, Williams PA (eds) Handbook of hydrocolloids. Woodhead Publ, Cambridge, pp 1–20
Yule AB, Walker G (1987) Adhesion in barnacles. In: Southward AJ (ed) Crustacean issues: barnacle biology, vol 5. Balkema, Rotterdam, pp 389–402