

## The Structure and Function of Adhesive Gels from Invertebrates<sup>1</sup>

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**SYNOPSIS.** Many marine invertebrates form strong, temporary attachments using viscoelastic gels. To better understand these adhesives, an analysis of what is known of gel structure and function was performed. There are different ways of making gels, ranging from entangling of giant glycoproteins to crosslinking of smaller proteins. The mechanics of the gel depend largely on the size of the polymer, its concentration, and whether it is crosslinked. Compared to gels such as mammalian mucus, the mechanics of adhesive mucous gels often appear to depend more heavily on relatively small proteins than on megadalton-sized glycoproteins. In addition, changes in concentration and the presence of specific proteins have been associated with the change from a non-adhesive to an adhesive form. The attachment strengths produced by different gels at different concentrations were compared with the changes in attachment strength seen in living animals. These data suggest that changes in concentration are not sufficient to account for adhesion. Thus, it is likely that the changes in protein composition may play a large role.

### INTRODUCTION

Many animals use viscoelastic gels such as mucus for adhesion. Gastropod molluscs depend on mucus to hold themselves to the substratum during locomotion. In particular, limpets are renowned for the adhesive strength they achieve using mucus as a glue (Grenon and Walker, 1981; Smith *et al.*, 1999a). Periwinkles also attach strongly using mucus (Denny, 1984). Many other gastropods use mucus to attach to the substratum with varying degrees of adhesive strength. Echinoderm podia and tentacles also adhere using viscoelastic secretions commonly identified as mucopolysaccharides (Flammang, 1996). Finally, a variety of tiny worm-like invertebrates adhere to sand grains with viscous secretions that are likely to be gels rather than solid (Hermans, 1983).

These adhesive gels can produce impressive performance. Limpet mucus is more than 95% water, yet the attachment strength per unit area, defined as the tenacity, can approach that of barnacles and mussels. When measured at times when they are likely to be glued down, the tenacity of different limpet species ranges from roughly 200 to 500 kPa (Branch and Marsh, 1978; Grenon and Walker, 1981; Smith, 1992). The tenacity of marsh periwinkles using dried mucus can be substantially greater (Denny, 1984). In addition to their strength, these glues are interesting because they are labile. Limpets can alternate between suction adhesion and gluing in a predictable pattern that corresponds with their activity and the tides (Smith, 1992). Marsh periwinkles similarly alternate between active and glued down states. Other animals such as echinoderms and interstitial worms can attach and let go on a time scale of seconds. The combination of strength and transience in a dilute gel makes these adhesives worthy of focused study.

In many cases, the adhesive gels secreted by invertebrates are described as mucus. The term mucus, however, is broadly used for any slimy secretion from an epithelial surface (Davies and Hawkins, 1998). This vagueness has covered over a number of interesting structural differences. The goal of this paper is to analyze the structure and function of these mucus-like adhesive gels. It will first delineate the basic principles of gel structure, identify the different ways of making gels and their impact on mechanics, and then review research on invertebrate adhesive gels in light of this information.

### STRUCTURE AND FUNCTION OF GELS

A gel is a dilute polymer network that takes on a somewhat solid form. In biology, most of the mass of the gel is water. The unusual characteristics of the gel result from interactions between the polymers. These interactions increase the stiffness and the viscosity of the gel so that it holds its shape. Gels can be formed from molecules that entangle or crosslink to form a network (Fig. 1).

#### *Non-crosslinked gels*

Non-crosslinked gels consist of giant molecules that entangle at low concentrations. For most gelling agents, the polymers begin to overlap and entangle at concentrations of 1% or less (Doi and Edwards, 1988; Williams and Phillips, 2000). These entanglements become more likely as the size of the polymer increases. Thus, gelling and thickening agents used in the food industry often have molecular masses of more than a megadalton. They often have an extended configuration, which also promotes entangling.

Deformation of the entangled network occurs by a process called reptation (deGennes and Leger, 1982; Doi and Edwards, 1988). In essence, one can imagine each polymer as fitting into an irregularly shaped tube bounded by its neighbors. The polymer must creep through this narrow, winding path for the material to deform; it cannot slide laterally through its neighbors.

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If a force is applied over a short time, the polymer might not break free of its entanglements. Given more time, the polymer's random brownian motion will allow it to work free and creep.

Doi and Edwards (1988) outline the factors that control the mechanics of an entangled polymer network. As described previously, a critical factor is the length of the polymers. As the polymer size increases, the extent of the entanglements increases and reptation becomes more difficult. In addition, branching of the polymer strongly impedes reptation. Finally, increasing the concentration of the gel increases the overlap and extent of entanglement. Changes in concentration are likely to control the mechanics of mammalian cervical mucus throughout the reproductive cycle (Tam and Verdugo, 1981; Silberberg and Meyer, 1982).

#### Cross-linked gels

Cross-links between polymers have a substantial effect on the polymer network. They greatly impede flow and cause the material to behave more elastically. The resistance to deformation will depend on the number and strength of the cross-links (Denny, 1983). The size and branching of the polymers do not play as large a role in the mechanics. In fact, the strength of cross-linked commercial gels typically reaches a maximum when the polymers are roughly 100 kDa. Larger molecular masses do not substantially increase the strength (Williams and Phillips, 2000).

Among commercially important gelling agents, there are several common crosslinking mechanisms (Williams and Phillips, 2000). Gels such as agar and gelatin are thermally reversible. As they cool, helices form in regions of the polymers and line up next to helical regions in neighboring polymers. Hydrogen and hydrophobic bonds maintain the links between helices. In contrast, gels such as pectin depend on ionic bonds with  $\text{Ca}^{++}$  bridging negatively charged groups on the molecule. Other gels often depend on some mixture of these mechanisms, such as aggregation of helical regions stabilized by cations.

For many animal glues, crosslinking appears to be of central importance. Mussel byssal plaques and threads depend on linkages involving the amino acid 3,4,-dihydroxyphenylalanine (Dopa) (Waite, 1990). Barnacle cement may be crosslinked by disulphide bonds and hydrophobic bonds (Naldrett, 1993; Naldrett and Kaplan, 1997). Such crosslinking is a common way for an adhesive to set and form a glue (Wake, 1982).

#### Common gel-forming molecules

There are three types of biological polymers that are typically used to form gels: glycoproteins/proteoglycans, polysaccharides, and proteins (Fig. 1). These form gels at similar concentrations, but the underlying structure and mechanics can vary widely.

Gel-forming glycoproteins and proteoglycans are giant molecules consisting of a protein with attached carbohydrate chains (Fig. 1A). They typically have mas-

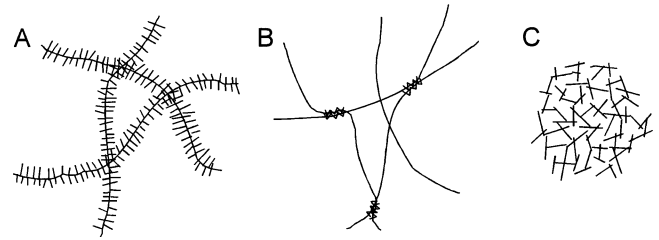


FIG. 1. Schematic diagram of three common gel types. A) Mammalian mucus is a non-cross-linked gel consisting of tangled megadalton-sized glycoproteins. The carbohydrates are the small chains attached to the protein backbone. B) A polysaccharide gel typically consists of megadalton sized, extended polysaccharides. These may be crosslinked as shown. C) A gelatin gel is a cross-linked network of proteins that are mostly in the 100–200 kD size range.

ses larger than a megadalton, and they are often linked together into even larger complexes. They are so large that they form gels solely by entangling. The best example of such a gel is mammalian mucus, which is based on the glycoprotein mucin. This has a high molecular weight protein core covered by hundreds of oligosaccharide chains (Silberberg and Meyer, 1982; Allen *et al.*, 1984; Perez-Vilar and Hill, 1999). The carbohydrates typically account for up to 90% of the mass (Perez-Vilar and Hill, 1999). Proteoglycans are also common gel-forming polymers. In this case, polysaccharides such as glycosaminoglycans are attached to a protein. Denny (1983) suggests that most invertebrate mucus polymers may fit on a continuum between mucin and proteoglycans.

Many commercially important gels are based on large polysaccharides (Fig. 1B) (Phillips and Williams, 2000). They range in size from roughly 0.1 to 15 MDa, and they usually take on an extended linear configuration. They form highly viscous solutions, but in order to form a gel, they usually crosslink. For example, guar gum has a mass of over a megadalton but it does not crosslink. A 2% solution forms a thick suspension that flows imperceptibly, but is not elastic. Mechanically, it resembles unusually thick ketchup. Agar is much smaller, but it forms crosslinks. A 2% solution of agar forms firm, elastic gels. This emphasizes the large role that crosslinking can play in gel mechanics. Incidentally, many traditional adhesives are large polysaccharides that are used in highly concentrated form. These are called gums (Wake, 1982).

The third way of making a gel with biological polymers is with relatively small, crosslinked proteins (Fig. 1C). Gelatin is the best example of such a gel. Gelatin consists of collagen fragments extracted from animal connective tissues. The majority of the protein in gelatin is roughly 100–200 kD, and it is these fragments that contribute the most to the mechanics of the gel (Ledward, 2000). While gelatin is well-known from the food industry, it also has a long history of use as an adhesive. Until the early 1900s, the most common adhesives were gelatin and starch (Nussinovitch, 2000).

Finally, gels can consist of combinations of different

polymers. Many loose connective tissues consist of space-filling proteoglycans intermingled with collagen fibers (Wainwright *et al.*, 1976). Furthermore, molluscan gels are often described as protein-polysaccharide complexes (Denny, 1983). Usually, this denotes giant glycoproteins or proteoglycans. It may also indicate polysaccharides that are non-covalently linked to proteins.

#### Analysis of biological gels

From the previous discussion, one can identify three critical features for characterizing adhesive gels: 1) the overall concentration of protein and carbohydrate, 2) the size of the polymers, 3) the presence of crosslinks.

There are a variety of assays for protein and carbohydrate concentration that are sufficient to distinguish between types of gels and to determine changes in relative concentration. Care should be taken in their use, though. Adhesive gels are likely to be relatively insoluble, necessitating the use of strong buffers which may interfere with the assays (Smith *et al.*, 1999a). In addition, carbohydrate assays are limited because there is no single method that works on all types of monosaccharide subunits (Labare *et al.*, 1989).

The size of the gel-forming polymers plays a large role in the mechanics of the gel. Despite this fact, very few studies attempt to measure size. Note that size is affected not only by polymer mass, but configuration and branching as well. One way to estimate the size of the polymers is gel filtration chromatography. For example, a common method of purifying the giant glycoprotein mucin is based on the fact that it is too large to enter the gel beads of Sepharose 4B, and thus elutes in the void volume (Allen *et al.*, 1984). Meikle *et al.* (1988) used column chromatography to characterize mucus from a variety of different corals (Fig. 2). They showed that one species of coral produces a mucus where all the protein and carbohydrate elutes together in a position indicating megadalton size. These mucus polymers had the characteristically high serine and threonine content of mucin. In another species, the mucus was made primarily of megadalton sized polysaccharides, and in another, it consisted primarily of proteins with a molecular weight of around 150 kD. The other three corals tested produced mucus with a mixture of these types of molecules.

Perhaps the most important feature to study is whether crosslinks are present, and if so, their extent and nature. Unfortunately, little work has been done demonstrating crosslinking in invertebrate gels. Denny (1983) analyzed data from mechanical tests on three molluscan species to calculate that there were crosslinks at roughly 400 kD intervals, and that these were not permanent. The effect of specific solvents can also indicate the presence of crosslinks. For example, in cartilage, proteoglycans are non-covalently linked to a polysaccharide core by link proteins. When guanidine hydrochloride is present these complexes dissociate into smaller units (Neame and Berry, 1993).

A promising technique for the study of adhesive

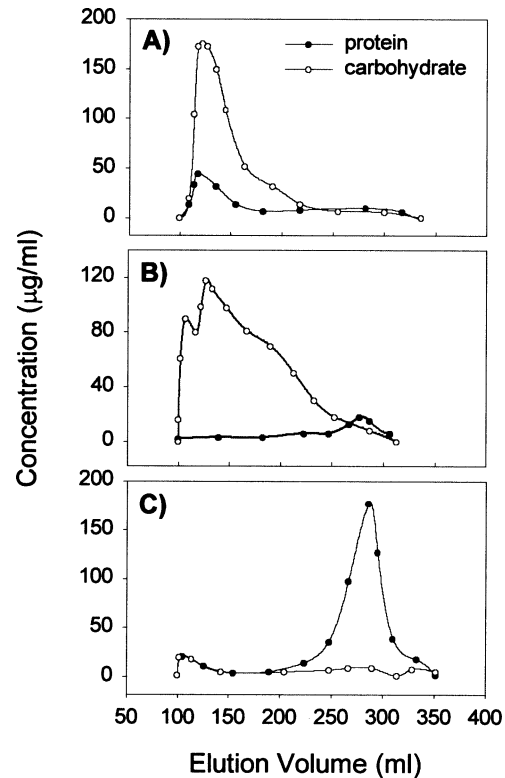


Fig. 2. Sepharose CL-4B elution profiles of reduced mucus samples from three coral species: A) *Acropora formosa*, B) *Cespitularia sp.*, C) *Fungia fungites*. The void volume was 100 ml in each case. Carbohydrate was measured by the phenol-sulfuric acid assay, and protein was measured by a modification of the Lowry method. Modified from Meikle *et al.* (1988).

gels is atomic force microscopy. This technique can visualize individual molecules and can even quantify changes in molecular shape in real time (Radmacher *et al.*, 1992, 1994). Thus, one can observe the size of the molecules directly and possibly distinguish among the different gel types identified in Fig. 1. This technique has been used successfully to look at gelatin, and to measure its elastic modulus (Radmacher *et al.*, 1995). It can also be used to measure the force of interaction between individual molecules (Florin *et al.*, 1994). Finally, Smith *et al.* (1999b) used atomic force microscopy to measure the force required to stretch fibers of the polymeric adhesive used to provide strength in snail shells. Their data gave insight into the molecular mechanism underlying the toughness of this adhesive.

Often, attempts to characterize mucus focus on detecting the presence of polysaccharides and characterizing their composition. Some studies identify the polysaccharides as acidic or neutral, for example, and others identify specific classes of monosaccharides, such as uronic acids, amino sugars, sialic acids, and neutral sugars (Denny, 1983). While this information is useful, particularly if it permits a more accurate measure of total concentration, it does not indicate what the mechanics of the gel will be like. For ex-

ample, Silberberg and Meyer (1982) note that there is considerable microheterogeneity in the carbohydrate side chains of mammalian mucin, but these differences do not affect the mechanics of the mucus. The nature of the sugars may affect their ability to cross-link, but not in a predictable way. The nature of the sugars can also affect the configuration of the molecule. For example, many polysaccharides carry substantial negative charge. In this case, the electrostatic repulsion leads to an extended configuration (Denny, 1983). It is also possible for neutral sugars to create an extended shape by having less flexible glycosidic linkages, as does cellulose (Williams and Phillips, 2000). Yet another way of achieving an extended shape is by having hundreds of small oligosaccharides along the polymer's length, as is the case for mucin. This creates steric inhibition of bending (Perez-Vilar and Hill, 1999). In summary, gross categorization of the monosaccharide subunits does not allow us to distinguish between different configurations, nor does it have much predictive value.

#### THE STRUCTURE OF ANIMAL ADHESIVE GELS

##### *Limpets*

Limpets are the best-studied example of an animal that uses an adhesive mucous gel. They produce mucus for locomotion and adhesion. Both types of mucus have a similar overall structure with specific changes that may be associated with function (Smith *et al.*, 1999a).

Grenon and Walker (1980) and Smith *et al.* (1999a) found that limpet mucus consists primarily of proteins ranging from roughly twenty to two hundred kilodaltons. Although giant glycoproteins may not show up in the polyacrylamide gels used in these studies, further work with gel filtration chromatography found no evidence of such glycoproteins (unpublished data, A.M.S.). Limpet mucus contains two to six times more protein than carbohydrate. The mucus of *Patella vulgata* consists of roughly 3% percent protein and 1.5% carbohydrate (w/w, Grenon and Walker, 1980; Davies *et al.*, 1990). The adhesive mucus of *Lottia limatula* has 1.8% protein and 0.3% carbohydrate. Connor (1986) reports values ranging from 2.4 to 3.9% protein and 0.8 to 1.8% carbohydrate for three other limpet species. The carbohydrate from *Patella* mucus appears to consist of acidic polysaccharides that are not covalently linked to any proteins (Grenon and Walker, 1978, 1980). In *Lottia* mucus, only a single protein with a mass of 140 kD protein is glycosylated (Smith *et al.*, 1999a).

Overall, while limpet mucus may contain some large polysaccharides or proteoglycans, in some ways it appears more similar to gelatin than mammalian mucus. Like gelatin, the main components seem to be proteins that are less than 200 kD. Like gelatin, non-covalent bonds appear important for crosslinking the proteins (Grenon and Walker, 1980; Smith *et al.*, 1999a). In column chromatography experiments using

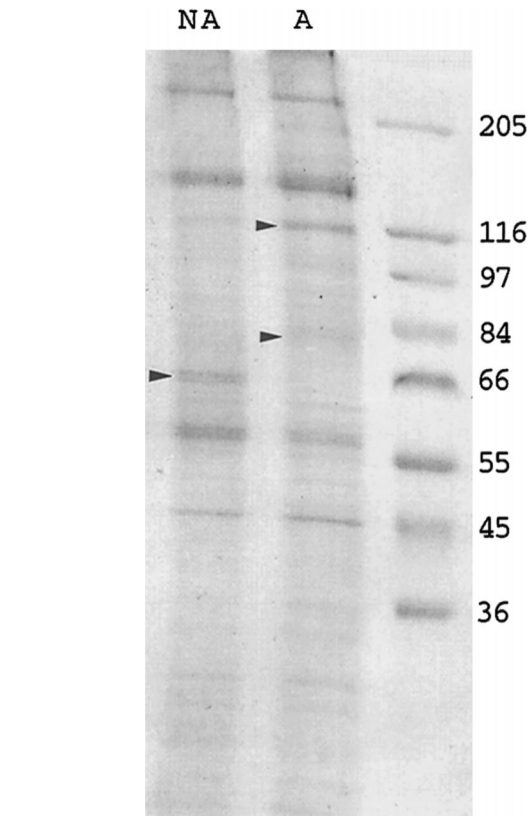


FIG. 3. An SDS-PAGE comparison of mucus from limpets. Similar quantities of non-adhesive trail mucus (NA) and adhesive mucus (A) were loaded. 118, 80 and 68 kD proteins characteristic of each form of mucus are identified by arrowheads. The right hand lane contains molecular weight markers. Reprinted from Smith *et al.* (1999a).

mild buffers, limpet mucus proteins aggregate into large complexes. When chaotropic agents and non-ionic detergents are present, the proteins disaggregate (unpublished data, A.M.S.). There are a few interesting differences from gelatin, though. First, carbohydrates are clearly present, though it is unclear how these carbohydrates affect the gel mechanics. Second, the crosslinks may be more stable; Smith *et al.* (1999a) found that limpet mucus is relatively insoluble without reagents that break crosslinks, even when heated.

A structure based on cross-linked proteins would seem more suited to an adhesive material than mammalian mucin. Eagland (1990) notes that polymeric adhesives generally must crosslink to provide sufficient mechanical strength. Furthermore, the ability to form non-specific crosslinks may aid bonding with the surface.

In addition to the differences from mammalian mucus, Smith *et al.* (1999a) found that the mucus used by limpets during adhesion differs from the mucus used during locomotion (hereafter referred to as trail mucus). Both forms of mucus have the same proteins in the same proportions except for a few notable differences (Fig. 3). There is a 118 kD protein and an 80 kD protein that are characteristic of the adhesive mu-

cus, and a 68 kD protein that is only found in the non-adhesive mucus. The adhesive mucus is also twice as concentrated. Given the change in function of mucus when the animal glues down, these are intriguing differences.

#### *Periwinkles*

Periwinkles are another type of mollusc that uses a mucous gel for adhesion. Marsh periwinkles, *Littorina irrorata*, climb marsh grass and glue themselves to the stems as the tide rises. In this way they avoid aquatic predators (Warren, 1985). Other intertidal periwinkle species also use mucus as an adhesive to cling to rocks. The reported values for the strength of this adhesive are surprisingly high. Denny (1984) reported a yield strength for fully dehydrated *Littorina aspera* mucus of roughly  $1 \times 10^8$  Pa. Measurements of the tenacity of marsh periwinkles adhering with hydrated mucus resulted in substantially lower values, yet still roughly as strong as limpet adhesion (Smith and Morin, 2002).

Smith and Morin (2002) studied the structure of marsh periwinkle mucus and found that, like limpets, specific proteins were correlated with the formation of the adhesive bond. The trail mucus appears to consist of large polysaccharides or proteoglycans with a smaller amount of proteins whose molecular weights range between 59 and 65 kD. The adhesive mucus has the same amount of polysaccharides, but the protein content is increased by a factor of two to three. This difference is due to the presence of two proteins with molecular masses of 41 and 36 kD. The correlation of specific proteins with adhesion was even more clear with periwinkle mucus than with limpet mucus. In the adhesive mucus, the putative adhesive proteins made up roughly half of the organic material.

#### *Other organisms*

Many echinoderms form temporary attachments using mucus (Flammang, 1996). The characterization of the adhesive, though, has been limited largely to histochemical staining. Thus, the presence of acidic or neutral polysaccharides is often detected, as is protein. Flammang *et al.* (1998) provide more detailed information for the adhesive secreted by sea star podia. By dry weight, 20% of the material is protein, and only 8% is carbohydrate. Much of the remainder of the dry weight is inorganic. The protein and carbohydrate values are similar to those for limpets. A similar proportion of inorganic material is also present in limpet and periwinkle mucus, and it presumably results from dried salts left over when the seawater in a gel evaporates (Smith *et al.*, 1999a; Smith and Morin, 2002).

In addition, a variety of algae adhere using gels. In the biofouling alga *Enteromorpha*, a 110 kD glycoprotein has been implicated in initial adhesion. This glycoprotein appears to crosslink to provide a strong adhesive (Finlay *et al.*, 2002). Finally, a variety of invertebrates secrete viscid secretions as part of a reported "duo-gland" adhesion (Hermans, 1983). These

secretions are likely gels, though the small quantities involved have prevented more detailed characterization.

#### LINKING STRUCTURE TO FUNCTION

Since there are clear structural differences among mucous gels, we can begin to look at the functional significance of these differences. The following are the key questions that need to be addressed. Is the overall structure of limpet or periwinkle mucus more suited for adhesion than that of mammalian mucus? Is mucus inherently adhesive, or are the identified structural modifications necessary to form a glue? If the modifications of mucus are necessary, which is most important: the change in concentration, or the presence of specific proteins? Finally, are there any changes in the extent and nature of crosslinking? Some answers to these questions are emerging, but a substantial amount of work remains to be done.

To answer these questions, it is first necessary to quantify the functional differences between different forms of mucus. Then we can attempt to determine which, if any, structural features may account for the differences. Although there is little such functional information available, there is enough to guide preliminary analyses. There is some evidence suggesting a substantial difference between mucus that is used for adhesion and mucus that is used for lubrication and protection, such as that produced by mammals. Grenon and Walker (1980) measured the mechanical properties of *Patella vulgata* mucus and found values of stiffness and viscosity that were several orders of magnitude higher than those typically reported for similar concentrations of mammalian mucus (Litt *et al.*, 1977). The limpet mucus was also freeze dried, so the tests may underestimate its mechanical strength (Denny, 1983). Furthermore, the method of collection described by Grenon and Walker (1980) suggests that the trail mucus was analyzed rather than the adhesive mucus.

In addition to the difference from mammalian mucus, there is a large functional difference between adhesive mucus and trail mucus from one animal. This implies a corresponding difference in mechanics. To gain a better comparison of the functional properties of non-adhesive and adhesive mucus from limpets, a comparison of the shear tenacity using both was recently carried out. When limpets are glued or moving on a smooth surface, the shear tenacity depends primarily on mucus. Under these conditions, suction does not generally augment the tenacity of limpets. The shear tenacity of the limpet *Lottia limatula* was measured with a more sensitive version of the strain gauge force transducer described by Smith (1991). Limpets that had been moving recently, and whose foot was in smooth, intimate contact with the glass aquarium wall were pulled across the glass with a loop of string attached to the force transducer. Limpets that were glued down, as judged by the patch of firm gel left stuck on the glass after detachment, were also tested in shear.

This was done using a wire loop and a spring scale with 0.1 kg gradations. The shear tenacity of the limpets that were not glued down was  $2.3 \pm 0.3$  kPa ( $n = 7$ ). The shear tenacity of limpets that were glued down was  $125 \pm 49$  kPa ( $n = 12$ ). The strongest tenacities were 192 and 210 kPa. Thus, the shear tenacity is almost two orders of magnitude higher when they are using adhesive mucus than when they are stationary and producing trail mucus. Smith and Morin (2002) found a similar pattern with periwinkle tenacity. Any proposed mechanism for adhesion must account for changes of this magnitude.

The most likely cause for such a substantial change would seem to be the structural changes in the mucus. Alternatively, the animal may increase its adhesion somehow by clamping down when disturbed. Clearly this improves suction adhesion. It has also been suggested to affect the tenacity via Stefan adhesion. In this mechanism, the viscous resistance to centripetal flow resists separation of rigid plates. The equation for Stefan adhesion predicts very large attachment forces for materials with the viscosity and stiffness of mucus (Grenon and Walker, 1981). It is not applicable to molluscan feet, though, since the assumptions of rigidity and uniform centripetal flow are violated. Furthermore, experimental results do not match the predictions of the equation (Grenon and Walker, 1981; Smith, 1991) and it can not account for the increase in shear tenacity or the resistance to peeling (Smith *et al.*, 1999a). The primary role for clamping may be to take advantage of the anchorage provided by a strong adhesive bond to pull the shell against the rock, creating better shear resistance (Ellem *et al.*, 2002). In summary, it appears likely that mucus is not inherently adhesive; either the change in concentration or the change in composition is likely to cause the large increase in tenacity.

As yet, there is no experimental work directly showing that addition of specific proteins causes adhesion. There is some evidence, however, that changes in concentration alone are not sufficient. To test the effect of concentration changes, a series of experiments were performed using different gels as adhesives. The concentration of each gel was manipulated, and the resulting normal tenacity measured in a standard system. Agar and gelatin were tested, as was guar gum, which forms a thick hydrocolloid but does not gel. Concentrations ranging from 0.5% to 5% were tested. Tenacity was measured between a Lucite plate and Lucite squares (1–2 cm<sup>2</sup>). Lucite (Plexiglass) is a moderately wettable surface and as such probably makes a fair comparison to the natural environment (Smith, 1996). Test pieces were connected by a wire hook to a strain gauge force transducer, as described by Smith (1991). The force transducer was attached to a metal bar that could be withdrawn by an electrical motor via a screw drive. Test pieces were glued to the plate with fifty microliters of freshly mixed gel solutions. This was enough to ensure that when the test piece was pressed firmly down, a small pool beaded around the edge. These were allowed to set for two to three hours at

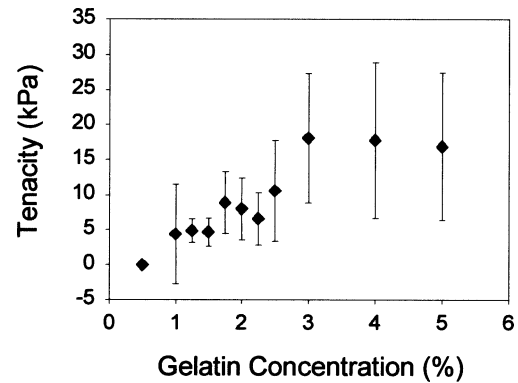


FIG. 4. The effect of gel concentration on normal tenacity for gelatin gels. The error bars show standard deviation. For comparison, limpet mucus is roughly 4% (w/w), and creates tenacities of several hundred kPa.

4°C. They were then detached by pulling at a rate of 1.2 mm/sec. Because of bending in the wire hook and Lucite plate, the exact strain rate on the adhesive was not known.

Concentration affected the tenacity of the test gels, but not enough to account for the tenacities of limpets or periwinkles. Gelatin showed a roughly linear increase in normal tenacity with concentration, leveling off at a tenacity near 20 kPa at a concentration of 3% (Fig. 4). Agar gave similar results. Guar gum did not produce significant adhesive forces. Ben-Zion and Nussinovitch (1997) found comparable results using a variety of high viscosity gums and gels at much higher concentrations. The concentration affected the adhesive strength, but only 13 of the 26 polymers that they tested were even effective adhesives. Among the natural polymers that were the best adhesives, typical maximum normal tenacities were 34 kPa for 15% (w/w) pectin, and 45–88 kPa for 65–75% gum ghatti, tapioca-dextran and arabinogalactan. Even for these concentrated gums, shear strengths were between 2 and 5 kPa. Therefore it seems unlikely that limpet and periwinkle mucus create shear tenacities near 100 kPa just because they are highly viscous gels, or their concentration increases a few percent.

Fully dehydrating mucus may be sufficient to create a strong bond, as suggested for periwinkles (Denny, 1984). Periwinkle tenacity changes from roughly 12 kPa when moving (Davies and Case, 1997) to possibly thousands of kilopascals when glued down (Denny, 1984). The stiffness of periwinkle mucus increases over roughly six orders of magnitude as it goes from fully hydrated to fully dehydrated. It should be noted though, that many periwinkles adhere equally well with hydrated mucus. In humid environments, the marsh periwinkle glue does not dehydrate. It forms firm gels containing roughly 95% water, which form strong attachments (Smith and Morin, 2002). Zebra periwinkles also adhere strongly to rocks using a hydrated mucus. Finally, increases in stiffness with drying may help up to a point, but then brittleness may

counteract the gains in stiffness, particularly since half the dried weight may be salt.

Changes in cross-linking could account for the large changes in tenacity, but there is little research on this. Calcium ions have been mentioned as a possible means of stiffening a mucus secretion (Hermans, 1983; Thomas and Hermans, 1985). This is certainly possible, given that many commercially important gels depend on calcium crosslinks. The only experimental evidence to date, however, showed that there is no change in calcium concentration between the adhesive and non-adhesive forms of mucus (Smith *et al.*, 1999a). This does not rule out a crosslinking role, though, since such crosslinks may be present in both forms of mucus. Another possible mechanism for crosslinking is through ionic bonds between proteins. Hermans (1983) noted that basic amino acids on a protein may mediate such linkages. This may be relevant given the correlation between specific proteins and adhesive function.

#### CONCLUSIONS

There are several ways of making a gel, and not all gels are equally effective as adhesives. This paper reviews the basic factors affecting the mechanics of gels, and then analyzes the structure of several different adhesive gels in this context. The adhesive forms of mucus that have been studied differ in composition from commonly studied mucus. They appear to consist of a much larger percentage of relatively small proteins. In order to contribute to the mechanics of the gel, these presumably crosslink. There is enough evidence emerging to suggest several hypotheses. One hypothesis is that relatively small, cross-linked proteins form gels that are more effective as adhesives than giant mucin-like glycoproteins. For limpets and periwinkles in specific, another hypothesis is that addition of specific proteins causes mechanical changes that increase the adhesiveness of mucus. Clearly much work remains to be done. The adhesive gels produced by other animals need to be characterized structurally. In addition, the functional importance of the structural differences needs to be tested.

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