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Molecular design of the α-keratin composite: insights from a matrix-free model, hagfish slime threads

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We performed mechanical tests on a matrix-free keratin model—hagfish slime threads—to test the hypothesis that intermediate filaments (IFs) in hydrated hard α-keratins are maintained in a partly dehydrated state. This hypothesis predicts that dry IFs should possess mechanical properties similar to the properties of hydrated hard α-keratins, and should swell more than hard α-keratins in water. Mechanical and swelling measurements of hagfish threads were consistent with both of these predictions, suggesting that an elastomeric keratin matrix resists IF swelling and keeps IF stiffness and yield stress high. The elastomeric nature of the matrix is indirectly supported by the inability of matrix-free IFs (i.e. slime threads) to recover from post-yield deformation. We propose a general conceptual model of the structural mechanics of IF-based materials that predicts the effects of hydration and cross-linking on stiffness, yield stress and extensibility.

Keywords: intermediate filament; wool; stratum corneum; protein structure; soft keratin; hard keratin

1. INTRODUCTION

Hard α-keratin is a tough composite material that forms structures such as hair, hooves and claws in mammals (Fraser & MacRae 1980). The composite consists of 7–8 nm microfibrils embedded in an isotropic, high-sulphur matrix (Feughelman 1959). It is now known that the microfibrils belong to the class of intracellular filaments known by cell biologists as intermediate filaments (IFs). Although current theories of keratin mechanics assert that the properties of hard α-keratins are dominated by the properties of the IFs (Wortmann & Zahn 1994; Hearle 2000), the intimacy of the IF–matrix interaction has made it extremely difficult to tease apart the mechanical contributions of the IF and matrix components.

In a previous study, we measured the tensile properties of hydrated hagfish threads, which consist of a nearly pure, solid bundle of axially aligned, keratin-like IFs (Downing *et al.* 1984; Spitzer *et al.* 1988; Koch *et al.* 1995; Fudge *et al.* 2003). The threads originate in the hagfish slime gland within specialized cells that assemble intracellular IFs into a 1–3 μm diameter thread that is several centimetres long (Downing *et al.* 1981; Fernholm 1981). Within mature thread cells, the thread is intricately coiled into a bundle or 'skein' that is ejected from the gland as part of the hagfish's defensive strategy. The threads lack the matrix proteins that exist in α-keratins such as wool, and they therefore represent a useful model for exploring the mechanics of IFs in the absence of a keratin matrix.

Interestingly, the properties of hydrated IFs suggested by hagfish thread mechanics are radically different from the properties of hydrated hard α-keratins (figure 1*a*). Specifically, hydrated hagfish threads are less than 1/300 times as stiff and almost five times more extensible than hydrated hard α-keratins such as wool. They also yield at a stress that is about 10 times lower, and a strain that is about 10 times higher (Fudge *et al.* 2003). In addition, matrix-free hagfish thread IFs do not recover from post-yield deformation (Fudge *et al.* 2003), whereas hydrated hard α-keratins recover completely (Hearle 2000). These properties of hydrated IFs thus contradict current theories of keratin mechanics (Wortmann & Zahn 1994; Hearle 2000), which assert that the mechanics of hydrated hard α-keratin are dominated by the IFs.

How can pure IFs differ so much in their mechanics from hard α-keratins? One possible explanation is covalent cross-linking. Hagfish thread IF proteins contain only one cysteine residue per IF dimer (Koch *et al.* 1995), whereas hard α-keratin IFs are known to have several disulphide cross-links (Wang *et al.* 2000). According to the model developed in Fudge *et al.* (2003), the soft elasticity exhibited by hydrated IFs is a consequence of the series arrangement of stiff coiled coils and soft, elastomeric terminal domains in IF dimers (figure 1*b*). Extension of this model to the higher-order half-staggered packing of dimers into IFs (Parry & Steinert 1999) suggests that cross-links between adjacent coiled coils within IFs should bypass the soft terminal domains, thereby giving the IFs an initial stiffness much closer to that of coiled coils, or *ca*. 2 GPa (Howard 2001).

While covalent cross-linking could explain the high initial stiffness of hydrated hard α-keratins, it cannot explain the high yield stress. The yield stress corresponds to the disruption of the *weakest* region of the coiled coils; thus increasing the yield stress requires stabilization of *all* portions of the coiled coils. Bridging adjacent coiled coils with cross-links should therefore have little effect on the yield stress. By contrast, removal of water from the IFs should *globally* stabilize the α-helix hydrogen bonds within the coiled coils, and therefore increase the stress at which they yield. In addition, water is a known plasticizer of proteins (Lillie & Gosline 2002), and its removal should drastically increase the stiffness of the elastomeric terminal domains. Dehydration will also increase lateral adhesion of coiled coils, thereby facilitating stress transfer between

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adjacent coiled coils. Dehydration should therefore increase the stiffness and the yield stress, and thus IF hydration could be a key parameter in determining the properties of IFs in hard α-keratins. We suggested, therefore, that the differences between the properties of wet IFs and hard α-keratins are due to dehydration of the IFs in hydrated hard α-keratins.

The dehydration hypothesis predicts that dry hagfish threads should have mechanical properties that are similar to those of hydrated hard α-keratins. The mechanical data presented here confirm this prediction. We also tested the prediction that pure IFs take up more water than the IFs in hard α-keratins, and found that hagfish threads indeed swell considerably more than hard α-keratins. Finally, we propose that swelling of IFs in hard α-keratins is resisted by elastic deformation of the keratin matrix, and we provide mechanical recovery data that are consistent with this idea.

2. MATERIAL AND METHODS

(a) *Experimental animals and slime collection*

Pacific hagfish (*Eptatretus stoutii*) were obtained and handled as described in Fudge *et al.* (2003).

(b) *Mechanical measurements*

The tensile properties of dry hagfish threads were measured with the glass microbeam apparatus described in Fudge *et al.* (2003) using a glass microbeam with a diameter of 124 μm. Hagfish thread skeins were unravelled and mounted in water, and the water was gradually replaced with anhydrous ethanol, resulting in a final ethanol concentration of *ca*. 95% (i.e. 26 changes). The lower surface tension and the dehydrating/stiffening effect of the ethanol allowed the threads to pass through the ethanol–air interface without significant deformation. Thread segments were secured to the testing apparatus using 5 min epoxy (Devcon, Danvers, MA, USA), and extended at a strain rate of 0.017 s^{-1}. Mechanical tests were conducted at room temperature (*ca*. 20 °C) and ambient humidity, which was *ca*. 40%.

Thread cross-sectional area was determined from the diameter of an adjacent piece of thread and measured using a Hitachi S-4700 scanning electron microscope (SEM). Samples were transferred to SEM stubs, secured with epoxy, and gold sputter coated for 200 s. Digital images were captured at an acceleration voltage of 5.0 kV. Initial stiffness (E_i) was calculated from linear regressions of stress–strain curves up to the yield point. Toughness was estimated from the energy to break, which was calculated as the area under the stress–strain curve.

(c) *Hagfish thread swelling*

Swelling was quantified by measuring the change in diameter and the change in length that occurs when hagfish threads are dehydrated. Diameter change was estimated by measuring the maximum diameter of threads within intact hagfish thread skeins in distilled water and following dehydration. A large number of skeins (30) were measured in both the hydrated and dehydrated state to reduce sampling error. For the measurement of hydrated thread diameter, stabilized skeins were wet mounted and three large drops of distilled water were drawn under the cover-slip using filter paper to ensure removal of the stabilization buffer. Diameter was measured with a ×100 interference contrast objective on a Leitz Orthoplan-pol microscope using a ×15 filar-micrometer eyepiece that was calibrated with a Bausch and Lomb calibration slide with 0.01 mm increments.

For the measurement of dehydrated diameters, hagfish thread skeins were isolated from slime gland exudate by filtering with 54 μm Nitex mesh and were then rinsed with anhydrous ethanol. A drop of skeins suspended in ethanol was placed on a glass slide, the ethanol was allowed to evaporate, and the dry skeins were covered with a drop of immersion oil. Maximum thread diameter was measured as described above.

Dehydration-induced length changes were measured by mounting 9–10 mm lengths of thread in distilled water onto the glass microbeam apparatus used for tensile tests. Thread length was measured to the nearest 10 μm by dialling the micrometer/traveller arm until the thread was just taut. The fibre was then slackened and the water in the 9 ml chamber serially replaced with anhydrous ethanol via 50, 1.0 ml replacements, resulting in a final water concentration of less than 1%. When the replacement was complete, thread length was measured again.

(d) *Recovery of hagfish threads and wool fibres*

We measured the recovery behaviour of a typical hard α-keratin fibre (Merino wool) and a matrix-free keratin (dry slime threads) over both short and long recovery times. Raw, lanolin-free Merino wool was obtained for these experiments from Birkeland Bros Wool, Ltd (Vancouver, BC). Short-term recovery was evaluated by performing 20 s load–unload cycles. Merino wool fibre recovery was measured using an Instron model 5500 universal testing machine. The ends of a single wool fibre were glued with epoxy between acetate sheets. Mounted samples were soaked in distilled water for 2 h before testing, and mechanical tests were performed at room temperature in distilled water at a strain rate of 0.017 s^{-1}. Load–unload cycles for dry slime threads were performed using the micromechanical apparatus described in Fudge *et al.* (2003) at a strain rate of 0.017 s^{-1}. Strain was measured simultaneously using a second video dimension analyser that tracked the movement of the traveller arm.

Longer-term recovery was measured for seven Merino wool fibres, with an average resting length in water of 8.1 mm. Fibres were mounted to a stretching apparatus described in Fudge *et al.* (2003) using epoxy. Mounted fibres were immersed in water at room temperature for 2 h before testing, and recovery was measured at 0.05 strain intervals. Fibres were extended at a rate of *ca*. 0.008 s^{-1} using a hand micrometer and held at the desired strain for 1 min. Threads were slackened and allowed to recover for 10 min, after which length was measured again to the nearest 10 μm. Data were collected for each thread in this way until failure.

Longer-term recovery was measured in dry hagfish threads at room temperature and relative humidity (RH = 40%) in a manner similar to that described above. Recovery from the following series of strains was measured in seven slime threads: 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90 and 1.0. Threads were stretched at a strain rate of *ca*. 0.008 s^{-1} to the desired strain, held for 1 min, and then allowed to recover for 10 min at ambient humidity, after which the recovered length was measured.

3. RESULTS

(a) *Dry hagfish thread mechanics*

Tensile tests on dry hagfish threads revealed a stress–strain curve resembling that of hydrated hard α-keratins,

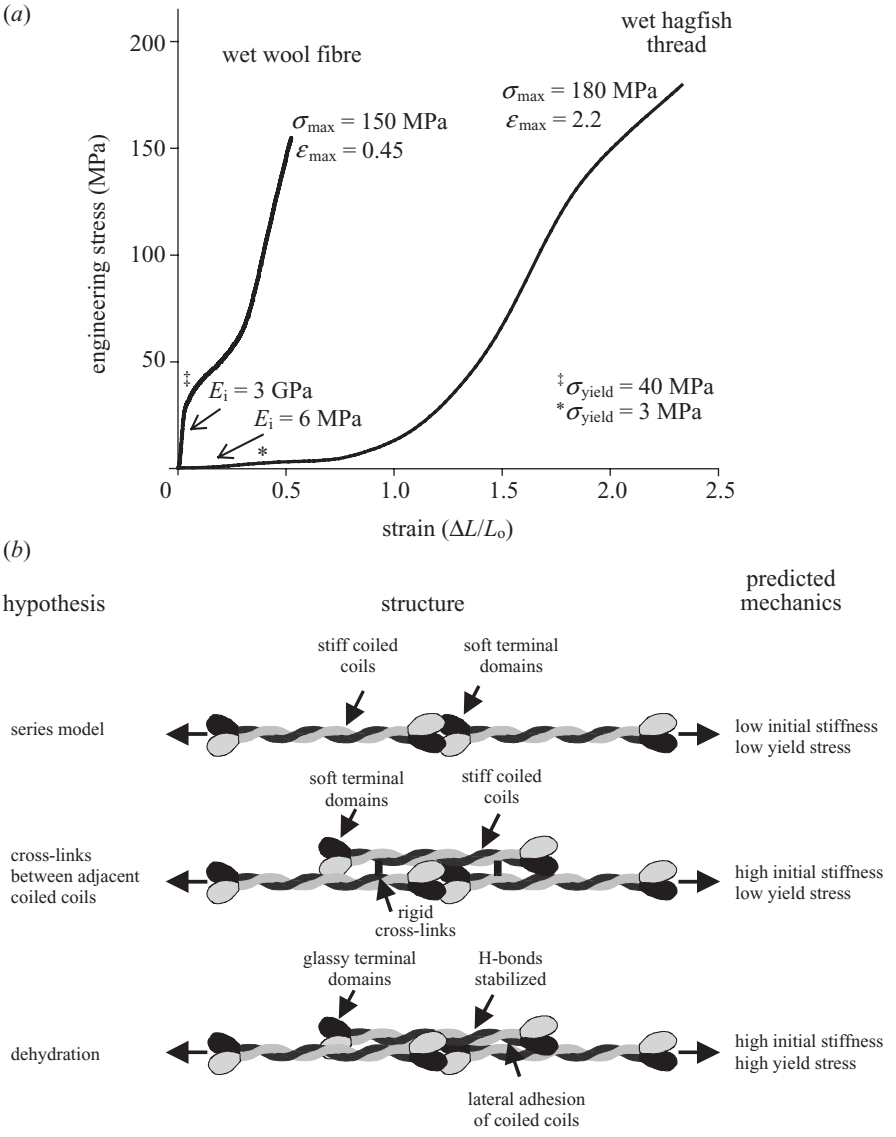

Figure 1. (*a*) Comparison of the tensile properties of hagfish threads in water (from Fudge *et al.* 2003) with hydrated hard α-keratins such as wool. σ_{max}, failure stress; ε_{max}, failure strain; σ_{yield}, yield stress; E_i, initial modulus. (*b*) Structural hypotheses that can account for some of the mechanical differences between hagfish threads and wool. In the series model proposed in Fudge *et al.* (2003), hydrated IFs possess low stiffness due to the series arrangement of soft terminal domains and stiff coiled coils, and a low yield stress due to the presence of water. Rigid covalent cross-links between adjacent coiled coils will bypass the soft terminal domains, resulting in a high initial modulus close to that of coiled coils (*ca*. 2 GPa), but should have little effect on the yield stress. Dehydration should have multiple effects on IF proteins, such as making the terminal domains glassy, increasing stress transfer among adjacent coiled coils, and stabilizing coiled-coil hydrogen bonds. Dehydration should therefore dramatically increase both the initial modulus and the yield stress.

with an initial stiff region, a long yield region and a final rise to failure (figure 2). Dry hagfish threads exhibited high initial stiffness ($E_i = 7700 \pm 500$ MPa, $n = 7$) and extensibility ($\varepsilon_{max} = 1.0 \pm 0.1$, $n = 13$), and yielded at a strain of 0.024 ± 0.001 ($n = 13$), and a stress of 150 ± 10 MPa ($n = 7$). Strength was 530 ± 40 MPa ($n = 7$), and toughness was 240 ± 20 MJ m^{-3} ($n = 7$). Note the dramatic differences in E_i and σ_{yield} between wet and dry threads (figures 1*a* and 2).

Compared with hagfish threads, the mechanics of hard α-keratins are much less sensitive to hydration (table 1), and this difference is consistent with the dehydration hypothesis. The initial tensile modulus (E_i) of dry hagfish threads decreased by a factor of 560 when they were hydrated, whereas E_i for hard α-keratins drops by a factor of only approximately 2.7 (Merideth 1956; Baden *et al.* 1974; Bendit 1976). These data suggest that the IFs in hard α-keratins remain at least partly dry even when the material is immersed in water.

Dehydration also has a strong effect on the yield stress of hagfish threads, with dry threads exhibiting a yield stress (150 MPa) that is approximately 50 times higher than hydrated threads (3 MPa). Hydrated keratins typically have a yield stress of *ca*. 40 MPa, which, for a typical keratin fibre composed of 30% matrix and 70% IFs by volume (Gillespie & Frenkel 1974), translates into a yield stress of *ca*. 60 MPa for the keratin IFs, assuming little contribution from the matrix. Because this value lies between the yield stresses of hydrated and dry IFs in hagfish threads, it is reasonable to conclude that the IFs in

Table 1. Effect of hydration on the initial tensile modulus of hagfish threads compared with hard and soft α-keratins (after Fraser & MacRae 1980).
(E_i = initial stiffness.)

material	dry E_i (GPa)	wet E_i (GPa)	ratio dry : wet	references	source
hagfish threads	3.6	0.006	560	this paper; Fudge *et al.* (2003)	hagfish slime
hard α-keratin	2.3	1.5	1.5	Baden *et al.* (1974)	human hair
	4.5	2.5	1.8	E. G. Bendit (unpublished data)	Lincoln wool
soft α-keratin	2.0	0.003	670	Park & Baddiel (1972)	stratum corneum

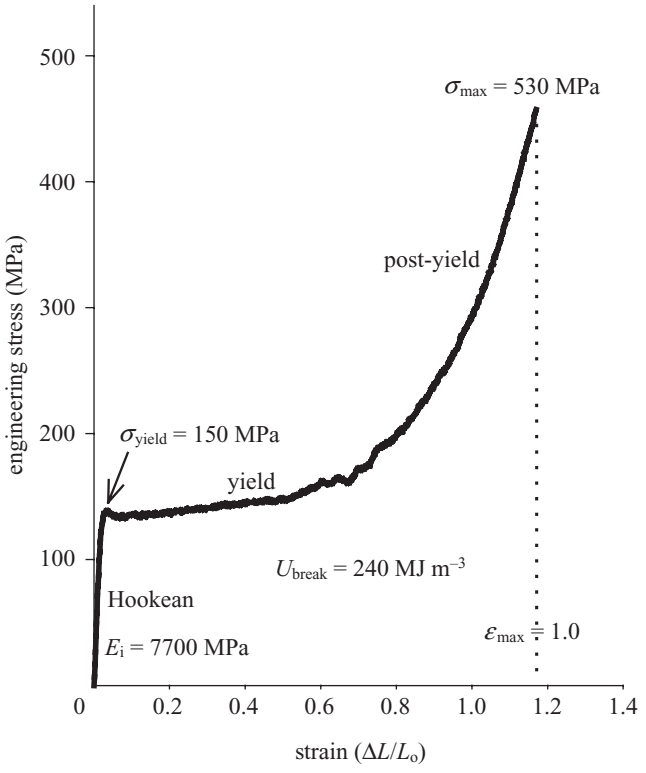

Figure 2. Typical tensile stress–strain curve for a hagfish thread tested in air. The three mechanical regions discussed in the text are labelled. Data provided are average values. E_i, initial stiffness; ε_{max}, extensibility; σ_{yield}, yield stress; σ_{max}, strength; U_{break}, energy to break, or toughness.

hydrated hard α-keratins are only partly dehydrated. This conclusion is also supported by the fact that the yield stress of keratin fibres such as wool increases steadily as RH decreases, with completely dry fibres (i.e. 0% RH) exhibiting a yield stress approximately four times higher than wet fibres (Feughelman & Robinson 1967).

(b) *Hagfish thread swelling*

Hydration caused significant changes in both diameter ($p < 0.001$, Mann–Whitney rank sum test) and length ($p = 0.009$, paired *t*-test) of hagfish threads. Diameter increased by 45% on average (2.2 ± 0.04 versus 3.2 ± 0.1 μm for dry and wet hagfish threads, respectively) and length *decreased* by 2.1 ± 0.8%. These values correspond to a 110% increase in cross-sectional area and a 106% increase in volume caused by hydration. By contrast, hydrated hard α-keratins increase in volume by only 35% (Feughelman 1987) to 43% (Fraser *et al.* 1972). These results also support the idea that IFs in wet hard α-keratins are not fully hydrated.

Because we do not know the contribution of the matrix to the swelling of α-keratins, it is difficult to quantify the difference in swelling between IFs in hagfish threads and IFs in hard α-keratins. However, hagfish threads swell so much more that it is almost impossible to imagine a scenario where IFs in hard α-keratins take up as much water as they do in hagfish threads. Consider a typical hard α-keratin fibre in which 30% of the volume is occupied by matrix, and the other 70% by IFs. If the IFs were to double in volume (as they do in hagfish threads) and the matrix showed *no swelling at all*, the fibre would still exhibit a 75% increase in volume, instead of 40%. In fact, in order for the IFs to double in volume and still result in only 40% overall swelling, the matrix would not only have to exhibit *negative* swelling, it would have to disappear completely. Clearly, this is not possible; thus, the water content of IFs in hydrated α-keratins must be lower than in hydrated hagfish threads.

(c) *Recovery of dry hagfish threads*

A primary difference between slime threads and hard α-keratins is the presence of an amorphous protein matrix in the latter. Therefore, we proposed that it is the matrix that opposes the swelling of IFs in hard α-keratins. We reasoned further that a protein network capable of resisting swelling should also resist longitudinal deformations and assist in the elastic recoil of hard α-keratins after deformation. Inversely, a matrix-free keratin (i.e. slime threads) should exhibit less recoil after deformation.

Load cycles revealed that even at relatively fast strain rates, wet Merino wool fibres recover almost completely from post-yield deformations, whereas dry hagfish threads do not (figure 3*a*). These data corroborate previous work on the recovery of hard α-keratins (Feughelman 1997). Recovery experiments over longer time-periods (10 min) revealed the same result, with wet wool fibres showing almost perfect recoil, and the dry slime threads showing mostly plastic deformation (figure 3*b*). When recovered strain is plotted against maximum strain, dry hagfish threads show a linear response with the relationship $\varepsilon_{recovered} = 0.86 \times \varepsilon_{max}$. This means that dry hagfish threads tend to recover only 14% of a given deformation after 10 min of recovery. The decreased ability of dry slime threads to recover implicates the keratin matrix as the source of longitudinal recovery in hydrated hard α-keratins and is consistent with our hypothesis that the matrix elastomerically resists IF swelling in hard α-keratins.

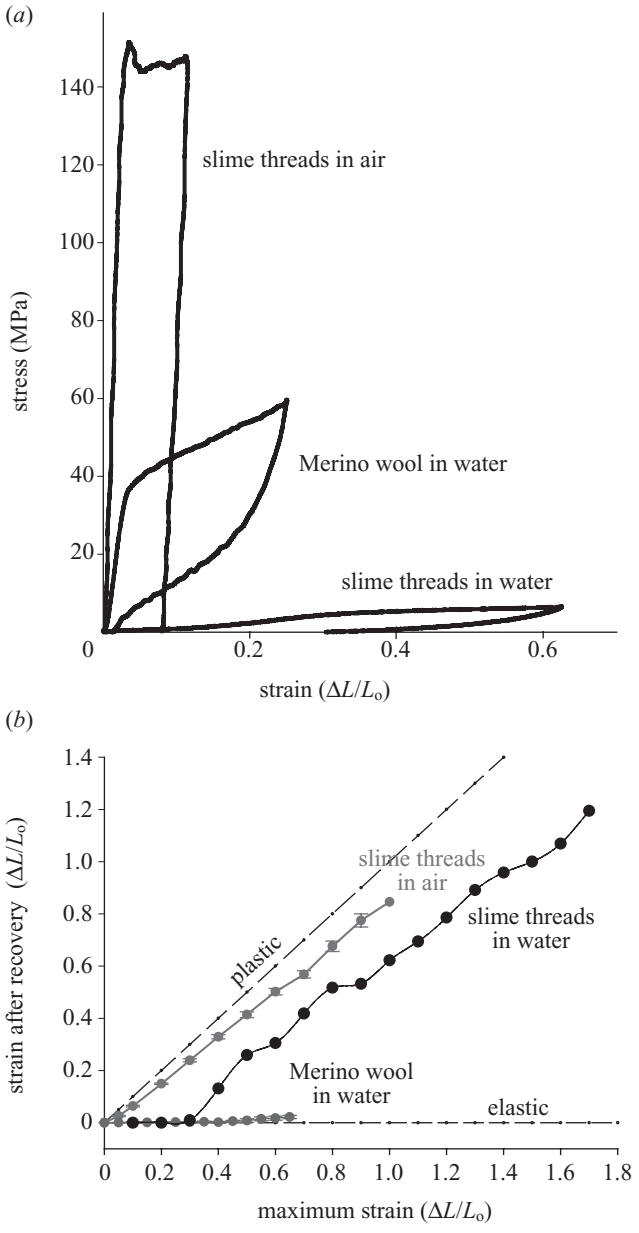

Figure 3. Short- and long-term recovery of wet and dry slime threads and wet Merino wool fibres. (*a*) Short-term recovery load cycles (*ca*. 20 s). (*b*) Long-term recovery. Dotted lines are curves for ideal elastic and plastic behaviour. Data for wet hagfish threads are from Fudge *et al.* (2003).

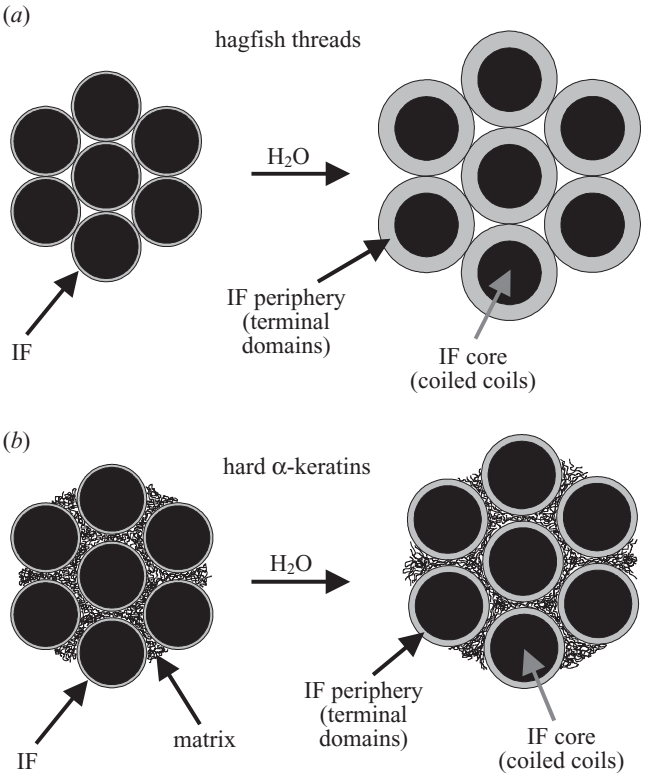

Figure 4. Schematic representation (cross-section) of swelling in matrix-free hagfish slime threads compared with swelling in hard α-keratins. Black circles represent the hydration-resistant central cores of the IFs, which are dominated by coiled coils. Grey outer regions denote more peripheral terminal domains. (*a*) Hagfish slime threads increase in diameter by 45%, most of which we attribute to swelling of the peripheral terminal domains. (*b*) Hard α-keratins swell considerably less than hagfish slime threads, a property we attribute to the presence of the keratin matrix. According to our model, keratin swelling is dominated by the swelling of peripheral IF terminal domains. IFs do not swell to the degree that they do in hagfish threads because further expansion of the IFs is opposed by deformation of the matrix. IF core size relative to inter-IF distance was based on X-ray diffraction data for porcupine quill from Fraser *et al.* (1972).

4. DISCUSSION

(a) *'Matrix squeeze' regulates IF hydration*

Matrix-free IFs are remarkably hydration sensitive, suggesting that the IFs in hard α-keratins somehow resist changes in hydration. Furthermore, the high E_i and σ_{yield} of hydrated hard α-keratins and dry hagfish threads suggest that IFs in hydrated hard α-keratins are maintained in a partly dry state. This conclusion is also supported by the fact that hard α-keratins do not swell nearly as much as hagfish threads when placed in water. We proposed that the hydration resistance of hard α-keratins is mediated by the keratin matrix, which elastomerically resists IF swelling. One prediction of this hypothesis is that an elastomeric matrix that resists circumferential expansion should also resist longitudinal deformation and impart the IFs with improved recovery. Recovery trials confirmed this prediction, demonstrating that matrix-free IFs exhibit poor recovery relative to hydrated hard α-keratins.

While one can imagine how an elastomeric protein network might resist IF hydration and swelling, it is less obvious how the IFs become dehydrated initially. In other words, how is it that IFs are assembled in an aqueous environment in developing keratinocytes, yet are partly dry when keratinization is complete, even when the cell is in equilibrium with water? One possibility is that dehydration occurs by air exposure, and subsequent cross-linking of the matrix locks the IFs into a dehydrated state. Another possibility is that cross-linking of the matrix occurs in an aqueous environment, and actually *causes* dehydration of the IFs. When covalent cross-links are introduced into an entangled polymer network, the network contracts to a smaller volume, forcing solvent out in

a process known as syneresis (Stropnik *et al.* 2000). Thus cross-linking of the keratin matrix could occur in the hydrated state, causing contraction of the matrix and water loss. Note that the result of both of the above mechanisms for the keratin composite in water is an outward pressure exerted by the IFs opposed by an inward pressure exerted by the matrix. The former mechanism requires an air dehydration step, which could help explain why many fur-bearing aquatic mammals such as seals confine their moults to brief periods on land. However, the development of chronically wet keratins, such as whale baleen, suggests that cross-linking must precede and in fact cause dehydration, at least in these structures.

The 'matrix squeeze' hypothesis described above explains not only the high initial stiffness and yield stress of hydrated hard α-keratins, but also a peculiar relationship that exists between keratin matrix content and hydration. In an interspecies comparison, Bendit (1980) demonstrated an inverse relationship between the swelling of hard α-keratins and matrix content. In other words, dehydrated keratins with a high proportion of matrix take up *less* water than keratins with a lower proportion of matrix. This result is especially puzzling given that the swelling of hard α-keratins is assumed to be dominated by swelling of the matrix, not the IFs (Fraser *et al.* 1972). Fraser & MacRae (1980) explain the inverse relationship between matrix content and water uptake as a problem of space constraints. They postulate that keratins with more matrix have a higher concentration of matrix protein in the inter-IF spaces, which leaves less volume for water to enter.

The matrix squeeze hypothesis provides an alternative explanation. If the keratin matrix mechanically resists IF swelling, then matrix-rich keratins should swell even less, and matrix-poor keratins should swell more. Curiously, this hypothesis hinges on the premise that swelling in keratins is governed by the swelling of IFs. How can this be, given the current understanding that the matrix swells much more than the IFs? Using X-ray diffraction, Fraser *et al.* (1972) demonstrated that swelling of porcupine quill causes a 13% increase in the distance between IFs, and only a 6% increase in the IF diameter. The authors concluded that swelling in quill thus occurs mostly in the interfibrillar space, which they assume is occupied by matrix. However, more recent measurements, using scanning transmission electron microscopy (STEM) suggest that some of this space may actually be occupied by IFs. Steven (1990) suggests that previous estimates of IF diameter are, in fact, estimates of the diameter of the IF *backbone*, which ignores the presence of a low-density halo that gives IFs an outer diameter that is 50% larger. Structural models suggest that the backbone consists of aligned coiled coils, while the halo consists of terminal domains clustered at the IF periphery (Steven *et al.* 1984). Thus, it is possible that the increase in interfibrillar space measured by Fraser *et al.* (1972) is actually an increase in the distance between IF backbones caused mainly by swelling of the IF protein terminal domains, with far less swelling of the matrix. We are not the first to suggest that IF swelling dominates the swelling of α-keratins. Zahn (1977) suggested that IFs should dominate the swelling of hard α-keratins because IF proteins are more hydrophilic than matrix proteins.

Previous models assumed that IFs are inherently hydration resistant, and that hard α-keratin swelling is dominated by matrix swelling (Fraser *et al.* 1972). The swelling data presented here for hagfish threads indicate the opposite; i.e. α-keratin hydration is dominated by IF swelling. In figure 4 we present a new three-phase hydration model, in which swelling is dominated by the IF terminal domains and resisted by deformation of an elastomeric matrix.

(b) *Implications for theories of keratin mechanics*

Currently, there are two dominant theories of hard α-keratin mechanics. While the theories are in agreement about most aspects of the hydrated hard α-keratin stress–strain curve, they differ in their interpretation of the upturn in the curve at the end of the yield region. One theory proposes that the stress increase is caused by strain hardening of the keratin matrix, which is modelled as an elastomer acting mechanically in parallel to the IFs (Chapman 1969; Hearle 2000). The other theory denies a significant role of the keratin matrix and attributes the increase in stress to the opening of α-helical domains stabilized by disulphide cross-links (Wortmann & Zahn 1994). Unfortunately, both theories predict a longer yield region for hagfish threads, albeit for different reasons. The first predicts a longer yield region due to the lack of a matrix in hagfish threads. The second theory also predicts a longer yield region, but in this case it is due to the paucity of cysteine residues in hagfish thread IF proteins. For this reason, the fact that the yield region of dry hagfish threads is about twice as long as it is in hydrated hard α-keratins (figures 1 and 2) does not help in distinguishing between the two models of keratin mechanics. However, the swelling and recovery data presented here do suggest that the keratin matrix can resist IF swelling and contribute to mechanical recoil after substantial deformations. These results are consistent with Chapman and Hearle's model and suggest that the matrix is robust enough to contribute to the post-yield rise in stiffness.

(c) *Implications for soft keratins*

The initial stiffness of soft keratins such as stratum corneum decreases by almost 700-fold when hydrated, whereas the stiffness of hair and wool decreases by only 2.7-fold (table 1). In addition, stratum corneum almost doubles in volume when it hydrates (Blank 1952), which far exceeds the volume change for hard α-keratins. This indicates that the IFs in stratum corneum hydrate more completely than the IFs in hard α-keratins, and therefore should mechanically resemble hagfish threads in water (figure 1) rather than hagfish threads in air (figure 2). Indeed, the E_i of hydrated stratum corneum is only *ca.* 3 MPa (Park & Baddiel 1972), which is very similar to the E_i of hagfish threads in water. Given its role as a flexible barrier, it is not surprising that the IFs in the stratum corneum are extensible and resilient like hydrated hagfish threads. If the IFs were dehydrated, this critical barrier would be awkwardly inflexible and prone to cracking. The matrix squeeze model presented here predicts that the hydration sensitivity of soft α-keratins is due to the presence of a compliant matrix that allows for more complete IF hydration and swelling. This is an intriguing prediction that we intend to explore further.

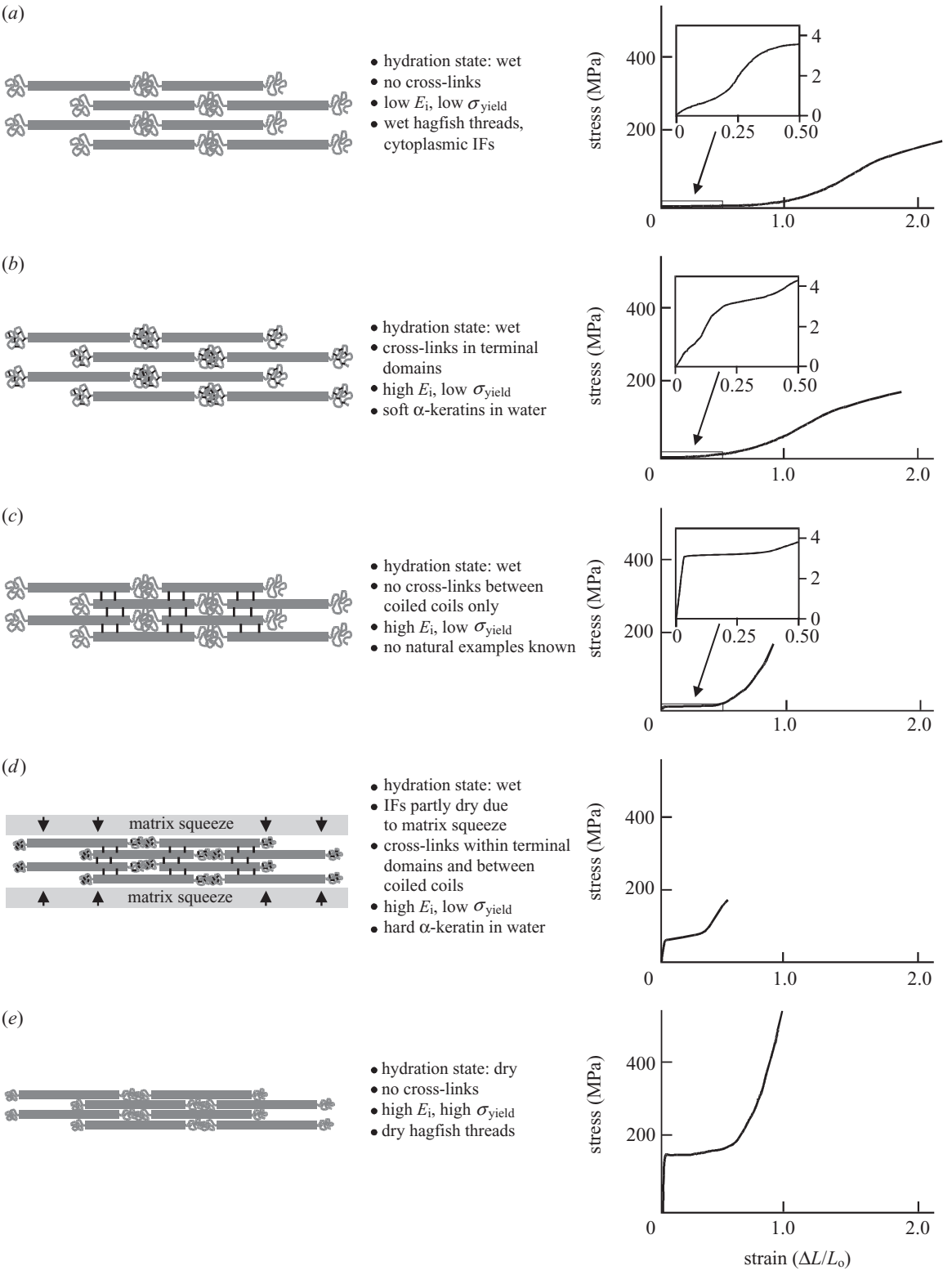

Figure 5. Summary schematic of the range of mechanical properties that can be achieved in IFs through variation in covalent cross-linking and hydration. Grey bars represent coiled coils, squiggles terminal domains, and black bars covalent cross-links. (*a*) In the absence of cross-links, hydrated IFs are soft and extensible with a low initial stiffness and yield stress. Inset shows a detail of the stress–strain curve up to a strain of approximately 0.5. (*b*) Introduction of cross-links within the terminal domains increases their stiffness, and therefore the initial stiffness of the IFs. Extensibility is slightly reduced, but the yield and ultimate stresses are mostly unaffected. (*c*) Cross-linking of adjacent coiled coils dramatically increases initial stiffness, but yield stress increases only slightly. (*d*) IFs in hydrated, hard α-keratins are partly dehydrated by the keratin matrix, and therefore possess both high stiffness and yield stress. (*e*) Dehydrated IFs in the absence of cross-links exhibit high stiffness, strength and yield stress.

(d) *Dry IFs are remarkably tough*

The high breaking strength of dry hagfish threads ($\sigma_{max} = 530 \pm 40$ MPa) and their high extensibility ($\varepsilon_{max} = 1.0 \pm 0.1$) combine to make them one of the toughest fibres known, with an energy to break of 240 ± 20 MJ m^{-3}. Only spider and insect silks possess comparable toughness. Mussel byssal threads, although as extensible as dry hagfish threads, are not nearly as strong, and therefore not nearly as tough. Collagen and wool are both less extensible and less strong, making them also far less tough than dry hagfish threads (see Gosline *et al.* 1999, 2002 for comparative data).

The fact that dry IFs in the absence of a matrix (i.e. dry hagfish threads) exhibit such impressive mechanical properties raises the question of why hard α-keratins have a matrix at all, when undiluted dry IFs are stiffer, stronger, tougher, and have a higher yield stress. The analysis provided above suggests that the hard α-keratin matrix performs two important functions, hydration resistance and recovery. Hydration resistance is critical for structures that require high stiffness and yield stress under a wide range of conditions. For example, horse hooves made purely of IFs would be of little use if they swelled and softened in water like hagfish threads. While plastic deformation is not a problem for disposable structures such as hagfish threads, more permanent structures such as hooves, horns and claws cannot afford such a luxury. The matrix is also likely to be important in preventing crack propagation and in allowing the keratin composite to better withstand compression loads. These positive contributions of the matrix to keratin mechanics probably balance the diluting effect it has on α-keratin toughness, strength and stiffness.

(e) *A general model for the mechanics of IF-based materials*

The data presented here and in Fudge *et al.* (2003) provide valuable insights into the mechanical properties of IFs in the absence of a keratin matrix. Most significantly, these results demonstrate that IF mechanics are remarkably sensitive to hydration, such that construction of IF-based materials possessing high stiffness and yield stress requires some dehydration of the IFs. By extending the model of IF dimer mechanics developed here and in Fudge *et al.* (2003) to higher-order structures within IFs, we have constructed a generalized molecular model of IF mechanics that takes into account the effects of covalent cross-linking and hydration. The schematic presented in figure 5 portrays half-staggered IF dimers as they might exist within intact IFs. Under hydrating conditions and in the absence of covalent cross-links (figure 5*a*) IFs are soft and extensible like hagfish threads in water. Introduction of covalent cross-links within the terminal domains (figure 5*b*) will serve to increase the stiffness and decrease the extensibility of the terminal domains, causing a modest increase in initial stiffness and a slight decrease in yield strain and strain at failure. Introduction of covalent cross-links between stiff coiled coils (figure 5*c*) will serve to mechanically bypass the soft terminal domains, dramatically increasing the initial stiffness. Under hydrating conditions, however, the effect on the yield stress will be modest. Partial dehydration of the IFs as occurs in hard α-keratins (figure 5*d*) will substantially increase the yield stress. Complete dehydration (figure 5*e*) results in IFs that are stiff, strong and remarkably tough, with a high yield stress. We are currently investigating some of the predictions of this model by measuring the mechanical properties of hagfish threads exposed to various kinds of cross-linking agents and/or infiltrated with artificial matrices.

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