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Rheology of Salmon Skin Mucus

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ABSTRACT

Studies of mammalian and molluscan mucus secretions have highlighted the wide variety of rheological responses these materials can exhibit dependent on the deformation conditions. Particularly, non-linear responses reported for mucus have been linked to diverse physiological functions. Here we investigate the rheology of mucus from salmon skin.

INTRODUCTION

In common with other mucus secretions, fish skin mucus is a hydrated gel of macromolecular glycoproteins[1, 2], mucins, which are structurally related to the mucins that compose mammalian[3] and molluscan mucus[4]. Compared to other mucus secretions, studies of fish skin mucus are limited, however the broad similarities between mucus secretion from very diverse sources[5-9] and a recently published article on the rheology of loach skin mucus[10] make salmon skin mucus an interesting focus for study.

Mucus secretions have diverse roles in mucosal protection, as both a barrier[5, 11] and a lubricant[5, 12], and in motility such a mucociliary clearance[8] and gastropod locomotion[4], and in all of these roles the rheology of the secretion is central to physiological function.

The matrix of the mucus hydrogel is comprised mainly of polymeric mucins, which interact with each other through a wide range of non-covalent intermolecular interactions[13, 14]. Mucus gels have a significant viscous component to their behaviour with typical phase angles in the region ~5° to 25°, corresponding to the viscous response having a magnitude between approximately half and one tenth that of the elastic modulus[5]. This inherent flow character of the mucus is important as it allows the mucus layer to adapt to the constant deformations of mucosal surfaces *in vivo*. Mucus gels are also rheologically reversible, transitioning to viscous dominant behaviour upon exposure to sufficient shear force and regaining elastic dominant behaviour upon removal of shear. This property is central to both lubricative[12] and gastropod locomotive functions[4].

Interestingly, in addition to strain softening and transition to flow, mucus gels have been shown to exhibit extensive and varied hardening behaviours under applied deformation in both the small and large deformation regime including strain hardening[6, 8, 15], stress hardening during repeated creep compliance testing[9] and rheopexy[10]. The variability of mucus rheological behaviour may go some way to explaining both why mucins are genetically so well conserved in nature and how mucus secretions can perform so many varied functions.

MATERIALS AND METHODS

Mucus was gently scraped from the skin of recently euthanized salmon, taking care minimise skin damage and the presence of scales in the mucus. Mucus was stored frozen at -20°C then thawed at 4°C prior to testing.

Rheological tests were performed on a Rheologica StressTech rheometer fitted with a cone and plate geometry (25mm, 1°) at 10°C. Initial strain sweeps were carried out at a frequency of 1Hz to determine a suitable strain within the linear viscoelastic region for frequency sweeps. This initial sweep was terminated upon signs of weakening of the gel structure or upon reaching a suitable strain with a good signal to noise ratio for further testing. Frequency sweep were carried out in the range 0.001-5Hz at a target strain of 0.005. Strain sweeps were carried out at frequencies of 1 and 0.1Hz in the strain range 0.001-10. The mucus was allowed a recovery time of 30 minutes between tests.

RESULTS AND DISCUSSION

The frequency sweep (Fig. 1) of salmon skin mucus shows typical mucus type behaviour[8, 10, 13] with elastic modulus G' being greater than the viscous modulus G'' over the entire accessed frequency range and both moduli showing similar frequency dependence with no tendency to flow at lower frequencies.

Strain sweeps at 1 Hz (Fig. 1A) and 0.1Hz (Fig. 1B) also show broadly typical mucus behaviour[5, 10] with a small plateau region in the moduli values with increasing strain before a relatively stable region with slight decreases in the moduli associated with a slight increase in the phase angle, and finally significant drops in the moduli values (particularly G'), significant increases in the phase angle and a transition to viscous dominant behaviour. The absolute moduli values are higher at higher frequencies as is to be expected from a mucus gel. In the strain sweep at 1Hz there is an apparent plateau in the phase angle at strains of 0.5-1before the phase angle again increases with the transition to viscous dominant (flow) behaviour.

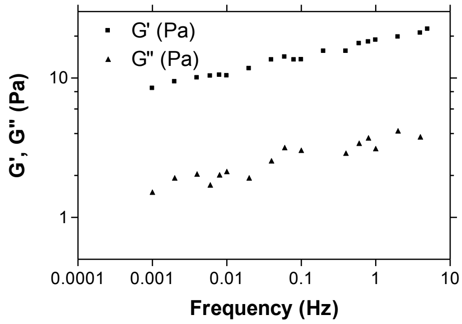
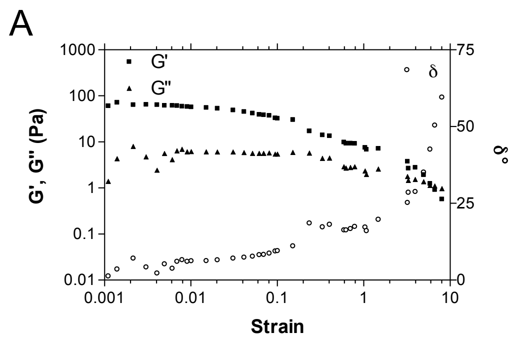


Figure 1. Frequency sweep of salmon skin mucus over the frequency range 0.001-5 Hz showing the elastic modulus (G') and the viscous modulus (G'').



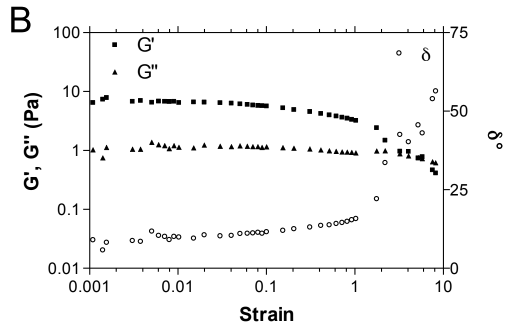


Figure 2. Strain sweep of salmon skin mucus in the strain range 0.001-5 at 1Hz (A) and 0.1Hz (B) showing the elastic modulus G', the viscous modulus G'' and the phase angle δ.

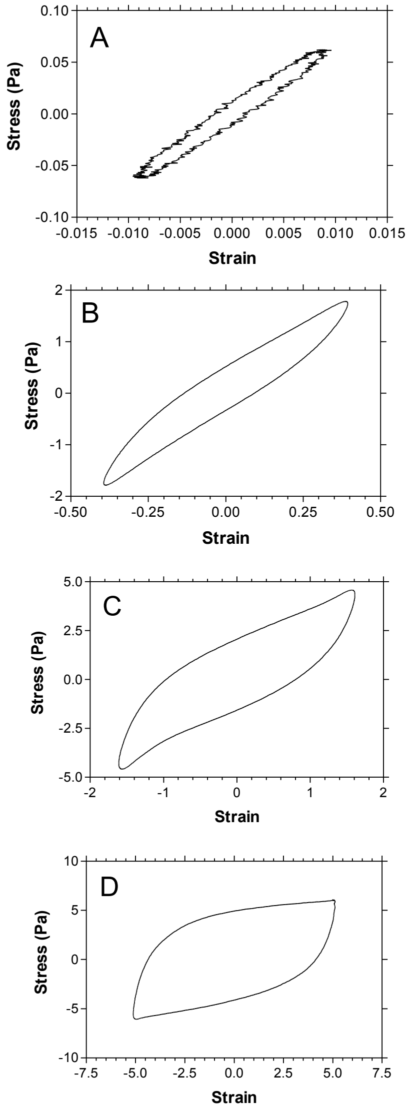


Figure 3. Lissajous figures showing the relationship between stress and strain during single oscillatory cycles of the strain sweep at 0.1Hz, for reference the measured phase angles for the data points were 10.1° (A), 13.6° (B), 22.1° (C) and 43° (D)

We can gain more insight into the behaviour during the strain sweep by considering the Lissajous plot of Stress against strain during single oscillatory cycles within the strain sweep. For the strain sweep at 0.1Hz, with the downward turn of the ends of the ellipse showing comparatively more stress is needed to induce unit strain than would be predicted from the central (smaller deformation) portion of the ellipse (Fig. 3B). As the strain increases the ellipse widens with the relative increase in viscous response but the stain hardening behaviour at the deformation maxima remains (Fig. 3C). Then as the strain increases further the ellipse widens further and hardening behaviour becomes less apparent (Fig. 3D).

This behaviour has been reported in mucus, particularly gastropod mucus[4], and appears to be a phenomenon that is observable as the frequency of oscillation is reduced below a critical level[4]. The requirement for a relatively low frequency of oscillation for this behaviour to be observed may suggest that either this behaviour is masked at higher frequencies by short timescale interactions, or that it requires molecular rearrangements which are only possible when the timescale of oscillation is sufficiently long and is thus only observed at lower frequencies.

The behaviour during individual cycles of the strain sweep at 1Hz is somewhat more complex. At low strains the stress-strain response during a single oscillation takes the form of an ellipse typical of a viscoelastic material (Fig. 4A) and as the strain increases the ellipse begins to show slight evidence of strain hardening towards the deformation maximums (Fig. 4B) as seen at 0.1Hz. This is followed by a transition to strain softening behaviour with the reverse deviation from the ellipse than seen earlier with the strain maxima showing comparatively less stress is needed to induce unit strain than would be predicted from the central (smaller deformation) portion of the ellipse (Fig. 4C). These first three Lissajous forms are typical for mucus strain sweeps at higher frequencies and presumably reflect the complex combination of intermolecular interactions with a wide range of lifetimes

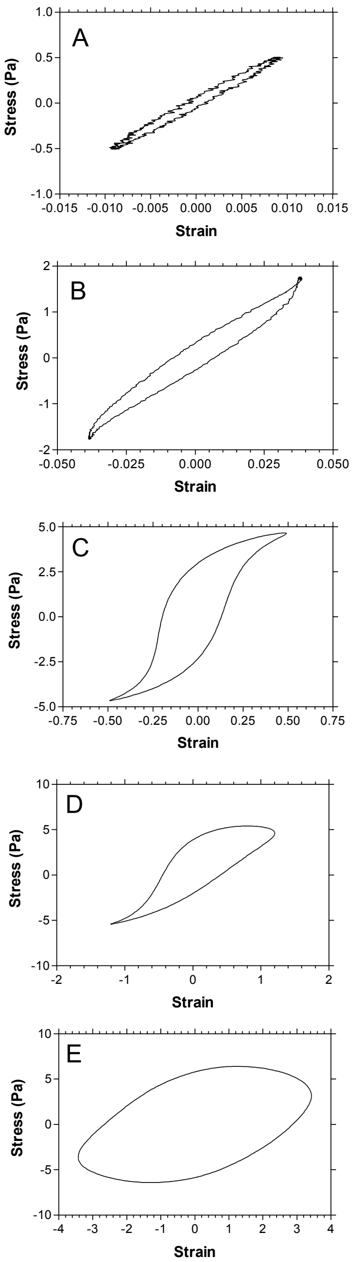


Figure 4. Lissajous figures showing the relationship between stress and strain during single oscillatory cycles of the strain sweep at 1Hz, for reference the measured phase angles for the data points were 6.0° (A), 7.5° (B), 18.1° (C), 17.2° (D) and 25.5° (E)

and the significant potential for molecular rearrangement in a gel matrix with such a significant viscous component. As the strain is increased further there is a further transition to anisotropic behaviour that has not previously been reported (Fig 4D). Here the opposing directions of the oscillatory cycle do not produce the same behaviour, and whilst the upper portion is similar to that seen at lower maximum strains the lower portion gives a relatively linear stress strain response. This is not a single event, but is seen in five consecutive oscillatory cycles, which rather interestingly correspond with the apparent plateau in the phase angle seen in the strain sweep (Fig. 2A). This unusual Lissajous stress strain profile suggests dynamic, and in this case directional, responses of the material to applied deformation. As the maximum strain is increased still further the behaviour returns to a more typical ellipse form and isotropy is restored (Fig. 4E).

CONCLUSIONS

Salmon skin mucus has been shown to have a rheological profile comparable to that of other mucus secretions of mammalian, gastropod and piscine origins. In common with other mucus secretions the salmon skin mucus demonstrates frequency dependent deviations from linear behaviour, including anisotropic behaviour not previously reported.

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