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Elastic contributions dominate the viscoelastic properties of sputum from cystic fibrosis patients

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Abstract

Sputum samples from cystic fibrosis (CF) patients were investigated by oscillatory, creep and steady shear rheological techniques over a range of time scales from 10^{-3} to 10^{6} s. The viscoelastic changes obtained by mixing sputa with the actin–filament-severing protein gelsolin and with the thiol-reducing agent dithiothreitol (DTT) were also investigated. At small strains sputum behaves like a viscoelastic solid rather than a liquid. A nearly constant steady shear viscosity at low shear rates is only observed after long shearing times which cause irreversible changes in the samples. Creep-recovery tests confirm that sputa exhibit viscoelastic properties, with a significant elastic recovery. The results suggest that measurements of elastic moduli, rather than viscosities are more closely related to the mechanical properties of sputum in situ. Severing of actin filaments lowers the elastic modulus by 30–40%, but maintains viscoelastic integrity, while reduction of thiols in the glycoproteins nearly completely fluidizes the samples.

Keywords: Rheology; Viscoelastic properties; Sputum; Cystic fibrosis; Actin filaments

1. Introduction

Biological materials and purified biopolymers have been the subjects of many rheological studies, both because biopolymers have viscoelastic properties that often differ from most synthetic materials and because the viscoelastic properties of soft biological materials are relevant to their physiological function. The pioneering studies of Ferry on the viscoelasticity of fibrin, reviewed in this issue by John Weisel, are a landmark example of how methods and concepts of polymer physics can help elucidate a polymer network structure as well as guide identification of molecular defects that cause altered fibrin clot structure in vivo. Another example in which viscoelasticity is directly related to a pathologic condition is the increased stiffness—both elasticity and viscosity—of lung airway fluid in patients with cystic fibrosis (CF). The increase stiffness of this fluid prevents proper clearance of fluid from the airway, and therapeutic methods are often directed to decreasing the viscoelastic parameters of this material to their normal levels. Design of agents capable of reversing the increased stiffness of CF sputum is a major goal for treatment of this disease, and rheological characterization of these materials can shed light on the structures responsible for the altered viscoelasticity in the diseased state.

Cystic fibrosis is inherited as an autosomal recessive gene defect. Disorders in ion transport channels in airway epithelium cells may contribute to abnormalities in the volume and composition of the biopolymers in airway secretions [1,2]. Increased sodium ion flow and reduced chloride permeability may be accompanied by a reduced water content of airway secretions which favor bronchial infections [3,4]. Associated with bacterial colonization of CF sputum are increased numbers of leukocytes in CF sputum and a high content of degenerated leukocytes and cell debris [5]. CF sputa are typically highly viscous and

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elastic [6] and are removed with difficulty by the ciliary and cough mechanism [3]. The abnormally thick consistency of CF sputum has been proposed to be due in part to the presence of DNA and actin filaments released from ruptured leukocytes. DNA and actin concentrations in sputum are typically in the mg/ml range and vary between patients [4,5,7–9]. Deoxyribonuclease I (DNase I) enzymatically hydrolyzes DNA, and was proposed in the 1950s for CF treatment [10], with current therapeutic use based on the improved purity of recombinant protein [11]. About 10% of the total leukocyte protein content is actin, a globular protein that polymerizes into long filaments (F-actin) that can strongly enhance viscoelastic parameters if they combine with the normal mucus network. Because the effects of polymers on solution viscosity depend on polymer length, a number of strategies aimed at decreasing DNA or actin fiber length have been devised.

The major constituent of both normal and CF sputum is a network of glycoproteins linked by disulfide bonds. This network contributes to the viscoelasticity of sputum, and its rheology can reasonably be compared with that of structurally similar mucins found in other tissues [12,13]. Alterations of the glycoprotein network and incorporation of abnormal constituents, such as those released from inflammatory cells, may alter strongly the rheology of sputum in a pathologic setting. Enhanced elasticity and viscosity of sputum in diseases such as CF have been proposed to be responsible for the difficulty of expelling this material from the airway, and some methods of treatment rely on the ability of agents to lessen the stiffness of the infected material and restore viscoelastic parameters that are appropriate for clearance. It is therefore important to obtain accurate quantitative measurements of the rheological properties of sputum, so that the treatment of cystic fibrosis and other respiratory ailments by these methods can be optimized.

The rheological properties of sputa have been investigated for several decades, and the characteristic elasticity of lung mucus at small deformations (strains) has been noted [14]. However, for technical reasons, many of the early rheological measurements performed on sputa have been carried out with relatively large deformations and forces. Consequently, much of the data on sputum rheology, including data showing the effects of potential mucolytics, has focused on the viscosity of the material after the initial disruption of its elastic component. To investigate the possibility that shear viscosity measurements damage networks or other elastic structures in the sputum, we have used a combination of oscillatory, creep-recovery and steady shear techniques. The results suggest that pure viscous flow only occurs at long deformation times in sputum samples and that methods designed to measure viscosities may destroy part of the biopolymer structures responsible for the abnormal rheology of CF sputum. In contrast, measurements of shear elastic moduli at low strains appear to preserve more of the native structure of the sputum and may be superior methods to evaluate the effects of potential mucolytic agents.

2. Materials and methods

Human blood plasma gelsolin was prepared by the method of Kurokawa et al. [15]. Dithiothreitol (DTT) was purchased from Sigma (St. Louis, MO). A total of 23 sputum samples from different CF patients were obtained from hospitals in Boston and Copenhagen, and in most cases, were stored frozen at −20 °C for up to 6 months prior to measurements on the rheometers described below. Before measurements the sputum was thawed, excess fluid was removed, and the sputum was transferred to the rheometers. Visual inspection of the sputum samples showed variations in consistencies ranging from fairly solid to nearly liquid samples. Each sample was therefore always measured first as a control and reference before addition of other agents. Gelsolin or DTT were subsequently added to final concentrations of 200 nM and 5 mM, respectively, and mixed with the sample in the rheometer, allowing 15 min of incubation time before subsequent measurements.

The rheological properties of a material are determined from the measured relationship between forces acting on the material and the resulting deformations. In a simple shear deformation, a cube is deformed by applying a tangential force to one side of the cube. Stress, σ, is defined as force per area of the cube, and strain, γ, is defined as the relative deformation ($\gamma=\Delta x/h$, where Δx is the tangential deformation of the cube and h is the height of the cube) [16]. Ideal elastic and viscous model systems are convenient references when more complex systems are analyzed. An ideal elastic system stores all energy during the deformation of the system, and for small deformations, there is a linear relationship, Hooke's Law, between stress and strain:

$$\sigma = G\gamma \qquad (1)$$

where G is the elastic modulus which is a material constant that is independent of time and strain for an ideal elastic system. For an ideal Newtonian liquid, the stress is independent of strain but proportional to the shear rate, $\dot{\gamma}$, which is the time derivative of the strain, and the stress is given by:

$$\sigma = \eta\dot{\gamma} \qquad (2)$$

where η is the shear viscosity which, for an ideal liquid, is independent of time and shear rate.

Most real materials are neither ideal elastic nor viscous but exhibit a mixture of both viscous and elastic properties. Such viscoelastic materials are conveniently studied by oscillatory techniques in which the material is either deformed using an oscillatory strain deformation or by applying an oscillatory stress to the material. When a viscoelastic material is subjected to a sinusoidal shear deformation, both the stress and the strain will oscillate in time, t, and the stress will be given by [16] :

$$\sigma(t) = \gamma_0(G'(\omega)\sin(\omega t) + G''(\omega)\cos(\omega t)) \quad (3)$$

where γ_0 is the strain amplitude and ω is the angular frequency in rad/s ($\omega=2\pi v$, where v is the frequency in Hz). G' is the elastic storage modulus and G'' is the loss modulus. For an ideal elastic system, $G'=G$ and $G''=0$, whereas $G'=0$ for an ideal viscous system and $G''=\omega\eta$ as seen from Eqs. (1) and (2), respectively.

The rheology of the sputum samples was measured using four different types of rheometers. The Bohlin VOR instrument (Lund, Sweden) and the Rheometrics RFS-II (Piscataway, NJ, USA) instrument are controlled strain rheometers, in which a sample is deformed and the stresses measured. The Haake RS100 (Karlsruhe, FRG) instrument and the torsion pendulum [17] are controlled stress rheometers, in which a stress is applied and the resulting strain is measured. All measurements were performed at 25 °C.

The VOR instrument was equipped with a parallel plate (diameter, 30 mm) measuring cell, and the RS100 instrument with a parallel plate geometry (diameter, 20 mm, and serrated plates). Both measuring cells with sputum samples were surrounded by covers containing droplets of water to maintain humidity. The VOR instrument was used to measure the frequency dependence of G' and G'' between 0.001 and 10.0 Hz and their strain amplitude dependence between 0.001 and 0.20 at a fixed frequency of 1 Hz. The stress was also measured as a function of time at constant steady shear rates of 4×10^{-3} s^{-1} and 2×10^{-2} s^{-1} and as function of shear rate between 1×10^{-3} and 200 s^{-1} at either increasing or decreasing shear rates. Samples were presheared for 20 s and then measured for 20 s at each shear rate.

The RS100 rheometer was used to perform creep and creep-recovery measurements. In a creep experiment, the sample is subjected to a constant stress (σ_0) and the strain γ is measured as a function of time. After a given time, this stress is removed, and the recovery of the strain is monitored with time. The creep and recovery parts were followed for 300 and 600 s, respectively, and stresses of 0.03–7 Pa were applied during the creep part. The shear modulus of an elastic material can be calculated from the measured strain in the creep part by use of Eq. (1), and the viscosity of a liquid from the shear rate by use of Eq. (2). The torsion pendulum with parallel plates (diameter, 18 mm) was operated in shear creep mode with stresses between 0.05 and 0.2 Pa. The small stresses enable measurements of viscosities at very low shear rates from 6×10^{-6} to 2×10^{-4} s^{-1}.

The RFS-II instrument with cone and plate geometry was used for measurements of shear viscosity at shear rates from 1 to 5000 s^{-1}. The sample was first deformed at a constant shear rate in a clockwise direction and then in a counterclockwise direction, and the calculated viscosity is the average of the two values. Measurements were started at the lowest shear rate for 20 s per direction.

3. Results

3.1. Oscillatory results

Fig. 1 shows the frequency dependencies of G' and G'' for a typical sputum sample. The figure illustrates that G' exceeds G'' at all measured frequencies, as was observed for all control samples. This result shows that sputa are viscoelastic solids at all examined frequencies, and it is seen that a plateau in G' may be approached at very low frequencies, which would then indicate a permanent network contribution. Fig. 1 shows furthermore that sputa have a broad distribution of relaxation times because G' gradually increases with increasing frequencies. The observed range of G' and G'' moduli for eight samples were 2.2–30 and 0.9–6.0 Pa, respectively, at 0.9 Hz and strain amplitudes below 0.05. The samples thus cover a wide range of moduli; however, the ratios G''/G', which is the loss tangent, showed much less variations. The loss tangent for the samples was 0.29±0.09 at 0.9 Hz which offers additional evidence for the dominating elastic properties.

Fig. 2 shows G' values at a frequency of 1 Hz measured at increasing strain amplitudes. Addition of gelsolin, which severs actin filaments, has a fairly large effect on G' (reduced by about 30%), but much smaller than the effect of DTT (more than 90% reduction), which breaks the bonds linking glycoprotein chains. A summary of the average relative reduction of G' values after addition of gelsolin and DTT is given in Table 1. The loss tangent, which, for control samples were 0.29±0.09, was 0.30±0.11 after addition of gelsolin. After addition of DTT, the value was 0.84±0.22. This shows that DTT makes the sputum more liquid-like, whereas gelsolin reduces both G' and G'' approximately to the same degree. The G' values for the control are seen to

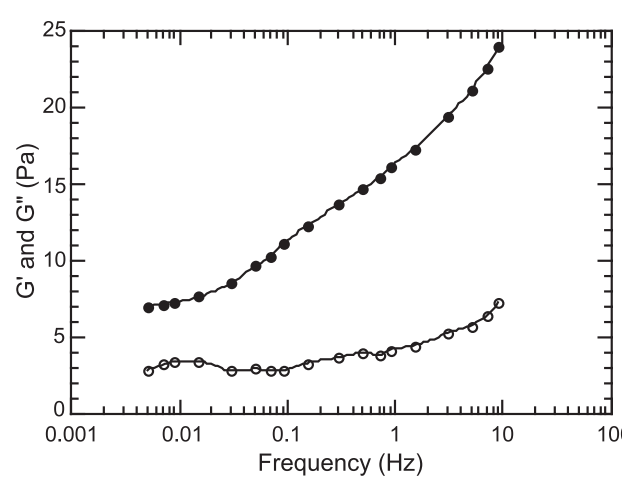

Fig. 1. Plot of G' (●) and G'' (○) against frequency for a typical control sputum sample.

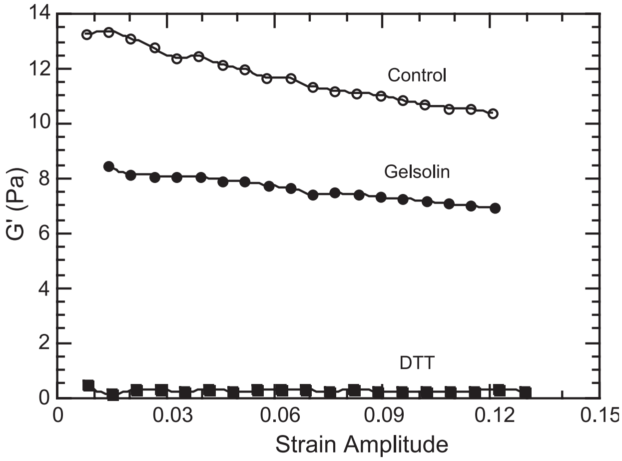

Fig. 2. Plots of G' against strain amplitude at 1.0 Hz for a control sputum sample (○) and for a same sample with added gelsolin (200 nM) (●) and DTT (5 mM) (■), respectively.

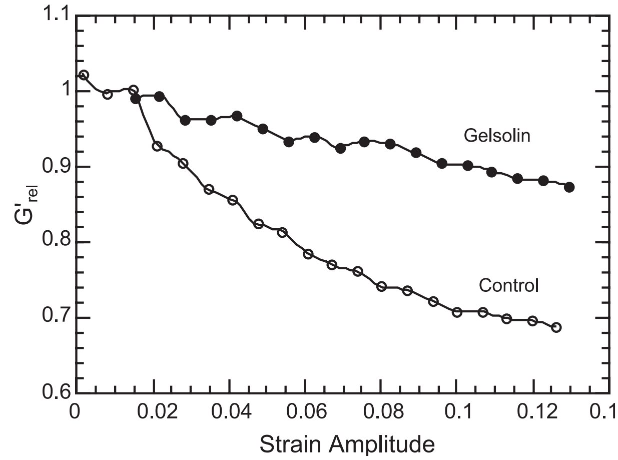

Fig. 3. Relative strain softening of sputum sample and effect of gelsolin. G' divided by the small strain value of G' is plotted against strain amplitude at 1.0 Hz. Results for control sample before (○) and after addition of gelsolin (●) are shown.

decrease with increasing amplitudes, and this decrease does not indicate a permanent damage of the sample because the same strain softening was observed in repeated measurements (data not shown). The sputum sample with gelsolin added shows a reduced degree of strain softening. This is more clearly seen in Fig. 3 which shows the strain amplitude dependence of G', divided by the G' value at small strain amplitudes, for a control sample and the same sample with gelsolin added. All samples investigated showed less pronounced strain softening after addition of gelsolin, and this suggests that at least part of the strain softening in sputum is related to the content of actin filaments.

3.2. Steady shear results

In contrast to oscillatory measurements of elastic moduli at low strains, steady shear measurements of viscosity require very large deformations before meaningful or reproducible data can be obtained. Fig. 4 shows how stress develops in a sample subjected to a constant rate (4×10^{-3} s^{-1}) of shear deformation. The figure shows the apparent viscosity defined as measured shear stress divided by the shear rate plotted against time. An ideal viscous system would give a constant viscosity on such a plot. The sputum sample does not behave as a simple liquid: the apparent viscosity increases to more than 2000 Pa s over the period of 10 min (curve 1), during which time the shear strain reaches 240%. At short times, the observed linear increase of the stress with strain is characteristic of an elastic material (see Eq. (1)). At larger times or strains, there is a sharp decrease in apparent viscosity to around 1000 Pa s. Some of the studied samples show such a sharp drop in viscosity, which may indicate rupture of structures in the sputum, but other samples showed a more slow gradual decrease of viscosity at long times. Subsequent measurements (curves 2 and 3) show similar viscosities at low strains, but reach a plateau at a lower level than the initial measurement and lack the large stress overshoot of the first measurement. Additional measurements at a higher shear rate (2×10^{-2} s^{-1}) show that lower stable viscosity levels are attained in a short time. Viscosities were smaller at high shear rates than at low rates for six samples. The maximal apparent viscosities observed for the sputum samples varied between 20–992 and 61–2300 Pa s, at the high and low shear rates, respectively. The results shown in Fig. 4 demonstrate that the sputum does not behave like a Newtonian liquid with a constant steady viscosity. The viscosity decreases both during repeated measurements at the same shear rate and with increasing

Table 1
Relative reduction of elastic and loss moduli at 0.9 Hz after addition of gelsolin (200 nM) and DTT (5 mM)

Additive	G'/G' (control)	G''/G'' (control)
Gelsolin	0.73±0.16	0.89±0.43
DTT	0.20±0.25	0.34±0.32

Mean and standard deviations for four samples are given.

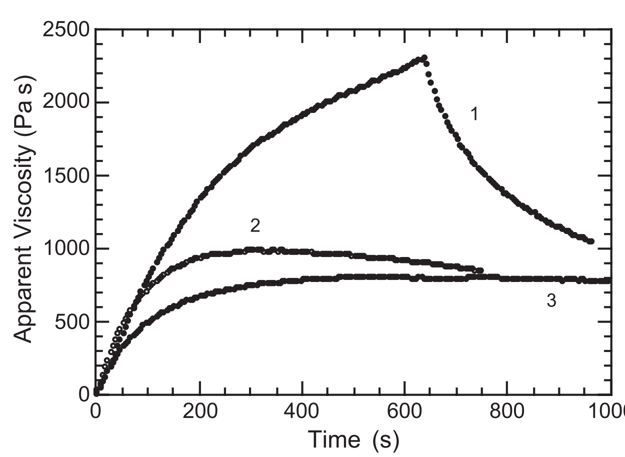

Fig. 4. Apparent viscosity, defined as stress divided by shear rate, plotted against time, in steady shear rate deformations. Rates are 4×10^{-3} s^{-1}. The measurements were performed consecutively on the same untreated sample from 1 to 3.

shear rates. It is therefore not possible to assign a well-defined viscosity to these sputum samples because the viscosity values depend strongly on the past history. This is illustrated in Fig. 5 which shows shear viscosities measured at increasing rates followed by measurements at decreasing rates. The sample was sheared for 40 s at each rate. The figure demonstrates that viscosities decrease with increasing rates and that measured viscosities depend on the strain history of the sample, and therefore exhibit thixotropy, as illustrated by the different values obtained at increasing and decreasing rates. Better agreement is obtained when samples were presheared at a high shear rate before measurements, but in this case, an irreversible change occurs that possibly obscures the physical properties of the native sputum.

3.3. Creep-recovery results

Additional evidence for the significant contribution of elasticity to sputum rheology is illustrated by the results in Fig. 6 obtained using a creep-recovery measurement. The compliance, $J(t)=\gamma(t)/\sigma_0$ is plotted against time during both the creep and the creep-recovery part. Results of three experiments on the same sample with stresses between 0.7 and 6 Pa are shown. The figure demonstrates that sputum is not in the linear range, where stresses are proportional to strain or rates, at the two high stresses because the compliance for such materials should be independent of stress. This creep curve is seen to consist of a rapid increase in compliance and strain when the stress is applied, followed by a more gradual increase at longer times. The rapid response is characteristic of an elastic material, whereas the gradual increase is characteristic of a viscoelastic material. The elastic response at short times, taken to be 1 s, corresponds to a J-value of 0.08 Pa^{-1}, and this corresponds to an elastic modulus of $G=1/J=12.5$ Pa. The creep measurements are consistent with the oscillatory measurements on the same sample which gave a G' of 12 Pa at a frequency of 0.1 Hz and a stress amplitude of 0.7 Pa. The creep-recovery parts, after 300 s of creep, show a rapid elastic retraction followed by a slower retraction characteristic of a viscoelastic system. At the end of the measured recovery, more than 75% of the strain is recovered at the low-stress experiment, and this again demonstrates that the elastic properties dominate in the sputum.

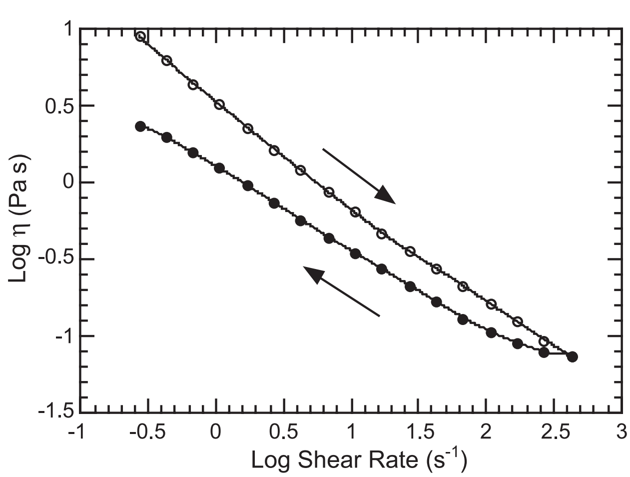

Fig. 5. Shear viscosity against shear rate for sputum sample measured first at increasing rates (○) followed by decreasing rates (●). Averaging time at each rate was 20 s after 20 s of preshearing.

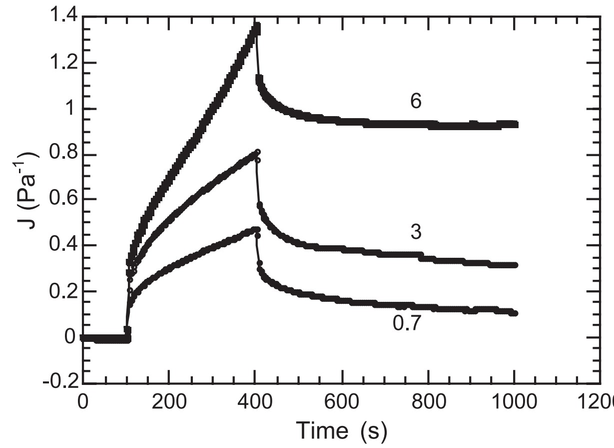

Fig. 6. Compliance plotted against time during a creep and a creep recovery experiment on sputum samples at the stresses shown at each curve. Stresses were applied to the sample at the time 100 s and removed again at time 400 s. The strain recovery was then monitored for 600 s.

The creep curve for the largest stress of 6 Pa shows a nearly linear increase of J with time. A linear increase of J or γ with time is characteristic for a viscous flow, and the viscosity can be calculated from the shear rate, by use of Eq. (2). The creep curves at the two low stresses in Fig. 6 do not reach a constant rate during the creep part and the rates are observed to decrease with time. However, the slopes just prior to the recovery part were used to calculate viscosities, and these are therefore estimates of minimum viscosity values. The viscosities of 12 sputum samples were determined in this manner from creep measurements by use of the torsion pendulum. The results are shown in Fig. 7.

3.4. Strain rate dependence of apparent viscosity

The contribution of elastic structures and the uncertainty as to whether or not steady flow is achieved without disruption of the samples suggest that there will be a wide variability in measured viscosities depending on the strain rate and the previous strain history of sputum samples. This expectation is verified by the data in Fig. 7 which shows experimental results on a number of different samples over a wide range of strain rates. The medium and high shear rate values up to 5000 s^{-1} were measured on the VOR and RFS-II instruments. The sample measured on the VOR instrument was presheared at 1 s^{-1} for 60 s. The straight line is a linear least squares fit to these data. The low shear rate values were obtained from creep experiments by use of the torsion pendulum as explained above. It is seen that these viscosities are in general below the fitted line most

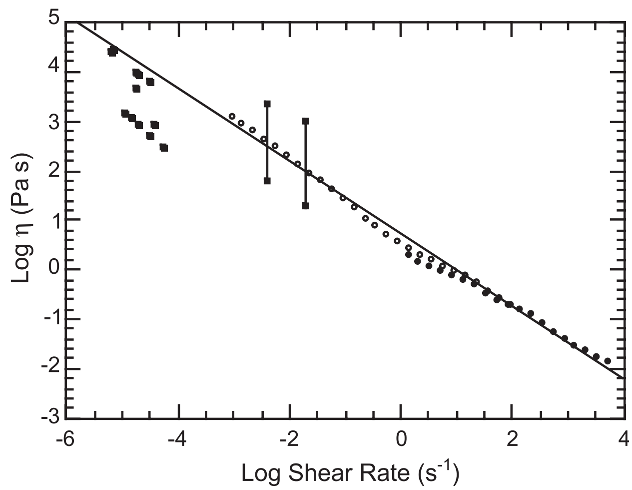

Fig. 7. Steady shear viscosities of sputum as a function of shear rate determined using different rheometers and techniques. Samples measured at a range of shear rates by use of a RFS-II (●) and a VOR instrument (○). A linear least squares fit to these data are shown with a line, which has a slope of −0.73. (□) Range of viscosities measured for seven samples at the two shear rates shown in Fig. 4. (■) Viscosities calculated from creep curves at low stresses for 12 samples.

likely due to an underestimate of viscosities from creep experiments as discussed above. There are two significant features of the results shown in Fig. 7. First, there is an approximately constant power–law dependence of viscosity on shear rate spanning nearly ten decades in shear rate. The absence of a rate-independent range of apparent viscosities shows that pure viscous flow has not been reached even at the lowest rates. Second, the slope of this log–log plot is approximately -0.73 ± 0.03 throughout a broad range. We cannot offer any theoretical explanation for this slope, but the rheology of this material resembles more closely a fragile solid in which flow occurs when fracture planes or other irreversible alterations of structure form after application of a critical stress. A power–law relationship has also been observed in viscoelastic studies on mucins isolated from CF patients [18].

4. Discussion

The abnormal viscoelasticity of infected sputum has been proposed to contribute to the clinical symptoms of respiratory diseases such as CF. Rheologic methods can directly test the efficacy of mucolytic agents in vitro, and these data may be useful for predicting their therapeutic value. Such methods have been applied to sputum samples for several decades, but the utility of these measurements has been questioned and the discrepancies in viscosity values reported by different groups are large. The lack of a coherent model for sputum viscoelasticity confounds efforts to determine the magnitude of effects that various potential mucolytic agents may have. In part, the variability of results is due to differences among samples and in methods of collection, but also, as shown in this work, to the inherent complexity of sputum rheology and on the emphasis on measuring viscosity rather than elasticity. As shown in Fig. 7, the values of sputum viscosity vary over many orders of magnitude, even when a single sample is measured at a range of shear rates. This extreme shear rate dependence suggests that sample rupture rather than viscous flow dominates the large strain deformation of sputum at high stresses. Whereas the complex flow properties of sputum complicate efforts to define an unambiguous viscosity in vitro, they are likely to be important for sputum function in vivo. In this context, elastic parameters such as the storage shear modulus may be a more useful measure of the properties of CF sputum and of the effects of mucolytics.

The results obtained in this investigation show a large variation among samples. This variation probably partly reflects variations in water content of the sputa, but part of it may also reflect variations in the content of actin, DNA and glycoproteins. The frequency dependency of G' in Fig. 1 shows that the sputum is characterized by a broad distribution of long relaxation times due to slowly relaxing structures. CF sputum contains a matrix of glycoproteins with long actin or DNA filaments imbedded in the glycoprotein network [19,20]. The slowly relaxing structures may be due to motions of these filaments. However, severing actin and DNA filaments do not completely eliminate the elasticity of the sputum or the frequency dependence of G'. The main part of the sputum elasticity is due to the matrix of glycoproteins, as demonstrated by the DTT results in Table 1 and in Fig. 2. The strain softening of sputum necessitates measurements at low-strain amplitudes. Severing actin filaments eliminates most of the strain softening as shown in Figs. 2 and 3, and this suggests that strain softening may be attributed to actin filaments. Gels consisting of actin filaments also show strain softening [21]. Additional studies are needed in order to show if there is a correlation between the DNA and actin contents of sputum and the rheological effects of additions of filament severing proteins.

The rheological properties are both frequency and strain dependent, and it is therefore necessary to know the precise experimental conditions used when comparing results. It is also important to develop methods that do not alter the rheological properties of sputum. We have performed repeated oscillatory measurements and obtained identical results on several samples. Strain sweeps as shown in Fig. 2 are also highly reproducible. Both frequency and strain measurements therefore serve as good control measurements when comparing samples and for investigations of the effects of mucolytic agents. Over the years, it has been attempted to characterize the rheological properties of sputum using steady shear viscosity measurements. We have summarized some of the viscosities reported in the literature in Table 2, together with shear rates used. The results from the different sources show clearly that the viscosity does not describe the rheological properties of sputum well, unless the shear rate is specified. The

Table 2
Viscosities measured at different shear rates from literature and this study

Shear rate (s^{-1})	η (Pa s)	Reference
1350	0.054	Charman and Ried [27]
1350	0.052±0.008	Picot et al. [9]
900	0.02–0.160	Feather and Russell [28]
Ca. 5	0.732	Shak et al. [11]
0.3	6–38	Puchelle et al. [3]
5×10^{-2}	8×10^{3}	Barnett and Dulfano [29]
2×10^{-2}	20–992	This study
8×10^{-3}	1.4×10^{4}	Barnett and Dulfano [29]
4×10^{-3}	61–2300	This study
$(3–16)\times10^{-4}$	322±199	Vasconcellos et al. [5]
1×10^{-4}	$(1–5)\times10^{4}$	Lethem et al. [4]

viscosities reported vary about 5–6 decades and are clearly dependent on the shear rate used in agreement with our results in Fig. 7. A similar log–log plot of the literature data in Table 2 shows a power–law slope of −0.80. Furthermore, the results in Fig. 3 demonstrate that the sputum only reaches a constant steady shear viscosity after very long times. The results in Fig. 1 demonstrate that if the sputum is a liquid, the longest relaxation times must be of the order of thousands of seconds, making sputum a viscoelastic solid at relevant physiological conditions. We can therefore only hope to get a zero-shear viscosity in a creep or very low shear rate experiment after several thousands of seconds, where drying and biochemical processes in the sputum may very likely become a problem.

The rheological properties of sputum are dominated by inter- and intramolecular interactions [20]. Some of the intermolecular interactions form a basic gel network which breaks during viscosity measurements even at very low shear rates, as seen in Fig. 4. It is, therefore, clear that the viscosity measurements cannot give direct information about the undamaged biopolymer structures in sputum samples. Several other studies have pointed out that sputum is irreversibly broken and gave altered rheological properties when subjected to a high shear rate [9,22]. Our results show that the apparent viscosity of sputum is reduced with increasing shear rates in agreement with a previous study [23]. It is therefore necessary to measure at the same shear rate to be able to compare results, and it is important to measure at a very low shear rate, to avoid or minimize irreversible breakdown of structures in sputum.

Davis and Dippy [24] and later, Mariott [25], emphasized that in order to determine the viscoelastic properties of intact sputum, creep or oscillatory techniques should be used in order to avoid irreversible breakdown of structures in sputum. Repeated creep or oscillatory measurements resulted in identical curves [24,25] in agreement with our findings. However, these authors did not observe a large elastic recovery for their samples. Our nearly complete recovery at low stresses, as seen in Fig. 6, may possibly reflect different stresses applied in our study relative to theirs. The most reliable information about the properties of intact sputum is therefore obtained by oscillatory techniques, which, in addition, operate in a biologically relevant timescale of about 10 Hz which is the beat frequency of the ciliary motions [26]. The viscoelasticity of sputum and its content of DNA and actin filaments also correlate with the transport and barrier properties of sputum [30–32].

5. Conclusions

The results demonstrate that sputum is a viscoelastic solid with a broad distribution of relaxation times. Some of these long relaxation times are probably due to slowly relaxing biopolymer structures; for example, long DNA or actin filaments embedded in the glycoprotein network. When gelsolin is added to sputum samples, the elastic modulus is lowered 30–40%, whereas addition of DTT causes a drastic reduction of the elastic modulus, to the point were the loss modulus exceeds the elastic modulus, and the sample is primarily fluid. These results suggest that the main contribution to the elasticity is due to the matrix of glycoproteins, and that the primary effect of cytoskeletal filaments is to increase this elastic component.

Repeated steady shear measurements lower the maximal viscosity. Therefore, viscosity measurements may be confounded by unknown and probably variable degrees of damage to the sample. The most relevant information about the rheological properties of native undamaged sputum is therefore obtained by use of oscillatory or creep techniques. These methods do not alter the rheological properties of sputum because repeated measurements give identical results.

Acknowledgments

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References

[1] C.F. Higgins, Cystic fibrosis transmembrane conductance regulator (CFTR), Br. Med. Bull., Cystic Fibros. 48 (1992) 754–765.
[2] T.F. Boat, in: J.F. Murray, J.A. Nadel (Eds.), Cystic Fibrosis, Textbook of Respiratory Medicine, W.B. Saunders Publ., Philadelphia, PA, 1988, pp. 1126–1152.
[3] E. Puchelle, J. Jacquot, G. Beck, J.M. Zahm, C. Galabert, Rheological and transport properties of airway secretions in cystic fibrosis—relationships with the degree of infection and severity of the disease, Eur. J. Clin. Investig. 15 (1985) 389–394.
[4] M.I. Lethem, S.L. James, C. Marriott, The role of mucous glycoproteins in the rheologic properties of cystic fibrosis sputum, Am. Rev. Respir. Dis. 142 (1990) 1053–1058.

[5] C.A. Vasconcellos, P.G. Allen, M.A. Wohl, J.M. Drazen, P.A. Janmey, T.P. Stossel, Reduction in viscosity of cystic fibrosis sputum in vitro by gelsolin, Science 262 (1994) 969–971.
[6] R.E. Wood, T.F. Boat, C.F. Doershuk, Cystic fibrosis, Am. Rev. Respir. Dis. 113 (1976) 833–878.
[7] W.S. Chernick, G.J. Barbero, Composition of tracheobronchial secretions in cystic fibrosis of the pancreas and bronchiectasis, Pediatrics 24 (1959) 739–745.
[8] J. Potter, L.W. Matthews, J. Lemm, S. Spector, The composition of pulmonary secretions from patients with and without cystic fibrosis, Am. J. Dis. Child. 100 (1960) 493–495.
[9] R. Picot, I. Das, L. Reid, Pus, deoxyribonucleic acid, and sputum viscosity, Thorax 33 (1978) 235–242.
[10] J.B. Armstrong, J.C. White, Liquefaction of viscous purulent exudates by deoxyribonuclease, Lancet 259 (1950) 739–742.
[11] S. Shak, D.J. Capon, R. Hellmiss, A. Marsters, C.L. Baker, Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 9188–9192.
[12] A. Silberberg, A model for mucus glycoprotein structure, Biorheology 24 (1987) 605–614.
[13] A. Silberberg, A model for mucus glycoprotein structure—comparison with submaxillary mucins, Biorheology 25 (1988) 799–801.
[14] R. Denton, Rheology of human lung mucus, Ann. N.Y. Acad. Sci. 106 (1963) 746–754.
[15] H. Kurokawa, W. Fujii, K. Ohmi, T. Sakurai, Y. Nonomura, Simple and rapid purification of brevin, Biochem. Biophys. Res. Commun. 168 (1990) 451–457.
[16] J.D. Ferry, Viscoelastic Properties of Polymers, 3rd ed., Wiley, New York, 1989.
[17] P.A. Janmey, A torsion pendulum for measurement of the viscoelasticity of biopolymers and its application to actin networks, J. Biochem. Biophys. Methods 22 (1991) 41–53.
[18] C.M. McCullagh, A.M. Jamieson, J. Blackwell, R. Gupta, Viscoelastic properties of human tracheobronchial mucin in aqueous solution, Biopolymers 35 (1995) 149–159.
[19] C.A. Sheils, J. Kas, W. Travassos, P.G. Allen, P.A. Janmey, M.E. Wohl, T.P. Stossel, Actin filaments mediate DNA fiber formation in chronic inflammatory airway disease, Am. J. Pathol. 148 (1996) 919–927.
[20] A.A. Bornstein, T.-M. Chen, M.J. Dulfano, Disulfide bonds and sputum viscoelasticity, Biorheology 15 (1978) 261–267.
[21] P.A. Janmey, S. Hvidt, J. Peetermans, J. Lamb, J.D. Ferry, T.P. Stossel, Viscoelasticity of F-actin/Gelsolin complexes, Biochemistry 27 (1988) 8218–8227.
[22] M. Litt, Mucus rheology. Relevance to mucociliary clearance, Arch. Intern. Med. 126 (1970) 417–423.
[23] S. Girod, J.-M. Zahm, C. Plotkowski, G. Beck, E. Puchelle, Role of the physicochemical properties of mucus in the protection of the respiratory epithelium, Eur. Respir. J. 5 (1992) 477–487.
[24] S.S. Davis, J.E. Dippy, The rheological properties of sputum, Biorheology 6 (1969) 11–21.
[25] C. Marriott, The viscoelastic nature of mucus secretion, Chest 80 (1981) 804–808.
[26] E. Puchelle, J.-M. Zahm, C. Duvivier, J. Didelon, J. Jacquot, D. Quemada, Elasto-thixotropic properties of bronchial mucus and polymer analogs: I. Experimental results, Biorheology 22 (1985) 415–423.
[27] J. Charman, L. Reid, The effect of freezing, storing and thawing on the viscosity of sputum, Biorheology 10 (1973) 295–301.
[28] E.A. Feather, G. Russell, Sputum viscosity and pulmonary function in cystic fibrosis, Arch. Dis. Child. 45 (1970) 807–808.
[29] B. Barnett, M.J. Dulfano, Sputum viscoelasticity: I. A new methodology, Am. Rev. Respir. Dis. 101 (1970) 773–776.
[30] M. Dawson, D. Wirtz, J.J. Hanes, Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport, J. Biol. Chem. 278 (2003) 50393–50401.
[31] B. Perks, J.K. Shute, DNA and actin bind and inhibit interleukin-8 function in cystic fibrosis sputa: in vitro effects of mucolytics, Am. J. Respir. Crit. Care Med. 162 (2000) 1767–1772.
[32] N.N. Sanders, S.C. De Smedt, E. Van Rompaey, P. Simoens, F. De Baets, J. Demeester, Cystic fibrosis sputum: a barrier to the transport of nanospheres, Am. J. Respir. Crit. Care Med. 162 (2000) 1905–1911.