Viscoelastic Properties of Sputum from Cystic Fibrosis Patients. Effects of Gelsolin, Dithiothreitol and DeoxyribonucleaseI.

Helle Jarden, Heidi Nielsen, and Søren Hvidt*

Department of Chemistry, Roskilde University, DK-4000 Roskilde, Denmark

ABSTRACT

Sputum samples from cystic fibrosis patients are studied by oscillatory and steady shear techniques. Sputum is shown to be a viscoelastic solid. Actin and DNA severing proteins reduce the elastic modulus moderately. The thiolreducing agent dithiothreitol lowers both the viscosity and the elastic modulus dramatically.

INTRODUCTION

Cystic fibrosis (CF) is inherited as an autosomal recessive gene defect. Disorders in the ion transport channels in airway epithelium cells may contribute to abnormalities in the volume and composition of airway secretions. Increased sodium flow and reduced chloride permeability may be accompanied by a water reabsorption resulting in a decreased water content of airway secretions, which favor bronchial infections[1].

Due to such infections, CF patients often produce sputum, which is highly viscous and elastic[2], and which is removed with difficulty by the ciliary and cough mechanism[1]. CF sputum has a high content of degenerated leukocytes, and a thick consistency[3] which may be due to the presence of DNA, actin filaments and glycoproteins. DNA and actin concentrations in sputum vary between 3-14 and 0.1-5 mg/ml, respectively[3,4]. DeoxyribonucleaseI (DNaseI) enzymatically severs DNA, and it has been used since the 1950s in CF treatment[4]. About 10% of the total leukocyte protein content is actin, which forms long actin filaments. Gelsolin can sever actin filaments, and it can reduce the viscosity of sputum *in vitro*[3]. Glycoproteins in sputum contain disulfide bonds and may also contribute to the viscoelasticity of the sputum. Disulfide bonds can be reduced by dithiothreitol (DTT), resulting in a lower viscosity *in vitro*[2].

Most of the sputum rheological studies reported in the literature are shear viscosity measurements, which likely will damage any network or elastic structure in the sputum. To investigate this possibility, we have used both oscillatory and steady shear techniques. The viscoelastic changes obtained by mixing sputum with gelsolin, DNase1, and DTT have also been investigated in order to obtain information about the relative contribution of actin, DNA, and glycoproteins to the elasticity and flow properties of sputum.

MATERIALS AND METHODS

Seven sputum samples from different CF patients were obtained from hospitals and stored frozen at -20°C. Before measurements the sputum was thawed and cut in suitable sizes, excess water was removed, and the sputum was transferred to a Bohlin VOR instrument, with a parallel plate geometry. The elastic storage modulus (G') and the loss modulus (G") were measured as a function of frequency (0.001-10.0 Hz) at 25°C and as a function of strain amplitude (0.001-0.460) at 1 Hz. The stress was also measured at constant steady shear rates ($4\ 10^{-3}$ and $2\ 10^{-2}\ s^{-1}$) as a function of time.

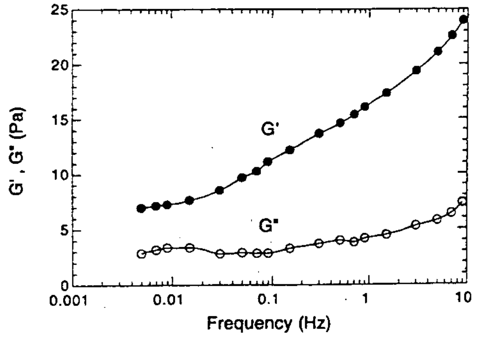

Figure 1. Plot of the frequency dependence of G' and G" for a control sputum sample.

An untreated sample was always measured first as a control and reference. Gelsolin (17 µg/ml), DNaseI (250 nM), or DTT (5 mM) were then subsequently added to and mixed with the sample in the rheometer, allowing 15 minutes of incubation before measurements.

RESULTS

Oscillatory results

The sputa from CF patients show great variations ranging from fairly solid to nearly liquid consistencies. Figure 1 shows the frequency dependence of G' and G" for a typical sample. The figure illustrates that G' exceeds G" at all frequencies. This was observed for all samples. We can therefore characterize the sputum as a viscoelastic solid material at all frequencies. The observed range of G' moduli was 4.4-26.4 Pa, and the G" moduli were 1.6-5.4 Pa, at 1 Hz and a strain amplitude of 0.1.

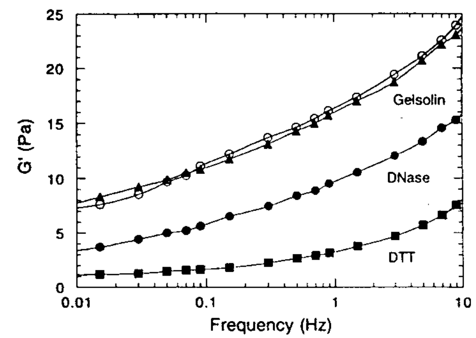

Figure 2. Plot of the frequency dependence of G'. An untreated sample is shown with open symbols. Sputum with gelsolin, DNaseI, and DTT are marked with filled symbols.

Figure 2 shows G' values after addition of gelsolin, DNaseI and DTT. Before any addition G' values of the 7 CF-sputum samples were 11± 6 Pa. The effects of the additives were quantified by calculations of the ratio of G', after addition, to G', prior to addition, at 1 Hz. The results are: Gelsolin 0.92± 0.32 (5 samples); DNaseI 0.65 ± 0.19 (2 samples); DTT 0.07 ± 0.08 (3 samples). It is seen that gelsolin and DNaseI only reduce G' moderately, whereas DTT has a dramatic effect resulting in very low elastic moduli.

The results in figure 3 illustrate the effects of gelsolin and DTT on the strain dependence of G' for a sample, where gelsolin is seen to have a fairly large effect on G' (reduced by about 30%) but still much smaller than DTT. A nearly linear strain dependence is seen for the gelsolin sputum in the measured strain range, whereas the control sputum shows a smaller strain softening. The figure also illustrates that a prior steady shear experiment (see below) only has a moderate effect on G'.

Steady shear results

Figure 4 shows results obtained on a series of repeated steady shear measurements on the same sputum sample at high and low shear rates. The order of experiments are indicated by 1 to 5. An apparent viscosity, defined as stress divided by shear rate, is plotted against time. The results demonstrate that the sputum does not behave like a Newtonian liquid with a steady viscosity. At short times, the observed linear increase is characteristic for an elastic material, and a constant shear viscosity is not even seen at longer times. The viscosity is seen to decrease with time and in the following repeat measurements. It is therefore not possible to assign a well-defined viscosity to these materials. The maximal viscosities observed for the sputum samples varied between 55-950 and 150-2300 Pa s, at the high and low shear rates, respectively. We have in general observed a decreasing viscosity of samples with increasing shear rates.

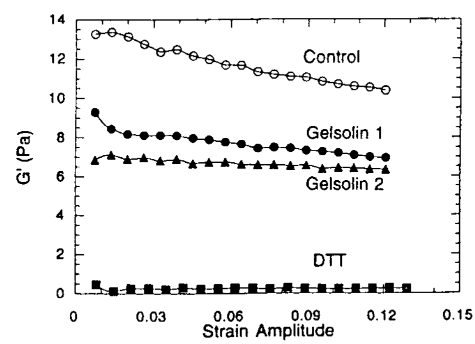

Figure 3. G' plotted against strain amplitude for an untreated control; after addition of gelsolin (1); after a steady shear experiment (2, see Fig. 4); after addition of DTT.

DISCUSSION

The results presented in Figs. 1, 2 and 4 demonstrate that sputa under physiologically relevant frequencies are viscoelastic solids. The results also demonstrate the great variation between samples. This variation probably primarily reflects variations in the water content of the sputa, but some of the variation may, in addition, also reflect variations in the content of actin, DNA, and glyco-proteins. The frequency dependencies of G' in Figs. 1 and 2 show that the sputum is characterized by a broad distribution of long relaxation times, probably due to slowly relaxing structures. These structures may potentially be attributed to long actin or DNA filaments embedded in a matrix of glycoproteins. However, severing actin and DNA filaments do not completely eliminate the elasticity of the sputum, so the main part of the elasticity is due to the matrix of glycoproteins, as demonstrated by the DTT results in Figs. 2 and 3. Additional studies will show if there is a correlation between the DNA and actin contents of sputum and the effects of DNase1 and gelsolin, respectively.

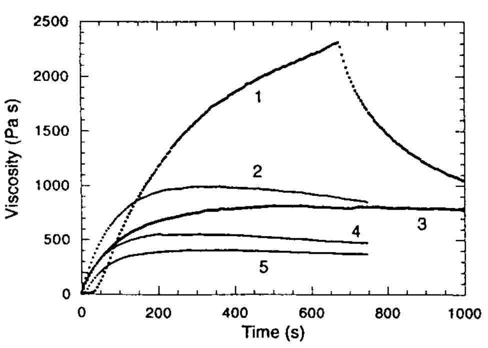

Figure 4. Apparently viscosity plotted against time, at low shear rate (4 10^{-3} s^{-1} (1 and 3)) and higher shear rate (2 10^{-2} s^{-1} (2, 4, and 5)). The measurements were performed consecutively on the same untreated sample from 1 to 5.

The rheological properties of sputum are dominated by the inter- and intra-molecular interactions[1]. Some of the inter-molecular interactions form a basic gel network which breaks during viscosity measurements even at very low shear rates (4 10^{-3} s^{-1}), as seen in Fig. 4. It is, therefore, clear that the viscosity measurements do not give direct information about the undamaged sputum samples. Furthermore the results in Fig. 4 demonstrate that the sputum never reaches a constant steady shear viscosity. The results in Fig. 1 show 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 most relevant information about the properties of sputum is therefore obtained by oscillatory techniques, which in addition operate in a biologically relevant time-scale.

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