Characterization of DNA Degradation Using Direct Current Conductivity and Dynamic Dielectric Relaxation Techniques

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ABSTRACT

The purpose of this study was to evaluate DNA degradation upon thermal heating using dielectric relaxation and direct current (DC) conductivity methods. Herring sperm DNA, human growth hormone (HgH) plasmid DNA, and secreted alkaline phosphatase (SEAP) plasmid DNA were used as the examples. DNA was heated at 80°C for 1 hour. The dielectric relaxation spectra as a function of the applied field frequency were measured for HgH DNA at 0.5 hours and at 1 hour. The frequency range covered was from 10 kHz to 100 kHz. The DC conductivity measurements were made for all 3 kinds of DNA at 4 time points: 0 hours, 0.5 hours, 0.75 hours, and 1 hour. At each time point the DC conductivity was measured for each sample as a function of concentration via water dilution. The results show that the dielectric relaxation method is less sensitive in characterizing heat-driven DNA degradation. Conversely, DC conductivity is very sensitive. The semiquantitative dependence of the conductivity upon heating suggests that DNA degradation involves more than plasmid DNA nicking. Double strand and single strand breaks may also occur. In addition, herring sperm DNA, HgH DNA, and SEAP DNA, though similar in their DC conductivity functional forms upon dilution, exhibit significant differences in their responses to sustained heating.

KEYWORDS: Conductivity, dielectric relaxation, DNA, degradation.

INTRODUCTION

DNA forms a molecular class by itself. Its unique double-stranded structure and the genetic code it carries intimately relate to human life cycles and in many cases dictate the well-being of cell cycles. Since the 1980s, the concept of embedding DNA into the cell nucleus to combat genetic diseases has drawn wide attention. As a result of the gene therapy concept, numerous efforts have been made to deliver DNA into the nucleus by injecting naked DNA, using nonviral vectors,[1] or using viral vectors.[2] A comprehensive review appeared in 2005.[3]

To construct such delivery vehicles, DNA molecules are often processed into various forms for better delivery and transfection efficiency. Some processes have been known to nick or degrade plasmid DNA.[4] Among them, magnetic fields,[5,6] ultrasonic waves,[7] heating,[8,9] high shearing forces,[10,11] and pH[12,13] are known to rapidly degrade DNA. Standard characterization of DNA degradation has been done using the gel electrophoresis method, where degraded DNA and intact DNA are separated by their different motilities in the gel. This method provides qualitative information about DNA degradation and to some extent differentiates the type of degradants. However, it does not provide good quantitative information or, in many cases, the true identity of the degradants.

In this study, for the first time, a novel approach was explored with an intent to quantitatively determine DNA degradation. The ultimate goal was to differentiate all DNA degradants in terms of their hydrodynamic sizes and to some extent their molecular weight distribution. As a starting point, dielectric relaxation and direct current (DC) conductivity methods were chosen. Both methods are based on the fact that DNA has a high surface charge density that is detectable by these methods. The dielectric relaxation method has been applied to study the counterion distribution around DNA to evaluate the theoretical model, such as Manning-Mandel-Oosawa's counterion fluctuation model.[14] Alternating current (AC) conductivity was also reported for studying electron transfer within the DNA helix.[15] This makes dielectric relaxation a candidate in this study.

Conceptually, if counterion condensation dictates the surface charge density, then shorter DNA fragments, after degradation, should have different counterion condensation distributions and thus should behave differently in either dielectric responses or DC conductivity properties. To further justify these methods, it was hypothesized that DNA degradation would result in molecular weight reduction and/or structural change that would lead to different diffusivity. This simple hydrodynamic diffusion argument should, in principle, allow the electric conductivity or dielectric relaxation method to quantitatively detect and differentiate the degradants. In this regard, obtaining a quantitative size

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(or molecular weight) distribution of the degradants is seemingly possible. However, the intent of this study was to explore the feasibility of using the surface charge as an indicator of DNA degradation. A quantitative analysis of the degradants was not the scope of this work.

Materials and Methods

Three DNA types were used as the model DNA: herring sperm DNA (<2000 bp), human growth hormone (HgH) plasmid DNA (~20 kbp), and secreted alkaline phosphatase (SEAP) plasmid DNA (~20 kbp). Temperature was the sole parameter used to initiate the degradation. The dielectric relaxation data were collected as a function of AC frequency, and the DC conductivity was measured as a function of concentration by water dilution at each selected time point after degradation was simulated. Data analysis involved the Cole-Cole plot[16] and the Argand plot[17] for the dielectric relaxation data. The DC conductivity data were analyzed by (1) plotting the conductivity as a function of concentration, (2) using the Kohlrousch plot, and (3) using the reduced conductivity plot by normalizing the conductivity by the unheated sample.

Presheared herring sperm DNA (<2000 bp) at 10 mg/mL was from Invitrogen (Cat 15634–017; Lot 1149916, San Diego, CA). HgH and SEAP plasmid DNA were gifts from Genteric Inc (Alameda, CA). Both HgH and SEAP have lengths of ~20 kbp. The deionized water has >18 MΩ of resistance. Polyethylene glycol 300 (PEG 300) from Sigma Aldrich (St Louis, MO) was used for dielectric constant measurements.

A Hewlett-Packard 4192A low-frequency impedance analyzer (Palo Alto, CA) was used to measure the dielectric relaxation. It has a frequency range from 5 Hz to 13 MHz. The cell used was a Rosemount 4-plate platinum black electrode from Beckman Industries (Model CEL-A, Fullerton, CA) with a cell constant of 0.01 cm^{-1}. A VWR Scientific conductivity meter (Model 2052, Westchester, PA) with a range from 0.001 μS to 99 mS was used for the DC conductivity measurements. The conductivity cell has a cell constant of 1 cm^{-1}. The temperature control for DNA heating was by a Lauda RM6 water bath (Long Island, NY) with ±0.1°C accuracy.

To perform dielectric relaxation measurements, known quantities of DNA were added to PEG to make a solution with a concentration of 6.66×10^{-6} mg/mL. The dielectric relaxation measurements were conducted as a function of frequencies from 10 kHz to 100 kHz. Dielectric storage, ε', and dielectric loss, ε'', were calculated from the measured capacitance between the electrodes. In DC conductivity measurements, a stock solution of ~0.01 mg/mL was prepared, split into 4 parts, and stored in 4 tightly capped 50 mL Falcon conical tubes (from BD Scientific, Franklin Lakes, NJ). Three of them were subsequently heated at 80°C. The DC conductivity of the unheated one was used as the control sample. It was measured at 25°C and at each dilution (by adding 1 mL of water at a time) until the concentration reached ~0.0016 mg/mL. The heated samples were withdrawn at 0.5 hours, 0.75 hours, and 1 hour to represent 3 time points of sustained heating. The conductivity of the retrieved samples was measured using the same diluting scheme as the unheated sample. The dielectric relaxation data and the DC conductivity data were analyzed using various available theories, to be described below. The temperature of 80°C and the maximum sustained heating time of 1 hour were chosen for convenience because the degraded DNA was measurable in the gel electrophoresis, making the comparison with the dielectric relaxation and the DC conductivity method possible. If the temperature was too high, the DNA would degrade too quickly, making differentiation between different degradation mechanisms more difficult. On the other hand, with too low a temperature, the DNA would take a longer time to degrade, which might not be practical.

Theoretically, dielectric relaxation spectroscopy (DRS) probes the interaction of a macroscopic sample with a time-dependent electric field. The resulting polarization of the material that responds to the electric field can be expressed by the frequency-dependent complex permittivity, the conductivity, or the impedance spectrum.

A DNA molecule with a given molecular weight should exhibit a characteristic motion in a liquid medium when an alternating electric field is applied. If the charge density of the DNA remains unchanged but the molecule weight varies because of degradation (eg, strands break), one expects the characteristic motion to change accordingly, and the variation should be reflected in the DRS spectrum. In this regard, the degradation can be obtained directly or indirectly from the DRS spectrum. The measurable quantities in DRS as a function of the frequency of the applied alternative field are dielectric storage ε', dielectric loss ε'', impedance Z, and the dynamic conductivity $G(\omega)$, where ω is the frequency of the applied alternating field. The permittivity can be expressed as follows[18]:

$$\varepsilon^* = \varepsilon' - i\varepsilon'' = \varepsilon_o\left[\varepsilon_r' - i\left(\varepsilon_r'' + \frac{\sigma}{\omega}\right)\right] \qquad (1)$$

where σ and ω are the static conductivity and the frequency of the applied electric field, respectively. ε' and ε'' behave differently at different frequency ranges. At substantially low frequencies, ε' is more or less constant, while ε'' acts like a power law of ω. At low frequencies both behave like a power law. Experimentally, these quantities

can be obtained by measuring the capacitance across the electrodes:

$$\varepsilon' = \frac{C\ell}{A} \tag{2}$$

where C is the capacitance, ℓ is the distance between electrodes, and A is the surface area of the electrode. The dielectric loss can be obtained from the measured low-frequency conductivity, G, as follows:

$$\varepsilon'' = \frac{G\ell}{A\omega} \tag{3}$$

Equations 2 and 3 are for low frequencies only, and the potential electrode polarization should be taken into account when measuring the capacitance and conductance. In this study, PEG 300 was used as the medium to avoid polarization. The frequency range was selected such that ε' and ε'' would fall into the power law range. This allows better differentiation of DNA molecular weight. Another quantity measured was the impedance, Z, which is related to the conductance.

The other technique used in this work is the static conductivity measurement. The conductance of an electrolyte solution depends on the concentration of the ions and the charges they carry. The dependence on concentration is different for strong and weak electrolytes. To differentiate them, a more fundamental quantity, equivalent conductance, Λ, was used. It represents the conductivity per unit concentration of the electrolytes. Using this quantity, one can characterize the properties of an electrolyte solution. For a strong electrolyte solution, Kohlrousch derived the following relation:

$$\Lambda = \Lambda_0 + K\sqrt{C} \tag{4}$$

If the DNA solutions are strong electrolyte solutions, the Kohlrousch plot (Λ as a function of $\sqrt{C}$) should show linearity.

Another way to analyze the DC conductivity data is to plot Λ as a function of the DNA concentration, followed by normalizing it by the similar plot of the unheated sample. This reduced equivalent conductivity can semiquantitatively reveal the DNA degradation response to the heating history.

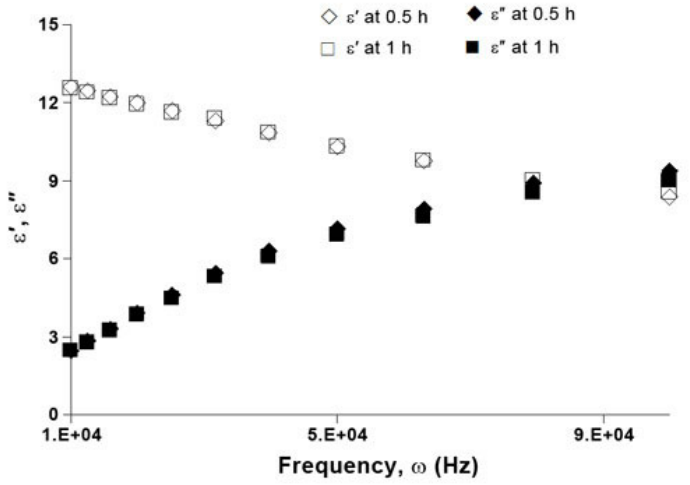

Figure 1. The dielectric storage, ε′, and loss, ε″, as a function of the frequency for the 4 μg/mL human growth hormone in polyethylene glycol 300 solution after 0.5-hour and 1-hour sustained heating at 80°C. No significant difference was observed between 0.5-hour and 1-hour heating, suggesting that the method is insensitive to the DNA degradation.

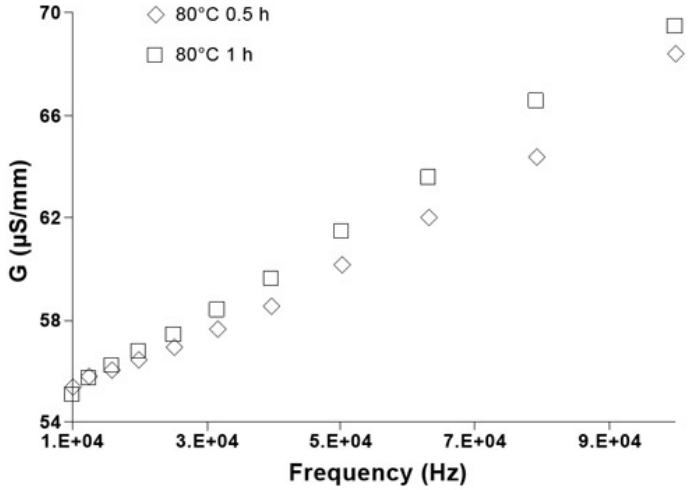

Figure 2. The dynamic conductivity, G, plotted as a function of the frequency for the 4 μg/mL human growth hormone in polyethylene glycol 300 solution, exhibits some difference between 0.5-hour and 1-hour heating.

Results and Discussion

Figure 1 shows the dielectric storage, ε′, and the dielectric loss, ε″, as a function of the applied alternating field for the HgH DNA systems at the 0.5-hour and 1-hour time points. The HgH concentration was 4 μg/mL, and the solvent used was PEG 300 to reduce the potential electrolyte deposition during measurements. The spectra are similar between the 2 time points, suggesting that the dielectric relaxation method is likely not a sensitive method for detecting DNA degradation. Figure 2 illustrates the behavior of the dynamic conductivity, G. There is an apparent difference in the spectra between the 2 time points, indicating the possibility of using conductivity to sensitively detect the DNA degradation. Figure 3 is the Cole-Cole plot, where the imaginary part of the impedance, Z″, was plotted against the real part, Z′, or ε″ plotted as a function of ε′. The difference between the 0.5-hour and 1-hour spectra is not significant enough to make this measurement a method

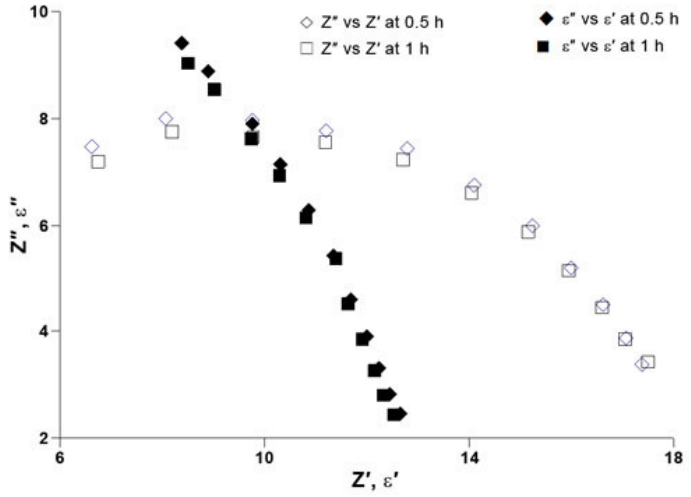

Figure 3. The Cole-Cole plots for the impedance, Z″ versus Z′, and the dielectric response, ε″ versus ε′. There is no appreciable difference between 0.5-hour and 1-hour sustained heating at 80°C. The Cole-Cole analysis apparently cannot enhance the sensitivity of the dielectric relaxation method for detecting heat-induced DNA degradation.

for characterizing DNA degradation. Among these plots (Figures 1-3), only the dynamic conductivity, G, seems to show some promise. These results more or less concluded that the dielectric relaxation method is not sensitive. However, the dynamic conductivity results prompted the second series of experiments using the static conductivity as a measure of DNA degradation.

Figure 4 shows the conductivity as a function of the DNA concentration for herring sperm DNA, HgH DNA, and SEAP DNA. Measurements were made for samples before heating (25°C) and after heating at 80°C for 0.5 hours, 0.75 hours, and 1 hour (except herring sperm DNA, where only 0.5- and 1-hour time points were taken). Clear differences among different time points were observed, indicative of severe DNA degradation during the heating process. This suggests that the DC conductivity method can be a candidate for detecting thermal degradation. The relative linearity as a function of concentration suggests that the interparticle interactions are not as significant even though the DNA molecules are charged.

Figure 5 contains Kohlrousch plots for all 3 types of DNA. None of the DNA solutions showed linearity. Thus, these solutions should not be considered strong electrolytes. However, it is interesting to note that the increase of the equivalent conductivity upon heating is not continuous. For example, the herring sperm DNA does not increase conductivity significantly during the first half-hour but increases it rapidly between 0 hours and 1 hour. On the other hand, the HgH conductivity increases drastically between 0.5 and 0.75 hours. Finally, most of the conductivity increase in SEAP DNA occurs between 0.75 hours and 1 hour. With a close look at the curves, it becomes evident that the herring sperm conductivity curve does not change its shape before 0.5 hours but undergoes significant change between 0.5 hours and 1 hour. In parallel to the significant change in curve shape, the DC conductivity also experiences a drastic jump. HgH experiences a similar change of the curve shape, but between 0 and 0.5 hours. The curve does not change shape

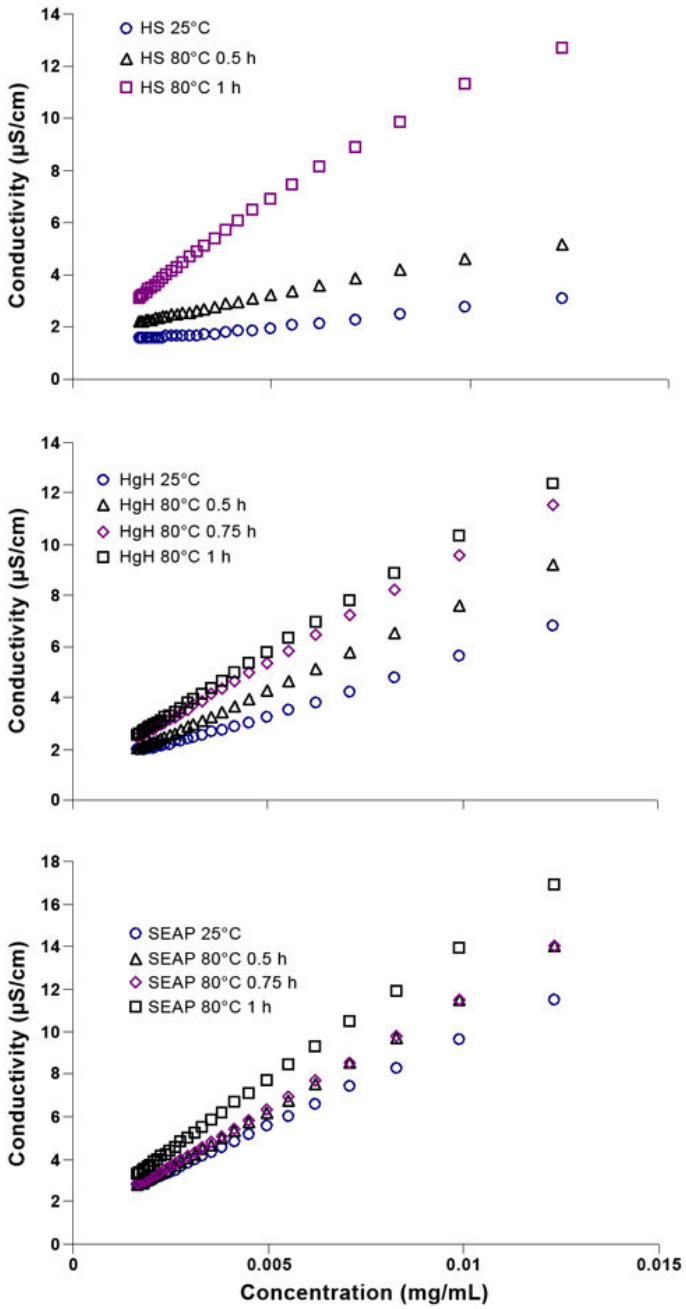

Figure 4. Equivalent conductivity, Λ, is plotted as a function of the DNA concentration for HS DNA, HgH DNA, and SEAP DNA. Clear distinctions were observed for 0.5-, 0.75-, and 1-hour time points of sustaining heating, indicating high sensitivity of this method for detecting DNA thermal degradation. HS indicates herring sperm; HgH, human growth hormone; SEAP, secreted alkaline phosphatase.

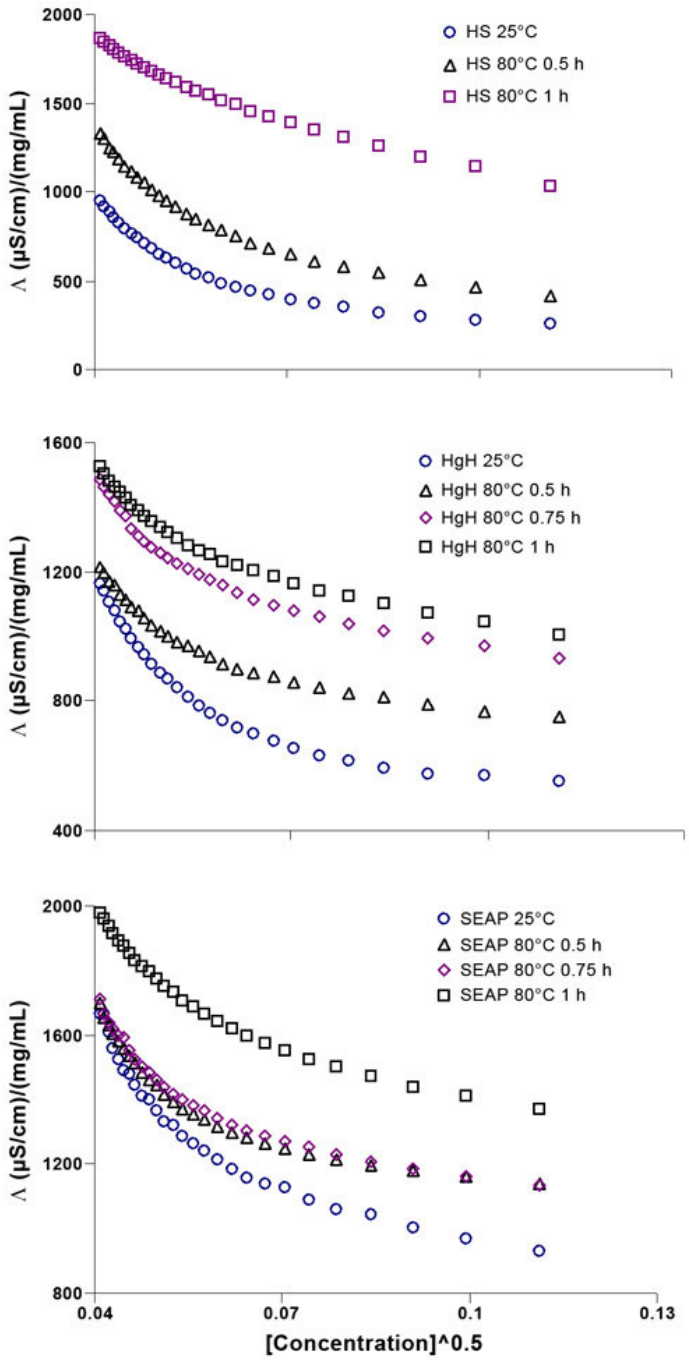

Figure 5. The Kohlrousch plot for HS DNA, HgH DNA, and SEAP DNA. Nonlinearity shows for all 3 DNA, indicating that they are not strong electrolyte solutions. HS indicates herring sperm; HgH, human growth hormone; SEAP, secreted alkaline phosphatase.

thereafter. In the case of SEAP, the shape change occurs within the first 0.5 hours; thereafter, the conductivity jumps but the curve shape remains the same.

To magnify the sensitivity to DNA degradation and exhibit the rate of change of the conductivity curve as a function of DNA concentration at different time points, the reduced conductivities (normalized by the unheated sample) were plotted (Figure 6). From Figure 6, herring sperm DNA appears to degrade within the first half-hour of heating (the reduced conductivity should maintain unity if the DNA molecules do not degrade). However, more significant degradation occurs between 0.5 hours and 1 hour, as indicated by the rapid increase of the conductivity. Obviously, there are parts of the DNA that are susceptible to heating and degrade within 0.5 hours, while other parts will not degrade until after 0.5 hours of sustained heating. Note also that the reduced conductivity, as a function of concentration, is not linear. This may be attributed to high polydispersity of this particular DNA. Herring sperm was in fact sheared to make the number of base pairs smaller as part of the process (see manufacturing process from Invitrogen).

HgH shows a different scenario (Figure 6). Its conductivity increases drastically within the first 0.5 hours of heating but increases more slowly thereafter. Apparently, more sites in the HgH DNA molecule are susceptible to heat and degrade quickly. Fewer sites are able to sustain the heat for longer than 0.5 hours. The HgH concentration dependence is also not linear in the low concentration region but seems to be consistent for all the measured time points, unlike for herring sperm. SEAP (Figure 6), on the other hand, shows linearity at all 3 time points. Moreover, SEAP DNA shows little degradation within the first 0.5 hours but suffers high degradation thereafter.

It was a surprise that the dielectric relaxation technique is not able to differentiate intact DNA from degraded DNA. The dielectric results obscured the original hypothesis that more pieces of smaller DNA (assuming the heat cleaves DNA into smaller pieces) would dielectrically relax differently from the original bigger piece at the frequency range selected in this study. This frequency range should be low enough to detect the DNA conformational change. One possible explanation is that the decreasing number of base pairs did not substantiate the charge polarization and thus the conformational relaxation.

The static conductivity, which depends heavily on the charge density and the movement of the charge carriers, did show differences between intact and degraded DNA. This is consistent with the hypothesis that a smaller charge carrier with similar charge density should move faster in the solution. Thus, the DC conductivity should increase, consistent with the experimental observations. The results are encouraging for potentially establishing a quantitative (or semiquantitative) DNA (or protein) degradation assay. From the static conductivity measurements, there are several points worth noting. First, the conductivity was found to be fairly linear as a function of DNA concentration. This indicates that DNA solutions, though similar to polyelectrolyte solutions, appear to behave like dilute solutions without long-range interactions. This is particularly true for the SEAP DNA solutions (Figure 4), where linearity is well preserved at all measured

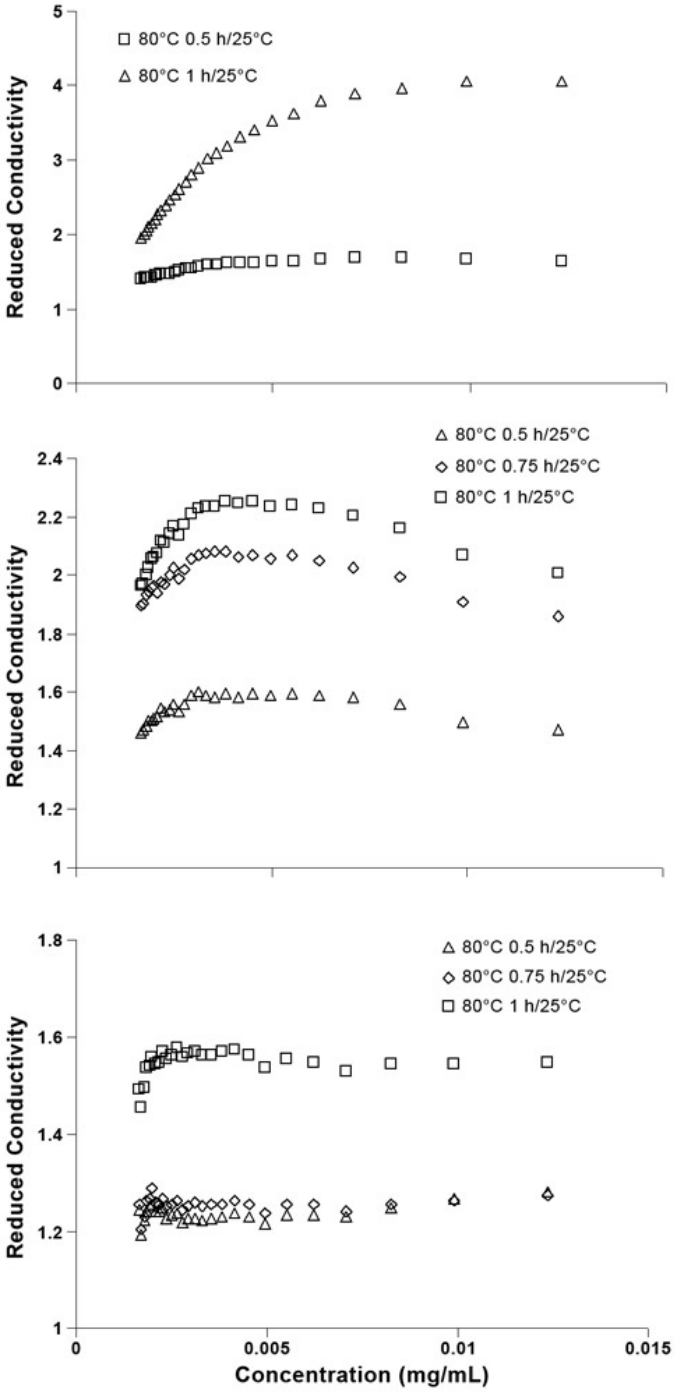

Figure 6. Reduced conductivity curves for HS DNA, HgH DNA, and SEAP DNA. Noncontinuous increasing rates suggest that there are different degradation mechanisms at different heating time points. HS indicates herring sperm; HgH, human growth hormone; SEAP, secreted alkaline phosphatase.

time points. Second, DNA solutions, whether before or after heating, are not strong electrolytes, based on Kohlrousch plots (Figure 5). This is consistent with the conductivity linearity observed in the concentration plots. Kohlrousch plots also provide valuable information about the heating effect on DNA. They indicate that the longer the heating time, the more the DNA molecules degrade. In addition, the time-degradation relationship was found to be more complex than a simple continuous degradation process in the Kohlrousch plots. Finally, the reduced conductivity plots (Figure 6) semiquantitatively demonstrate that each DNA type has sites that are more susceptible to heating than other sites. At 80°C each site has its critical heating time, beyond which the site is broken. For herring sperm DNA, the first half-hour does not degrade many sites. The conductivity increases ~30% to 40%. The next half-hour, on the other hand, heavily damages most of the sites, and the conductivity of the sample increases to up to 4 times that of the unheated sample. The critical time for herring sperm DNA is ~0.5 hours. The HgH DNA increases in conductivity by ~40% to 60% within the first half-hour, then increases to nearly 200% in the next 15 minutes, and further increases to ~220% at 1 hour. The critical time is between 0.5 hours and 0.75 hours. SEAP increases only 20% up to 0.75 hours but then rapidly increases to ~60% at the 1-hour point. Its critical time is between 0.75 hours and 1 hour. If one uses the fractional conductivity increase rate as a measure of the durability of the DNA, SEAP appears to be more durable than herring sperm and HgH.

A crucial objective of this study was to compare the conductivity results with those of gel electrophoresis, which is the technique most commonly used for detecting DNA degradation. Figure 7 shows the electrophoresis results (in 0.7% agarose gel) of SEAP DNA heated at 80°C for 0.5 hours

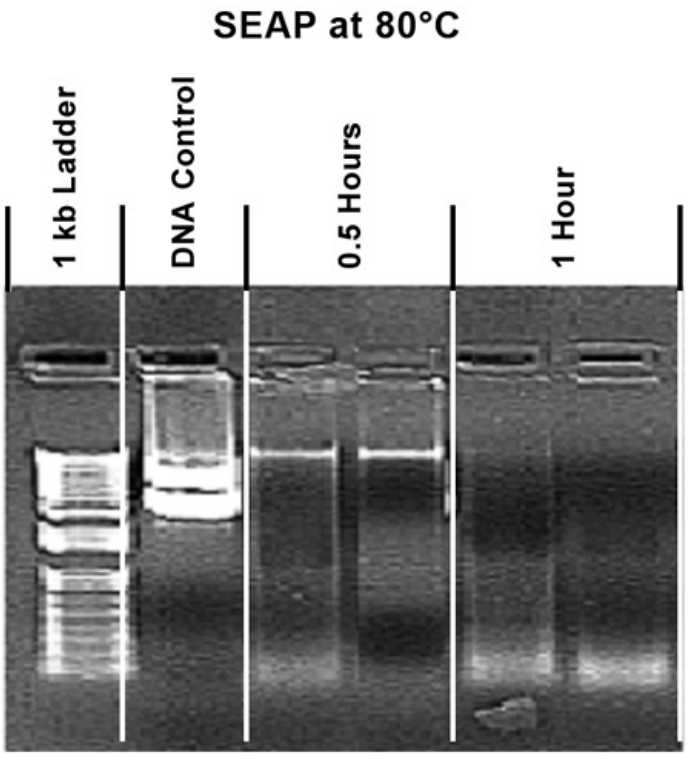

Figure 7. Gel electrophoresis of SEAP DNA degradation at 80°C at 0.5 hours and 1 hour in 0.7% agarose gel. SEAP indicates secreted alkaline phosphatase.

and 1 hour. Degradation can be seen at 0.5 hours and is much greater at 1 hour. Electrophoresis semiquantitatively indicates the molecular weight of the degradants and to some extent their amount but is not conclusive. It is worth noting that the degradations seem to break the DNA into 2 groups of smaller molecules. This confirms the noncontinuous degradation process as observed in the DC conductivity measurements.

Gel electrophoresis is a direct method and can provide qualitative results much more quickly than the conductivity method. However, the conductivity method may be able to be used for quantitative analysis. The method proposed here does not provide quantitative measurement yet. A proper scheme will need to be developed to make quantitative analysis possible in the future.

CONCLUSIONS

Static conductivity was demonstrated to be a viable parameter for detecting DNA degradation upon heating. It could semiquantitatively characterize the DNA degradation and may become a quantitative method. Herring sperm DNA, HgH DNA, and SEAP DNA degrade under sustained heating at 80°C. The static conductivity not only characterizes the degradation but also differentiates the degradation mechanisms and relative robustness against thermal heating. The dielectric relaxation method, though sophisticated in determining molecular dynamics, was found not to be sensitive for detecting DNA degradation.

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