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ORIGINAL RESEARCH ARTICLE

Thermal Degradation of DNA

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In this article, we investigate the thermal degradation of deoxyribonucleic acid (DNA). We find that under dry conditions, complete DNA degradation occurs at above 190°C. In addition, as the boiling temperature of water is pressure dependent, we have investigated the thermal degradation of the DNA in water for different applied partial pressures. This information is important for fundamental understanding of DNA structure and energetics, and can be useful for biomedical applications such as thermal targeting of DNA in cancer cells, as well as for basic research.

Introduction

Deoxyribonucleic acid (DNA) constitutes the genetic material of every living organism on earth, and is therefore the focus of many physical, chemical, and biological studies. Structural properties such as DNA folding probabilities or melting curves of double-stranded DNA into single-stranded DNA have been extensively described in the literature (Deininger and Schmid, 1976; Yakovchuk *et al.,* 2006; Nisoli and Bishop, 2011). However, the degradation of DNA at high temperature, that is, breaking the covalent bonds within each DNA strand, has not been thoroughly investigated before. Here we measure, for the first time, the thermal degradation of the DNA molecule. This information could be extremely important for studies that use or target DNA, as well as for biomedical applications such as thermal targeting of DNA in cancer cells. DNA could be thermally targeted using, for example, gold nanoparticles that would adhere to the target DNA. Gold nanoparticles can be heated to very high temperatures in a highly localized, noninvasive manner, using laser. Currently, research on targeting of DNA in cancer cells is done using low-intensity ultrasound (Furusawa *et al.,* 2012), ionizing radiation (Costes *et al.,* 2010; Löbrich *et al.,* 2010), or targeting the DNA break-repair machinery (Shaheen *et al.,* 2011). In this article, we aim to propose a simpler way to do so.

DNA is usually found in aqueous solutions both *in vivo* and *in vitro*. Since DNA is stable at temperatures below 100°C [as evident in polymerase chain reactions (PCR), for example (Bartlett and Stirling, 2003)], its degradation at higher temperatures cannot be easily tested in aqueous solutions. We therefore conducted our measurements both under dry conditions, and in water, using a pressure system that prevented evaporation of the water. We found that under dry conditions, at gradual temperatures, DNA degradation occurs in a linear manner, with complete degradation at around 190°C. In addition, we found that pressure itself negatively affects DNA thermal degradation in water.

Again, we would like to stress that we do not discuss the denaturation of DNA, that is, separation between the two strands by breaking the hydrogen bonds between them, but rather we refer to DNA degradation, that is, breaking the covalent bonds within each strand.

Results and Discussion

Thermal degradation of dry DNA

To determine DNA degradation at high temperatures, we used a controlled system to heat DNA to different temperatures and visualize its integrity. DNA was loaded on a soldering station and heated to the desired temperature (Fig. 1a). Since the chosen temperatures were higher than 100°C, the water that was used to dissolve the DNA had evaporated during the heating period. After 5 min of heating, the soldering system was switched off, and the DNA was collected by redissolving in water using repeated pipetting. The sample was then separated by agarose gel electrophoresis, and the DNA was visualized by ethidium bromide staining under UV light. Agarose gel electrophoresis separates charged molecules according to size, with smaller molecules migrating faster than larger molecules. Intact DNA appears in the gel as a strong band that can be single, double, or triple depending on the presence of different folding topologies corresponding to supercoiled, linear, and nicked circles. DNA degradation can result in weakening of the band intensity, faster migration of the band (meaning that it appears lower in the picture), or both. Degradation of the DNA starts already at 130°C, as seen by weakening of the band (Fig. 1a, b), and the DNA is gradually degraded until complete degradation around 190°C. Quantification of the degradation is presented in Figure 1b. The experiment was repeated with similar results using a different DNA sequence (plasmid

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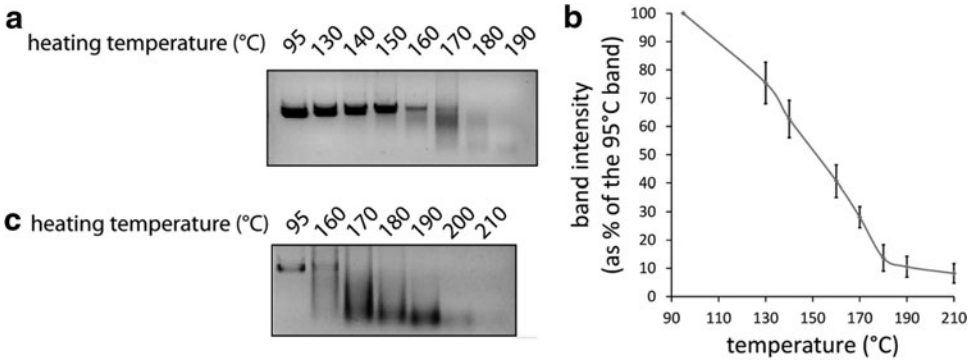

FIG. 1. **(a)** About 0.75 μg of plasmid deoxyribonucleic acid (DNA) pUC19 was incubated at the indicated temperatures for 5 min. DNA was resuspended and separated by agarose gel electrophoresis. **(b)** Densitometry of the gels. The graph shows an average of six independent experiments with standard error of the mean (SEM); **(c)** 0.75 μg of plasmid DNA pGY1 was incubated at the indicated temperatures for 5 min. DNA was resuspended and separated by agarose gel electrophoresis.

pUC19 in Figure 1a, b, and plasmid pGY1 in Fig. 1c). For plasmid pGY1, accumulation of DNA lower in the gel is observed at the temperature range 170°C–200°C that disappears at 210°C (Fig. 1c). This is also observed for plasmid pUC19 in Figure 2. This lower band is likely composed of aggregates of short DNA fragments, resulting from incomplete degradation. At 210°C, these fragments are destroyed, and therefore no aggregates can be formed.

To determine the amount of time the DNA requires for degradation, we heated the DNA at 170°C or 200°C for several time durations (Fig. 2). At 170°C (Fig. 2a), degradation of the DNA (weakening of the band) begins at 2 seconds. After 4 min, some intact DNA is still observed, but at 7 and 9 min, almost all of the DNA migrates very quickly through the gel, meaning that it is degraded. At 200°C (Fig. 2b), weakening of the band is already apparent at 1 s, and most of the DNA is degraded after 20 s, and complete degradation is observed after 60 s.

Thermal degradation of DNA dissolved in water

Naturally DNA is found in aqueous solutions both *in vivo* and *in vitro*. Since DNA is stable at temperatures below 100°C (as evident in PCRs, for example), its degradation cannot be easily tested in aqueous solutions. To determine DNA degradation at high temperatures under more natural conditions, we decided to measure the degradation in water, using a pressure system that prevented evaporation of the water (see the Experimental Section for full description of the system). In this case, DNA degradation occurs between 100°C and 110°C (Fig. 3), much lower than under dry conditions. This observation is surprising, since degradation in this experiment was observed at a lower temperature than the boiling temperature of water, a temperature range in which DNA does not degrade when pressure is not applied. This result indicates that applying pressure to a DNA solution greatly increases the sensitivity of DNA to heating.

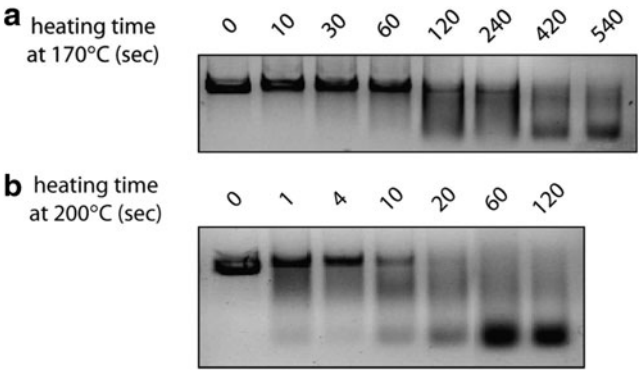

FIG. 2. **(a)** About 0.75 μg of plasmid DNA pUC19 was incubated at 170°C for the indicated time periods. DNA was resuspended and separated by agarose gel electrophoresis; **(b)** 0.75 μg of plasmid DNA pUC19 was incubated at 200°C for the indicated time periods. DNA was resuspended and separated by agarose gel electrophoresis. An irrelevant lane was digitally removed between lanes 5 and 6 (from the left) in this figure.

Experimental Section

DNA plasmids

pUC19 (2686-base-pair plasmid) was purchased from Invitrogen. pGY1 (4077-base-pair plasmid) was received from

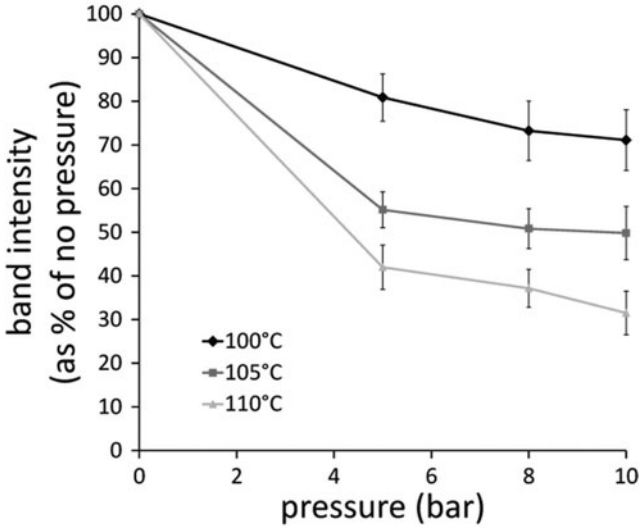

FIG. 3. About 1.5 μg of plasmid DNA pUC19 was incubated in an aqueous solution at the indicated temperatures and pressures for 5 min. DNA was separated by agarose gel electrophoresis.

Dr. Gal Yerushalmi (Yerushalmi *et al.*, 2008). About 0.75 μg of DNA were used for each reaction.

Gel separation

The DNA was separated using 1% agarose gel, for 30 min at 100 V. Staining was done with ethidium bromide.

Densitometry

Densitometry was done using ImageJ software.

Measurement of dry DNA degradation temperature

DNA was loaded on a soldering station's (Weller WSD81) modified tip (see below). The soldering iron was precoated with thermal grease (Zalman ZM-STG1) to maximize heat transfer between the iron body and the tip (Fig. 4a). To avoid a loss of material, for experiments in which the final temperature used for DNA degradation measurement was higher than 110°C, the soldering station was first warmed to 110°C; the aqueous solution was allowed to evaporate in a controlled manner; and only then, the temperature was further increased to the endpoint temperature. After the heating period, the DNA was collected from the soldering station by redissolving in water using repeated pipetting. The sample was separated by agarose gel electrophoresis at 150 V for 20 min, and the DNA was visualized by ethidium bromide staining under UV light.

Please note that the degradation under dry conditions was measured for a very short time, so we believe that the dry aspect did not have enough time to affect the DNA. The solution was passed through 100°C to investigate the thermal- and pressure-related effects in a more isolated and controlled manner, but the DNA itself was in this phase for short time, and thus we believe that the products are similar to the case of wet conditions. This applied procedure that we used is very different from the case of storage of dehydrated DNA in which the dry conditions are applied for a very long period of time.

Measurement of DNA degradation temperature in solution with pressure

A modified tip was made in a cup shape, to hold the sample on the soldering iron (Fig. 4b). The outer face of the tip was threaded, to attach it to a pressure system during

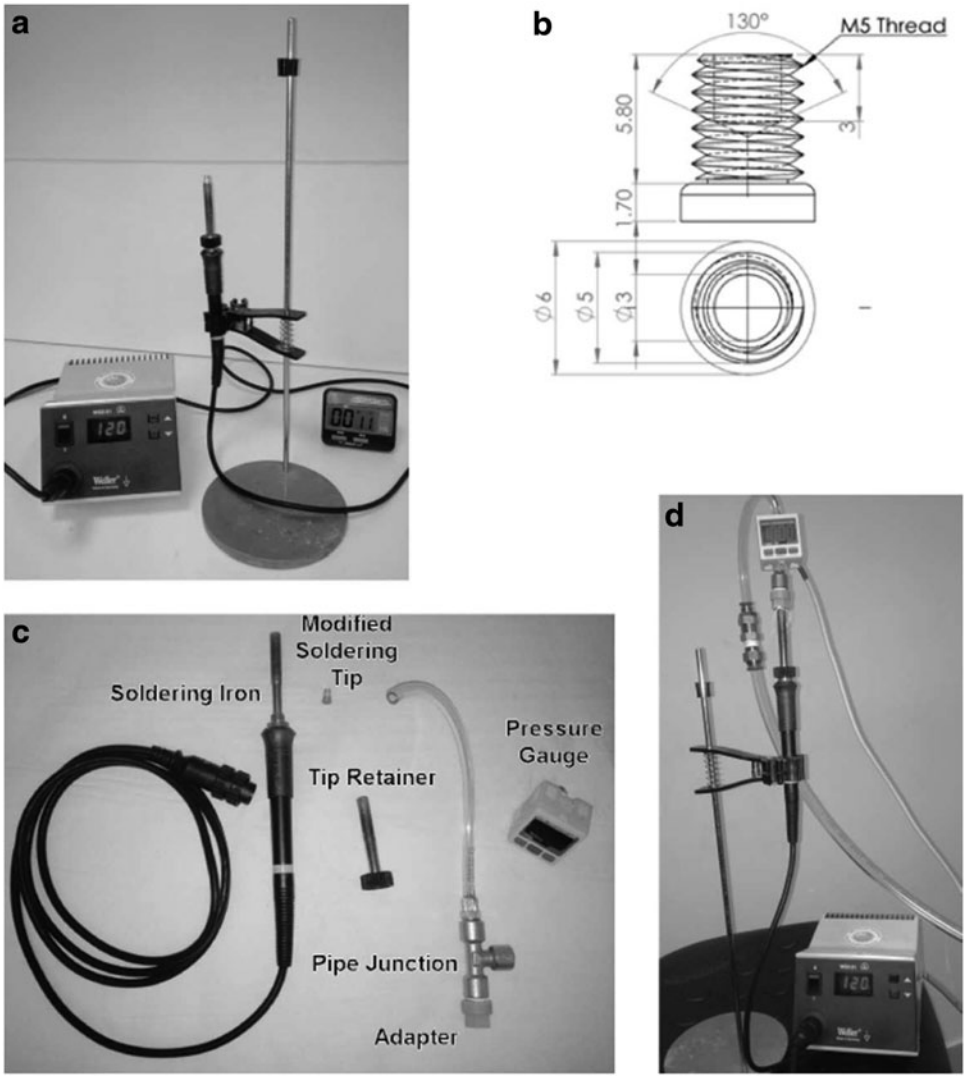

FIG. 4. **(a)** Picture of the system used to measure dry degradation temperature. **(b)** Illustration of the modified tip used to heat the DNA. **(c, d)** Pictures of the system used to measure the wet degradation temperature.

the wet experiments. A custom adapter was made from polyether ether ketone (PEEK), which provides both the thermal isolation and excellent mechanical and chemical resistance properties, to connect the iron to the pressure system and to thermally isolate the iron. Another adapter was used between the soldering iron tip and our pressure system. The adapter was made of PEEK. The bottom has an M5 inner thread that fits our tip, and the upper has a 1/8 NPT outer tread that fits our pressure gauge and tube fitting. The bottom part was shaped as a hex nut to allow easy and robust threading capability. The system is shown in Figure 4c and d.

The setup was similar to the dry measurements, with the addition of the pressure system. To create high pressure on the sample, compressed air was used. The tank pressure was regulated using a simple pressure regulator to the test pressure (13 or 10 bar). The tank pipe was connected to the pipe junction with a polyurethane tube using a touch fitting. The soldering iron was fixed using a boiling flask stand. Twenty μL plasmid (pUC19, 75 ng/μL) was loaded into the modified cup. The cups' thread was wrapped with a polytetrafluoroethylene (PTFE) film to seal the system. The pipe junction was screwed to the tip. The pressure was increased slowly to the target pressure. Then, the soldering iron was switched on and set to the target temperature. The sample was held at the target temperature for 5 min, and then the soldering iron was switched off and allowed to cool to room temperature. About 15 μL was collected from the tip and mixed with 3 μL of loading dye (Invitrogen). The sample was separated by agarose gel electrophoresis at 100 V for 45 min, and the DNA was visualized by ethidium bromide staining under UV light.

In order to calibrate the temperature of the soldering station. We used an external thermometer with a flexible head that could measure the temperature of the soldering station. Temperature measurements were identical to the measurements taken with the internal thermometer of the soldering station. Also, the tank pressure was calibrated. We used both the analog pressure meter of the compressed air balloon and an external pressure meter that can be seen in Figure 4c and d (marked as pressure gauge).

Conclusions

We found that under dry conditions, DNA degradation begins at 130°C, and continues in a linear manner until complete degradation occurs around 190°C. In an aqueous solution, it is not possible to determine the degradation temperature using our method, since applying pressure on the DNA solution causes the DNA to be more sensitive to heat, and therefore the DNA degrades already above 90°C. This observation is very interesting and may be useful by itself. It is possible that the pressure weakens the chemical bonds between the atoms within the DNA molecule. Another possibility is that the exposure to oxygen within the compressed air that was used to create the pressure damaged the DNA and made it more sensitive to heat-induced degradation.

Our findings provide important information for fundamental understanding of DNA structure and energetics, and may be useful in the future for basic research, as well as for biomedical applications such as thermal targeting of DNA in cancer cells. Such targeting can be achieved, for example, by using localized heating with nanoparticles.

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Disclosure Statement

The authors state that they do not have any conflict of interest with this article.

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