The effect of microwave irradiation on DNA hybridization†

Wesleigh F. Edwards, Douglas D. Young and Alexander Deiters*

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The effect of microwave irradiation on DNA/DNA hybridization has been studied under controlled power and temperature conditions. It was discovered that microwave irradiation led to the melting of double-stranded deoxyoligonucleotides well below their thermal melting temperature and independent of the length of the deoxyoligonucleotides. These observations indicate a specific interaction of microwaves with DNA, and have important implications in the chemical or enzymatic processing of DNA under microwave heating.

Despite its biological importance, the effects of microwave irradiation[1–7] on DNA structure, processing, and replication have been virtually unexplored. Of the few studies conducted under microwave irradiation, two investigated the cellular effects of irradiation leading to sterilization,[8,9] and were performed in traditional household microwave ovens. Two investigations were conducted on the genetic damage caused by microwave irradiation.[10–12] Herein, we describe the effects of microwave irradiation on the hybridization of DNA oligomers. Hybridization is a key property of DNA, and the ability to modulate hybridization with microwave irradiation provides several applications, including studies involving DNA hybridization probes, interactions with proteins, and experiments utilizing hybridization for materials science purposes. Additionally, the susceptibility of DNA to microwave irradiation due to its large dipole moment[13] has implications in microwave-mediated oligonucleotide synthesis, similar to the effects observed in the synthesis of peptides,[14,15] as well as enzymatic reactions involving the processing of DNA, *e.g.* in a microwave-assisted polymerase chain reaction (PCR).[16,17]

To investigate the effects of microwave irradiation, a viable assay to detect DNA hybridization needed to be established. While many real-time protocols[18–20] exist for the detection of double-stranded DNA (*e.g.* real-time PCR using intercalating fluorescent probes), these methods are not applicable to the closed environment of a microwave reactor. Thus, an offline assay was required to determine if the DNA is melting while being subjected to microwave irradiation. In order to achieve this, an end-point assay was designed that relies upon fluorescence quenching to detect the melting and annealing of DNA in a microwave synthesizer (Fig. 1). This assay uses two sets of double-stranded DNA. One of the pairs possesses a 5′ fluorescein modification which hybridizes in close proximity to a 3′ dabcyl modification, and the other pair consists of the identical sequences with no modifications. Due to the proximity of the fluorophore and quencher on the hybridized modified oligomer pair, very little background fluorescence should initially be present. Upon microwave irradiation of the system and melting of the dsDNA, the ssDNA will subsequently anneal in a statistical fashion to either its previous partner or to the non-modified complement. If the fluorescein-modified strand anneals to its non-modified complement, the quencher is removed, leading to an increase in the fluorescence signal. A similar result is observed if the DNA melts, but does not re-hybridize. If microwave irradiation is not capable of inducing DNA melting, then no change in fluorescence should be observed.

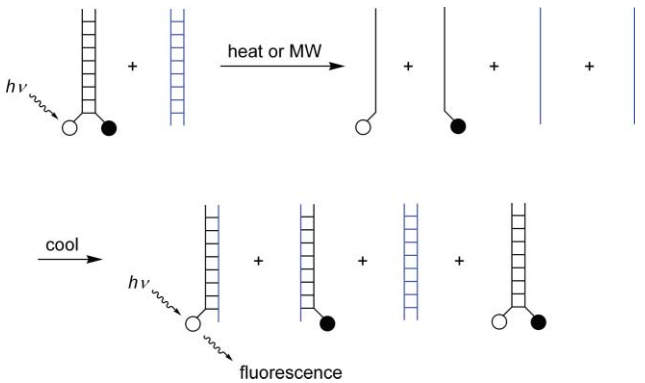

Fig. 1 Schematic of the fluorescence quenching assay used to determine if microwave irradiation (MW) is capable of melting dsDNA. Open circle = fluorophore (fluorescein); closed circle = fluorescence quencher (dabcyl).

In order to probe the viability of the assay, we designed dsDNA covering T_ms from 50–80 °C, and obtained the complementary fluorophore- and quencher-modified (**D1F*** & **D1R***) and unmodified oligomers (**D1F** & **D1R**)(Alpha DNA Technologies) (Table 1). Initial hybridizations were conducted thermally (Eppendorf Mastercycler, 95 °C (5 min), 37 °C (30 min), 4 °C (30 min)) to generate the two sets of double stranded DNA. A 1:1 ratio of dabcyl-modified oligomer to fluorescein-modified oligomer was employed to sufficiently afford hybridization and effectively suppress the fluorescence signal in **D1F***:**D1R*** (Fig. 2, column 1).

Table 1 Sequences and melting temperatures of DNA oligomers

DNA	Sequence	T_m/°C
D1F*	5′-CGCACCCAGGCTTAGCTACAAACAT-3′	81.5 ± 0.4
D1R*	3′-GCGTGGGTCCGAATCGATGTTTGTA-5′	
D2F*	5′-CCCAGGCTTAGCTACA-3′	66.2 ± 0.3
D2R*	3′-GGGTCCGAATCGATGT-5′	
D3F*	5′-AGGCTTAGCTACA-3′	51.5 ± 0.3
D3R*	3′-TCCGAATCGATGT-5′	

F* contains a fluorescein label at the 5′ end; R* contains a dabcyl label at the 3′ end. T_ms were determined in triplicate on a BioRad MyiQ RT-PCR thermalcycler.

Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA. E-mail: alex_deiters@ncsu.edu; Fax: +1-919-515-5079; Tel: +1-919-513-2958

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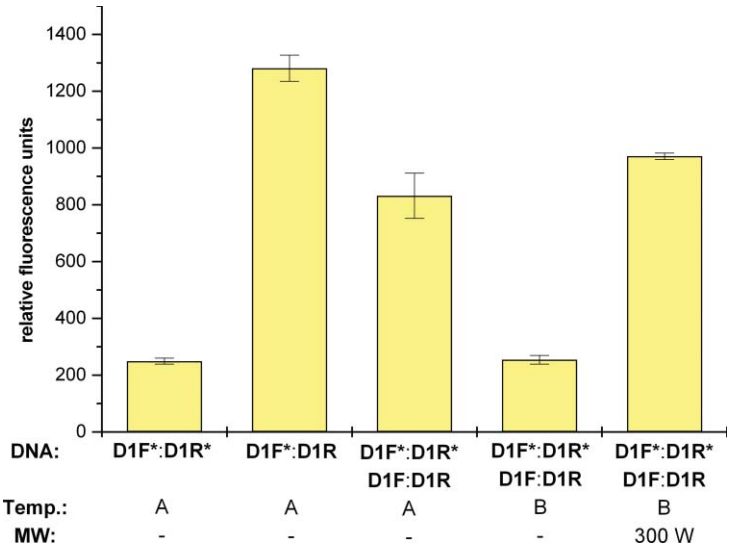

Fig. 2 Fluorescence signals arising from various conditions employed in the DNA melting experiments with **D1F** and **D1R**. All assays were conducted in triplicate and error bars represent standard deviations. Temperature profiles: A) 95 °C to 4 °C; B) –20 °C to 20 °C to –20 °C. **D1F*** = 5′ fluorescein-labeled **D1F**; **D1R*** = 3′ dabcyl-labeled **D1R**. Initially the **D1F*/D1R(*)** dsDNA was added, followed by the addition of non-labeled **D1F/D1R** to monitor the changes in hybridization under the various experimental conditions.

A positive control experiment was conducted by hybridizing **D1F*** and **D1R** to determine the maximum attainable fluorescence signal (Fig. 2, column 2). Additionally, a thermal comparison control experiment was performed in which we employed a two-fold excess of the non-labeled dsDNA **D1F**:**D1R** to the labeled dsDNA **D1F***:**D1R***. This ensured that an adequate amount of non-labeled DNA was present for hybridization when **D1F*:D1R*** melted, and was based upon initial results (data not shown). The resulting fluorescence signal (Fig. 2, column 3) is lower than that of the positive control experiment (**D1F***:**D1R**), as upon melting and subsequent hybridization, the labeled DNA can reanneal to its labeled complement forming **D1F***:**D1R*** and thus quench fluorescence (Fig. 1).

To differentiate between microwave effects and thermal effects on the melting of dsDNA, the experiments were conducted in a CEM Discover system equipped with a jacketed reaction vessel enabling temperature control through the continuous flow of coolant while precisely measuring the temperature using a fiber optics probe (CEM Coolmate), in a fashion analogous to our work with hyperthermophilic enzymes.[21] By attempting to melt DNA at temperatures well below the calculated melting point (e.g. –20 to 20 °C), it can be demonstrated that the melting was a direct effect of the microwave irradiation, and not due to a thermal influence that could convolute the results. Thus, the pre-hybridized oligomers were mixed and subjected to various conditions to probe hybridization. The mixture of dsDNAs **D1F***:**D1R*** and **D1F**:**D1R** was either heated (95 °C (5 min), 37 °C (30 min), 4 °C (30 min)), subjected to purely thermal conditions mimicking the microwave temperature profile (–20 °C to 20 °C to –20 °C, 0 W), or microwave-irradiated (–20 °C to 20 °C to –20 °C) at 300 W while coolant at –60 °C was simultaneously circulated through the jacketed reaction vessel. A temperature profile starting at –20 °C was necessary due to the non microwave-transparent hybridization buffer which would otherwise reach high temperatures in only a few seconds at a high power of 300 W. All reactions were subsequently equilibrated at 4 °C to ensure hybridization.

As expected, the thermal heating from –20 °C to 20 °C without microwave irradiation (Fig. 2, column 4) did not result in any increase of fluorescence, delivering the identical readout as the initially hybridized **D1F***:**D1R*** pair (Fig. 2, column 1). However, heating the dsDNA mixture from –20 °C to 20 °C using microwave irradiation of 300 W (Fig. 2, column 5) yielded a fluorescence signal identical with the positive control under thermal conditions (95 °C, Fig. 2, column 3). This indicates that microwave irradiation is capable of inducing complete DNA melting at temperatures well below the 81.5 °C melting point of the **D1F**:**D1R** DNA duplex.

Based on the apparent influence of microwave irradiation on DNA hybridization, this phenomenon was further probed by employing different DNA/DNA duplexes based on the same sequence, but shortened to afford lower melting temperatures of 66.2 °C (**D2F**:**D2R**) and 51.5 °C (**D3F**:**D3R**) (Table 1). The melting temperatures of all oligonucleotide pairs (**D1F***:**D1R***, **D2F***:**D2R***, and **D3F***:**D4R***) were determined on a BioRad MyiQ RT-PCR thermocycler by conducting a sequence of three heating and cooling cycles, while measuring the increase of fluorescence due to the loss of the quencher in the melting process.

All oligomer pairs were subsequently used in the described melting assay following the identical temperature profile as before (–20 °C to 20 °C to –20 °C), but varying microwave power (0, 25, 50, 75, 100, 125, 150, 175, and 200 W) in order to generate microwave melting curves in an analogous fashion to thermal melting curves (Fig. 3).

Interestingly, all of the DNA sequences have similar inflection points at 111 W (**D1**), 117 W (**D2**), and 100 W (**D3**), as determined by differentiation of the sigmoidal curves shown in Fig. 3. Although each oligo pair possesses a different thermal melting temperature, they all melt at virtually the same microwave power (109 ± 8 W). This result was unexpected and indicates that the microwave irradiation specifically interacts with the DNA. This

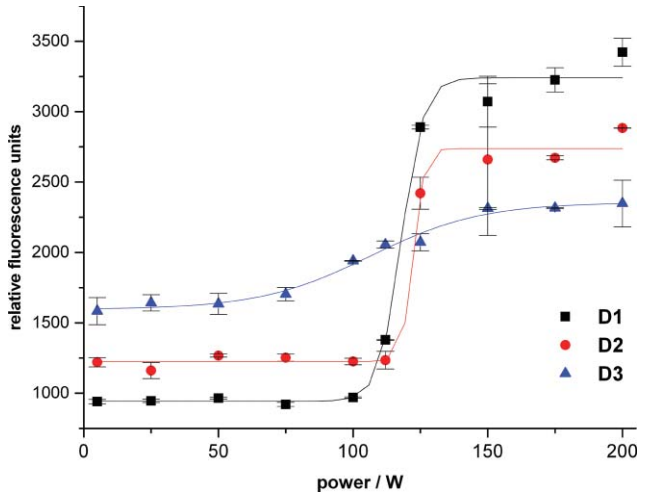

Fig. 3 Microwave melting curves indicate that microwave irradiation induces DNA melting at a specific power regardless of the duplex length. Oligomers were annealed and subjected to increasing microwave power under identical temperature profiles (–20 °C to 20 °C to –20 °C). All assays were conducted in triplicate and error bars represent standard deviations.

is in contrast to thermal heating, where the bulk solvent initially absorbs the energy, thus delivering a thermal melting point that is dependent on the number of base pairs providing hydrogen bonding interactions.[22] We speculate that there are several possible sources of this unexpected microwave effect. First, it is possible that the direct energy transfer into the oligonucleotide increases with the number of nucleotides, thus maintaining the melting temperature at the same microwave power despite the increased degree of hybridization in longer dsDNA sequences. Secondly, the microwave irradiation could disrupt cation binding to the negatively charged DNA, as cations and anions align with the electromagnetic field, thus moving them in opposite directions.[5] Thus, at a specific microwave power the cations become completely dissociated from the anionic phosphodiester backbone, leading to the repulsion of the negative charges on the two DNA strands. This may destablize the duplex and facilitate DNA melting to minimize the negative charge repulsion.[23] Finally, it is known that the dielectric permittivity of single stranded DNA is greater than that of dsDNA.[24] Consequentially, when the double-stranded DNA begins to unravel, forming single strands, a greater coupling with the electromagnetic field occurs providing increasing energy transfer to overcome the hydrogen bonding interactions of the remaining dsDNA. The observed effects may be one or a combination of these possibilities. Microwave systems enabling online analyses of DNA melting will be required to elucidate the true mechanism.

In conclusion, we have demonstrated the disruption of DNA hybridization at temperatures much lower than thermally measured *via* the implementation of microwave irradiation. Interestingly, the microwave irradiation leads to a different DNA melting mechanism than thermal heating, as complementary DNA oligomers with varying lengths all melted at identical microwave powers. This finding suggests a specific interaction of microwave irradiation with deoxyoligonucleotides. These discoveries have important implications in the future development of microwave-assisted DNA technologies.

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