DNA Denaturing through UV-C Photon Dissipation: A Possible Route to Archean Non-enzymatic Replication

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Abstract

The results of a series of experiments are presented which demonstrate that the absorption of UV-C light by DNA at temperatures below the melting temperature leads to complete and reversible denaturing for small synthetic DNA of 25 base pairs (bp), and to partial and reversible denaturing for the 48 bp DNA and for the large salmon sperm and yeast DNA of average size 100 kbp. This result supports the thermodynamic dissipation theory of the origin of life in which non-enzymatic replication and the coupling of replication to dissipation are two important aspects. It also supports corollaries of this theory which provide an explanation for the homochirality of the molecules of life and for the beginnings of information accumulation in RNA and DNA through the thermodynamically driven process of photon dissipation.

Introduction

Many of the fundamental molecules of life, those common to all three domains (bacteria, eukaryote, and archea), including RNA and DNA, amino acids, enzymes, vitamins, cofactors, and protoporphyrins, are pigments that absorb photons in the UV-C (Michaelian and Simeonov, 2014d). Alone, or in mutual interaction, when in water , these molecules dissipate the resulting photon induced electronic excitation energy through internal conversion rapidly into molecular vibrational energy, i.e. heat.

Most of these molecules have conjugated carbon bonds which not only gives them their UV-C absorption properties but also their planer aromatic-like structures and thus chemical affinity to RNA and DNA by intercalating between consecutive base pairs. Many also have chemical affinity to the secondary or tertiary structures of RNA and DNA (Govil et al. 1985). The aromatic amino acids (tryptophan, tyrosine, phenylalanine) and other UV-C absorbing amino acids (histidine and cystine) even have particularly strong chemical affinity to their DNA codons or anti-codons and this fact not only hints at a stereochemical era for the beginnings of life (Yarus,

1988; 2009, Yarus and Christian, 1989) but also a UV-C dissipative era for the beginnings of life. When in such complexes and in water, RNA and DNA act as quenching molecules, providing the pigment molecule with an extremely rapid, sub picosecond, dexcitation channel (Pecourt et al. 2000) for passing electronic excitation energy to the vibrational modes of the nucleic acids through internal conversion and subsequently to the surrounding water molecules.

Because these pigment-RNA/DNA complexes dissipate UV-C photons extremely efficiently into heat, their production and proliferation to well beyond expected equilibrium concentrations during the Archean, before the existence of biological metabolic pathways, can be explained through non-linear, non-equilibrium thermodynamic theory, as an autocatalytic photochemical reaction process which arose spontaneously to dissipate the solar UV-C photon potential (Michaelian, 2013). Indeed, this thermodynamic explanation of proliferation remains valid even today under visible light, irrespective of the complicated nature of the contemporary metabolic pathways to the production of these molecules necessitated by the utilization of photons of lower available energy. The cosmic ubiquity of aromatic organic molecules probably can also be explained by this non-linear, non-equilibrium thermodynamic imperative (Michaelian, 2014b).

In the "Thermodynamic Dissipation Theory of the Origin of Life" (Michaelian,2009; 2011; Michaelian and Santillan, 2014a) it was conjectured that the origin and evolution of life was contingent on increases in the entropy production of the biosphere through increases in the dissipation of the photons in the prevailing solar spectrum at Earth's surface (Michaelian, 2012b; 2014d). In particular, it was suggested (Michaelian 2009, 2011) that RNA and DNA evolved to optimize the absorption and dissipation in the wavelength region of 230 to 290 nm, which corresponds to a window existent in Earth's atmosphere during the Archean (Sagan, 1973; Michaelian and Simeonov, 2014d), and that the locally deposited heat resulting from the dissipation of these photons could have been utilized to denature RNA and DNA without the need for enzymes, allowing for subsequent extension during overnight dark periods facilitated by Mg^{++} ions incorporated into a hypothetical but simple organic molecule, and UV day-activated phosphorylated nucleotides (nucleotide triphosphates). This primitive reproduction process was named ultraviolet and temperature assisted reproduction (UVTAR) and has similarity to polymerase chain reaction (PCR - Mullis, 1990) but where the heating and cooling cycle is partially substituted by a day/night UV-C light cycling at a time when the ocean surface temperature descended to somewhat below the melting temperature of the relevant RNA and DNA segments, and in which Mg^{++} ions, or their complexes with other simple molecules, played the role of the extension enzyme polymerase.

The UVTAR mechanism (Michaelian, 2009, 2011) has been shown to provide a plausible explanation of the homochirality of life (Michaelian, 2010) as a result of the positive circular

dichroism of RNA and DNA around 260 nm which corresponds to the peak in the UV-C solar spectrum reaching Earth's surface during the Archean (Michaelian and Simeonov, 2014d), and a small prevalence of right over left handed circularly polarized submarine light in the late afternoon (Angel et al., 1972; Wolstencroft, 2004) when surface water temperatures are highest and thus more conducive to denaturing. The UVTAR mechanism also provides an explanation for the beginnings of information storage in RNA and DNA (Michaelian 2009, 2011) since the aromatic amino acids absorb in the UV-C and have known chemical affinity to their codons or anti-codons (Majerfeld and Yarus, 2005; Yarus, 2009) and thus may have played the role of antenna molecules for greater local heating. RNA or DNA segments coding for these amino acids would thus have had greater reproductive success through the UVTAR mechanism in increasingly colder seas. The "thermodynamic dissipation theory of the origin of life" proposes, in fact, that all characteristics of the process of life, in the past and today, including its origin, proliferation and evolution, are driven by increases in the entropy production of the biosphere through greater photon dissipation of the prevailing surface photon spectrum.

In this letter we present experimental evidence for the reversible UV-C light denaturing of DNA for four different DNA samples; relatively large (~100 kbp) salmon sperm and yeast DNA, and short 25 bp and 48 bp synthetic DNA. A quantitative analysis of hyperchromism and dispersion in our extinction data suggests that UV-C light induced denaturing is significant and therefore could have promoted DNA amplification in the Archean through the proposed UVTAR mechanism, even in the absence of a diurnal variation in the sea surface temperature (mainly caused today by absorption of solar infrared wavelengths). The measured effect is also sufficiently large to support the explanations for homochirality and the beginnings of information coding in DNA through the dissipation mechanisms mentioned above and described in greater detail in the cited references.

Method

Pure salmon sperm and yeast DNA samples of varied lengths of average 100 kbp were obtained from the Institute of Cellular Physiology at the National Autonomous University of Mexico (IFC-UNAM). Synthetic DNA complementary oligonucleotides of 25 and 48 bases were synthesized at the IFC-UNAM. The complementary oligos were designed to be free of adjacent thymine and to have convenient denaturing and priming temperatures in order to facilitate the UVTAR process under the simulation of only very small diurnal surface temperature variation.

To form the double helices of 25 and 48 bp, complementary oligos at equal concentration were mixed in a Dulbecco PBS buffer (pH 7.3) solution containing 2.7 mM potassium chloride (KCl), 136.9 mM sodium chloride (NaCl), 1.5 mM potassium phosphate monobasic (KH_2PO_4) and 8.9 mM sodium phosphate dibasic (Na_2HPO_4) , then heated to 85° C and kept there during 10 min and finally brought to ambient temperature at a rate of 1° C/min.

The yeast DNA was dissolved in a Dulbecco PBS buffer and the salmon sperm DNA dissolved in purified water (Mili-q). The resulting concentrations of double helix DNA were determined from their absorption spectrum to be 2.2, 0.7, 0.0015 and 0.00023 µM for the 25 bp synthetic, 48 bp synthetic, yeast and salmon sperm DNA respectively (assuming average lengths of 100kpb for the yeast and salmon sperm DNA).

One ml of the corresponding solution was placed in a standard quartz cuvette of 1 cm light path length. The cuvette was placed in a precise (± 0.01°C) temperature control unit purchased from Ocean Optics[@] operating via the Peltier effect with water flow stabilization of the temperature; important for precise temperature control when removal of heat from the unit was required. The temperature was monitored via a probe located in one of the four supporting towers of the cuvette and in direct contact with it. A magnetic stirrer provided assurances of temperature equilibration throughout the sample, uniform mixing of the DNA sample, and homogeneous passage of the DNA through the approximately 3.7 mm diameter light beam at the sample.

UV light from a 3.8 W (9.4 µW on sample) deuterium source, covering continuously the range 200 to 800 nm, or light from a 1.2 W (7.0 µW on sample) tungsten-halogen source, used as a control, covering 550 to 1000 nm, was defocused onto the DNA sample via UV light resistant 30 cm long optical fiber of 600 µm diameter and a quartz lens. After passing through the sample, the surviving light was collected by a second lens and focused onto a similar optical fiber which fed into an Ocean Optics[@] HR4000CG charge-coupled device spectrometer. The spectrometer covered the range 200 to 1200 nm with a resolution of 0.3 nm. Spectrometer integration times were of the order of 1.5 s and data are plotted below are averaged over five wavelength channels (1.5 nm).

After allowing approximately 1/2 hour for lamp stabilization, sample extinction spectra $E(\lambda)$ were obtained by comparing the measured intensity spectrum $I(\lambda)$ of the light through the DNA sample with a reference spectrum $I_R(\lambda)$ obtained at room temperature with a similar quartz cuvette containing either purified water or PBS buffer but no DNA. A dark spectrum , $I_D(\lambda)$ obtained with a shutter blocking the deuterium light to the reference sample at room temperature, was subtracted from the spectra to correct for stray light and electronic noise. Extinction was thus calculated as;

$$E = -log_{10}\left(\frac{I-I_D}{I_R-I_D}\right). \quad (1)$$

The synthetic DNA samples were heated rapidly (rate 5°C/min) to 90 ° and held there for approximately 10 minutes to ensure complete denaturing. The temperature of the sample was then lowered to that desired for the particular run. The longer DNA samples were brought directly to the temperature of the run from room temperature (~24 °C). Runs consisted of allowing the sample to equilibrate (between denatured single strands and natured double

strands) for approximately 1/2 hour at the given temperature and then inserting and retracting a shutter to cycle the deuterium light on and off through the sample for different time periods while continuously monitoring the extinction (Eq. (1), absorption plus dispersion) of the sample over all wavelengths detected by the spectrometer during the light on sample periods.

Results

Figure 1 shows a typical extinction spectrum using the deuterium light source for (a) 48 bp synthetic DNA and (b) yeast DNA, at three different temperatures. The extinction peak at 260 nm is due predominantly to absorption on the nucleic acid bases but also includes a small amount of Rayleigh and Mie scattering. The inset shows an amplified view of the longer wavelength region for the three different temperatures. The observed spectrum in this region is due entirely to Mie scattering dominating in the short wavelength region (~200 to 350 nm) and Rayleigh scattering dominating in the long wavelength (~ >350 nm) region for the 25 bp and 48 bp synthetic DNA (of physical size of approximately 2 nm diameter and 17 nm length). For the larger yeast and salmon sperm DNA, the scattering spectrum is mainly Mie scattering (inset, Fig. 1(b)).

The actual form of the scattering is a complicated function of the number of scattering centers, the ratio of the indices of refraction (DNA/buffer), and shape of the scatter (Johnsen and Widder, 1999). However, it can be seen from the inset of figure 1(a) that for the small 48 bp and 25 bp (not shown) DNA the scattering increases with denaturing (correlated with temperature) while the scattering is constant or only slightly increasing with denaturing for the large yeast (figure 1(b)) and salmon sperm DNA (not shown). This result, interpreted as being due to the fact that that complete denaturing occurs for the small synthetic DNA (even at low temperature) which then act as separate scattering centers, but only partial denaturing occurs for the large DNA, allows us to use scattering as an additional (to hyperchromicity) sensitive indicator of denaturing for the small synthetic DNA but of little utility for the large DNA except at the high temperatures where complete denaturing also occurs. For the small synthetic DNA, therefore, a sensitive measure of photon induced denaturing was composed out of a linear combination of the hyperchromicity of absorption peaking at 260 nm and the scattering measured over longer wavelengths of up to about 500 nm.

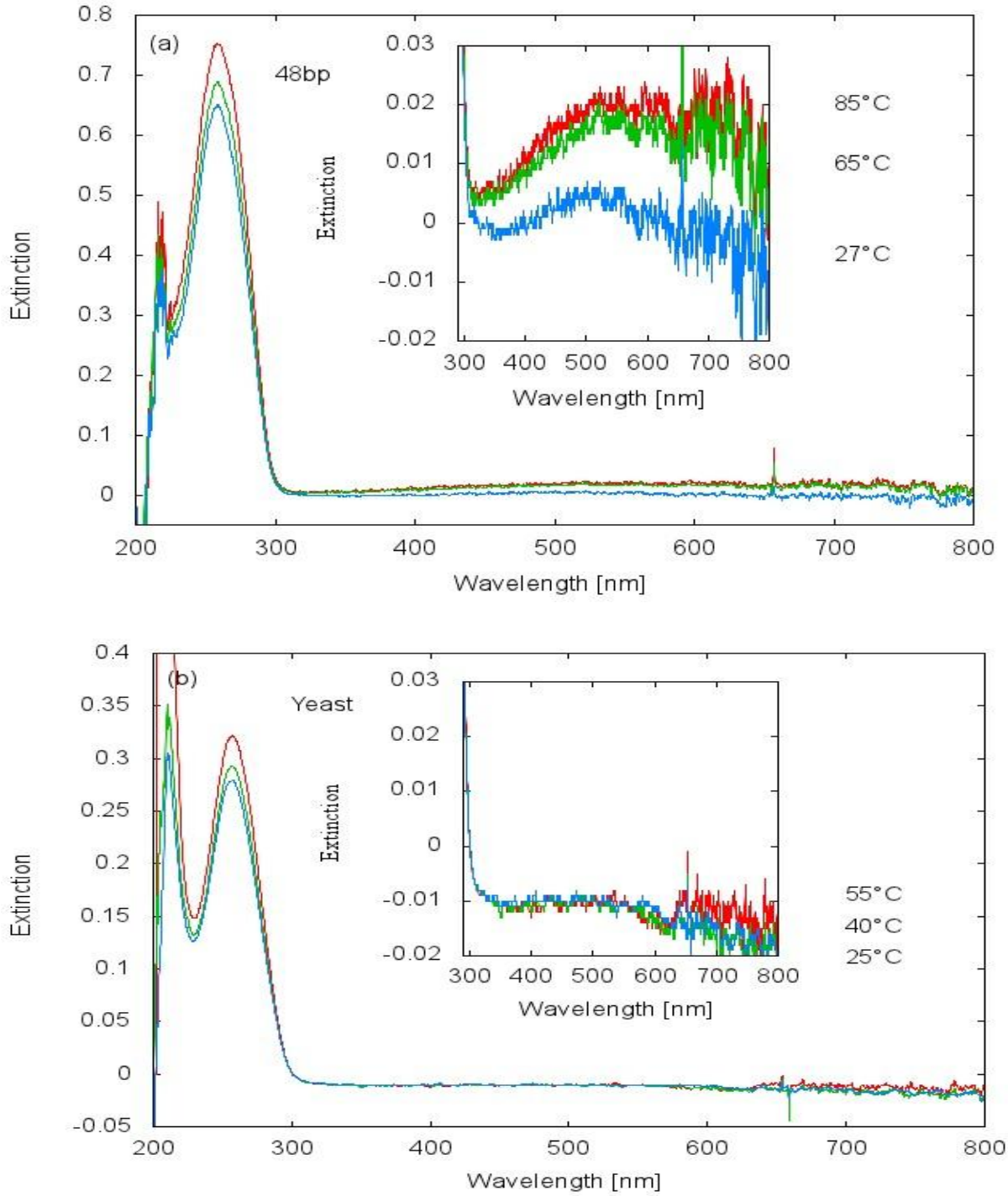


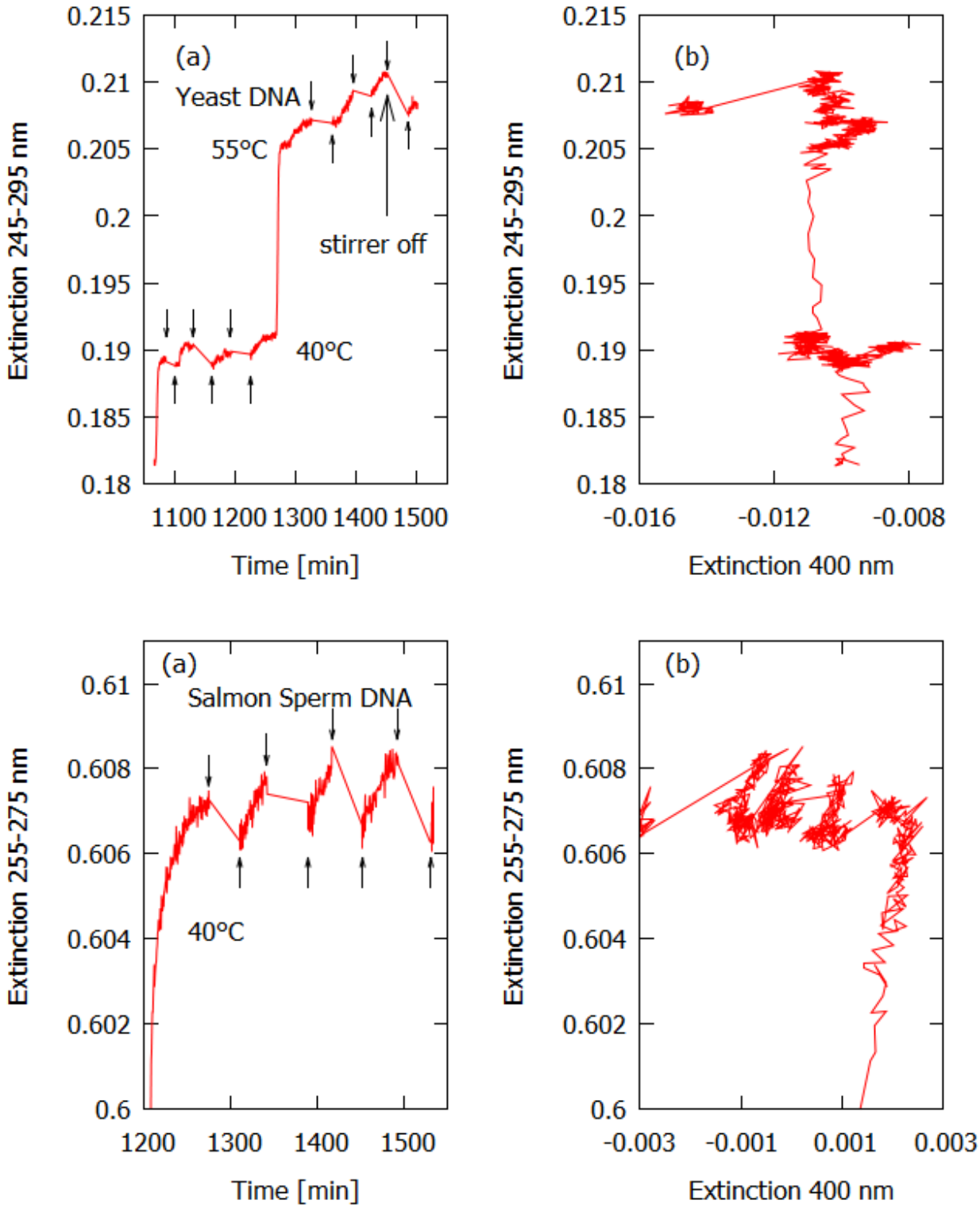
Fig. 1. Extinction spectrum of DNA. a) 48 bp synthetic DNA. Beyond 350 nm the extinction is mainly due to Rayleigh scattering. b) 100 kbp average size yeast DNA. Extinction is mainly due to Mie scattering. Hyperchromism is observable at 260 nm in both samples. The insets show an expansion of the scattering regions at three different temperatures. Scattering is obviously temperature dependent for the short synthetic DNA but not for the longer DNA, suggesting complete denaturing for the short DNA but only partial denaturing for the longer DNA until the highest temperatures.

Figure 2(a) shows the extinction as a function of time over the run, integrated over wavelengths 245 to 495 nm for the short synthetic DNA samples, and integrated over 245-295 nm, or 255-275 nm, for the yeast and salmon sperm DNA respectively. The graphical segments of interest for each run correspond to deuterium light cycling on and off, noted on the graphs by up and down arrows respectively , at the given fixed temperature. It can be observed that in all cases, as a result of UV-C light induced denaturing by photon dissipation, the extinction rises during the light on periods, due to absorption hyperchromicity and increased Rayleigh or Mie scattering of the denatured strands .

Figure 2(b) shows the correlation spectrum of the extinction peaks versus only scattering at 400 nm for all DNA samples during the runs. From these figures, particularly that for 48 bp DNA, it can be deduced that while the heating or cooling is slow (0.2 °C/min) there is strong correlation between the absorption and dispersion, but little correlation during rapid heating or cooling. This may be interpreted as being attributed to the fact that heat separation of the DNA bases at hydrogen bonding sites and the consequent liberation of the bases to take on arbitrary orientation around the axis of the DNA, which gives rise to hyperchromicity, does not imply the physical separation of the complementary strands to an extent (at least one wavelength) that would make them separate Rayleigh or Mie scattering centers. Thus, the increase in scattering lags behind the increase in absorption through a diffusion time constant since it requires substantial separation of the liberated strands. The time constant is dependent on factors such as the salt concentration and the stirrer setting.

Figure 2(b) indicates that Mie scattering increases only slightly with denaturing for the long salmon sperm and yeast DNA (~100 kbp) as compared to the synthetic 25 bp DNA. This is because photon dissipation induces only partial denaturing in these long segments while usually complete denaturing in the short synthetic DNA (particularly the 25 bp DNA). Partial denaturing does not allow the complementary segments to separate sufficiently to act as

independent Mie scattering centers.



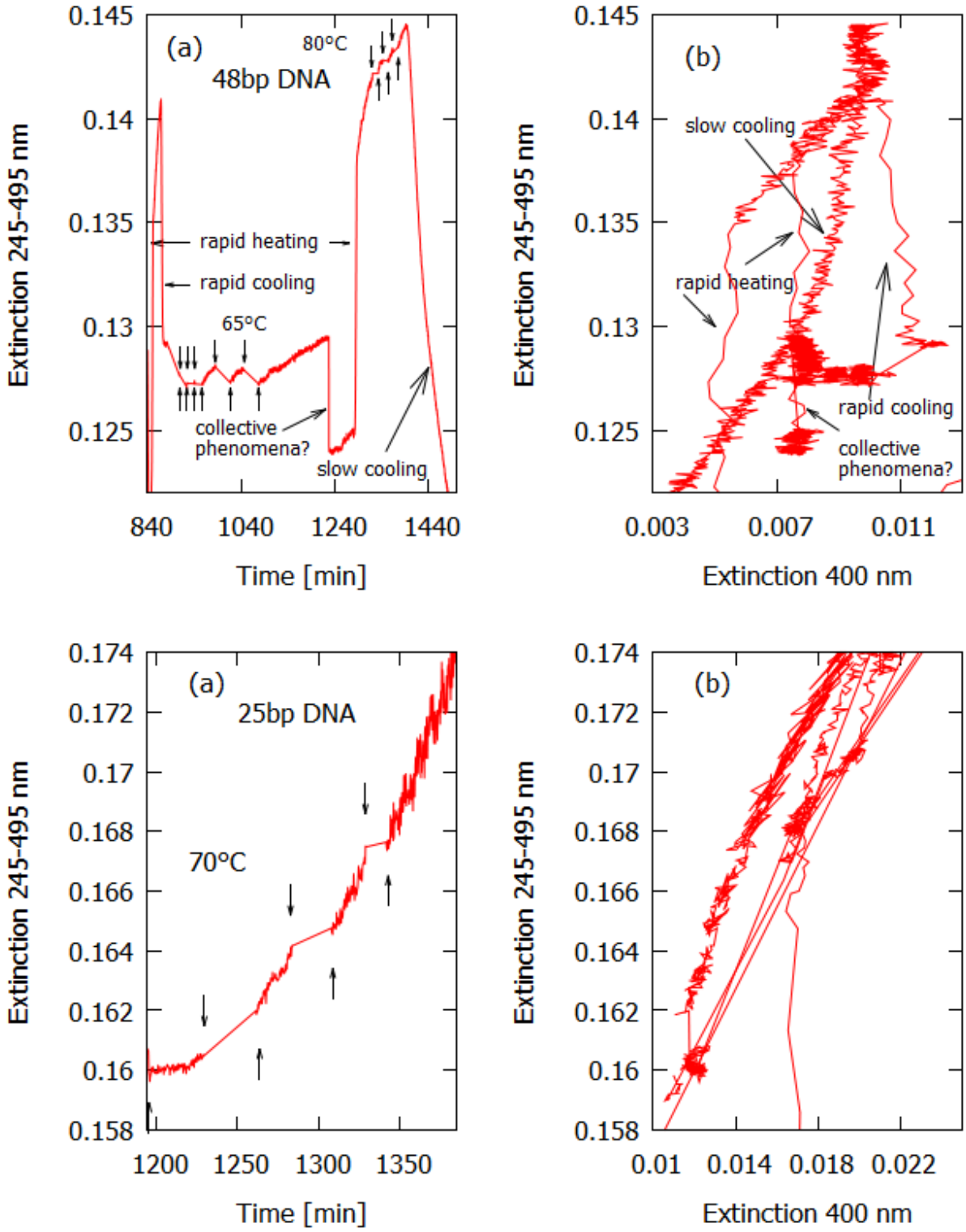


Fig. 2 a) Extinction (mainly absorption) as a function of time in minutes for the four different DNA samples. Deuterium light on an off changes are noted on the graphs by up and down arrows respectively. A change in the stirrer condtion is noted on the graph for yeast DNA. b) Correlation

between extinction (mainly absorption) and Rayleigh or Mie scattering at 400 nm. Scattering lags behind absorption during rapid heating and cooling stages due to a diffusion time constant of the single strands, but correlates strongly during slow cooling. Note that during the light off periods, extending for approximately 1/2 hour, the large yeast and salmon sperm DNA (and, to some extent the 48 bp DNA) readily re-unite with their complementary starnds (renature) and thus extinction decreases to levels before the light on period. However, the 1/2 hour light off periods are not sufficient for most short 25 bp synthetic DNA to renature since they are completely separated and for that reason extinction does not return to pre light-on values.

The renaturing of completely separated DNA proceeds via chance encounter of a few complementary base pairs and then rapid re-zipping of the remaining pairs. It is thus second order in the concentration but there is also a volume or steric effect since in longer DNA the folding of single strands implies that some base pairs are inaccessible to first encounter. Renaturing is thus a diffusion plus steric limited effect but has some peculiarities that depend on the nucleotide complexity of the strand, the ionic nature of the solvent, and also the viscosity of the solvent (Wetmur and Davidson, 1968). Renaturing is readily observed as a decrease in absorption after the end of the light off period in the photon induced partial denaturing of salmon sperm and yeast DNA (and to some extent in the 48 bp DNA) but not observed, as expected, for the completely denatured short 25 bp synthetic DNA, where it even appears that denaturing continues during the short light off periods; probably due to heat denaturing once UV-C light induced partial denaturing has reduced the melting temperature of the remaining double strand (see Fig. 2(a) for the 25 bp DNA).

"Collective phenomena" marked on the 48 bp DNA graph makes reference to unexpected, and still unexplained, sudden decreases (within 30 seconds) of the extinction at a particular time during the run, found usually after an extensive light on period for both the synthetic 25 (not shown) and 48 bp samples, but not for the longer DNA. Our initial suspicion is that they may be attributed to sudden and collective form changes from the canonical B-stacking geometry, for example, a B-Z transformation, perhaps a result of UV light ionization of DNA reducing the electrostatic repulsion of the phosphate residues (Rich and Zhang, 2003). The fact that there is no change in the scattering observed during the sudden drop in extinction (see corresponding Fig. 2(b) for the 48 bp DNA) indicates that this phenomena is probably not agglomeration of the DNA strands.

A quantitative measure of the UV-C light induced denaturing can be obtained by comparing the increase in hyperchromicity during the light on periods with the temperature induced hyperchromicity when completely denatured at 90°C and assuming a linear relation between hyperchromicity and denaturing. For the salmon sperm DNA in purified water at 40 °C, a half hour light on period is sufficient to increase the extinction (255-275 nm) by 0.002 units (see Fig. 2(a)) which corresponds to 1.7% of the total increase in extinction obtained from room

temperature to complete denaturing at 90°C. Therefore, the rate of UV-C light induced denaturing for salmon sperm DNA in purified water at 40 °C (at the beginning of the denaturing curve) is approximately 3.4% per hour. A similar value was found for yeast DNA. An analogous calculation for the 48 bp DNA in pBS buffer (see Fig. 2(a)) shows that at 65 °C (at the beginning of the denaturing curve) the rate of denaturing is approximately 4.6% per hour. For the 25 bp DNA in PBS buffer (see Fig. 2(a)) at 70 °C (somewhat into the denaturing curve) the UV-C light induced denaturing is approximately 20.1% per hour.

In order to verify that the increases in extinction observed for all samples during the light on periods was due to UV-C light induced denaturing and not to some other mechanical or electronic artifact, we carried out an identical experiment with 48 bp DNA replacing the deuterium UV-C light with the control halogen-tungsten visible light. In this case (data not shown), no increase in the extinction (scattering) was observed during the light on sample periods, thereby suggesting that the observed increase in extinction with the deuterium light is, indeed, due to DNA denaturing by UV-C photon dissipation.

Finally, it is pertinent to compare our experimental conditions of light, temperature and DNA concentrations to those probably existent during the Archean at the beginning of life on Earth (~3.8 Ga). Sagan (1973) has calculated an integrated UV-C flux during the Archean over the 240-270 nm region where DNA absorbs strongly (see Fig. 1) of 3.3 W/m^2 while we have estimated a somewhat larger flux 4.3 W/m^2 (Michaelian and Simeonov, 2014). Our light on sample was estimated to give a flux in this same wavelength range (240-270 nm) of 2.9 W/m^2 . However, the beam volume on sample was 0.107 cm^3 while the total volume of the sample was 1.0 cm^3 and uniform mixing of the DNA sample due to the magnetic stirrer ensured homogeneous passage of the DNA through the whole volume, giving an average UV-C light flux of only 0.31 W/ m^2 during light-on periods, less than 1/10 of what it may have been in the Archean.

Isotopic geologic data suggest that at 3.8 Ga the Earth was kept warm by CO_2 and CH_4, maintaining average surface temperatures around 80°C (Knauth, 1992; Knauth and Lowe, 2003) and falling to 70 $\pm$ 15 °C at 3.5–3.2 Ga (Lowe and Tice, 2004). Of course, polar regions would have been colder and equatorial regions warmer. The data presented here were obtained within this temperature range.

Miller (1998) has determined adenine concentrations of 15 µM in the prebiotic soup at the beginnings of life by using calculations of photochemical production rates of prebiotic organic molecules determined by Stribling and Miller (1987). Although these estimates are considered overly optimistic by some, it is noted that Miller was not aware of non-equilibrium thermodynamic routes to the nucleotides based on photon dissipation (Michaelian, 2014b), for example, the recently discovered route to pyrimidine ribonucleotide production utilizing UV-C light (Powner et al., 2009), nor of the existence of an organically enriched sea surface skin layer

(Hardy, 1982; Grammatika and Zimmerman, 2001) so these determinations of nucleotide concentrations at the beginnings of life may, in fact, be overly conservative. Adenine concentrations in our sample DNA were of the order of 50 µM.

The above considerations, particularly our lower on-sample UV-C light flux as compared to estimates of the flux at Earth's surface during the Archean, indicate that our UV-C induced denaturing results should probably be taken as a conservative lower limit to what could have occurred at the beginnings of life.

Conclusions

Through an analysis of extinction (absorption and scattering) at UV-C wavelengths and only scattering at longer wavelengths, and considering the characteristics of hyperchromicity and Rayleigh and Mie scattering of DNA, we have demonstrated that absorption and dissipation of UV-C light in the range of 240 to 270 nm denatures DNA in a completely reversible and benign manner. Analysis of the scattering component suggests complete denaturing for short 25 bp synthetic DNA while partial denaturing for long salmon sperm, yeast DNA, and 48 bp DNA. As far as the authors are aware, there has been only one previous indication of possible denaturing of DNA with UV light given in a much different context (Hagen et al., 1965). Our results demonstrate clearly that UV-C light denatures DNA and we have quantified this effect under conditions that may be considered conservative with respect to those expected at the beginnings of life in the Archean.

These results provide evidence for the plausibility of the ultraviolet and temperature assisted reproduction (UVTAR) mechanism for enzyme less amplification of DNA at the Archean sea surface when temperatures began to descend below their natural melting temperatures and when there existed an atmospheric window permitting solar UV-C light to reach Earth's surface. The UVTAR mechanism is an integral component of the thermodynamic dissipation theory of the origin of life (Michaelian 2009, 2011) which proposes that life arose, proliferated and evolved to dissipate the prevailing Earth surface solar spectrum. The coupling of growth or replication with dissipation is a necessary prerequisite of any proliferating irreversible process and in this context our result could reconcile "replication first" with "metabolism first" scenarios concerning the character of the origin of life. The results presented here also lend plausibility to the corollary theories on the acquisition of homochirality and information content for DNA or RNA (Michaelian 2009, 2010, 2011).

Since RNA has similar optical and chemical properties as DNA, it is almost certain that such UVTAR, homochirality, and information acquisition mechanisms, driven by photon dissipation, would also have acted over RNA in a similar manner, and we are presently conducting analogous experiments to test this. DNA and RNA may thus have been contemporaries in the

primordial soup and evolved with other fundamental UV-C absorbing molecules independently towards increased photon dissipation before finally forming a symbiosis which augmented the dissipation. Evidence for a stereochemical era of DNA and RNA association with amino acids (Yarus et al., 2009) supports this assertion as does evidence of chemical affinity to RNA and DNA of other fundamental molecules of life such as cofactors, vitamins and protoporphyrins, which could have acted as light antenna pigments in the UV-C (Michaelian and Simeonov, 2014d). This would have allowed continued reproduction and proliferation, and thus increases in photon dissipation, despite the continued cooling of the ocean surface and the eventual attenuation of the UV-C light brought on by the accumulation of oxygen and the delegation of UV-C dissipation to life derived ozone in the upper atmosphere. The thermodynamic dissipation theory for the origin of life suggests that all biotic and coupled biotic-abiotic evolution in general, would have been, and still is, driven by increases in the entropy production of the biosphere through increasing the global solar photon dissipation rate.

We are currently engaged in experiments to verify the second part of the UVTAR mechanism, that of enzyme less extension during dark, overnight, periods.

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