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Lambda Exonuclease Digestion of CGG Trinucleotide Repeats

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Abstract

Fragile X syndrome and other triplet repeat diseases are characterized by an elongation of a repeating DNA triplet. The ensemble-averaged lambda exonuclease digestion rate of different substrates, including one with an elongated FMR1 gene containing 120 CGG repeats, was measured using absorption and fluorescence spectroscopy. Using magnetic tweezers sequence-dependent digestion rates and pausing was measured for individual lambda exonucleases. Within the triplet repeats a lower average and narrower distribution of rates and a higher frequency of pausing was observed.

Introduction

In the past two decades, at least eighteen genetic neurodegenerative diseases have been linked with the expansion of repeating DNA triplets, resulting in disorders which include myotonic dystrophy, Huntington's disease and Fragile X syndrome (Gatchel and Zoghbi 2005). DNA triplet repeats, for example (CGG)_m or (GAA)_m where $m = 4 - 50$, occur naturally throughout the genome, primarily in non-coding regions. Fragile X syndrome was the first identified and most commonly studied triplet repeat disease (Kremer et al. 1991). The original fragile site, FRAXA, is in the 5' untranslated region of the FMR1 gene and the repeating CGG codon is subject to expansion from the normal range of 6 – 60 repeats to more than 200 repeats in the pathogenic state. Although these repeats have a B-DNA structure, their persistence length has been measured to be 60% less than random DNA (Bacolla et al. 1997). These repetitive sequences can also give rise to secondary structures, in particular hairpins depending on DNA density, pH, ionic strength and superhelical density (Usdin and Grabczyk 2000).

Both the causes and mechanisms by which the expansion of the triplet repeats occur are not resolved though a number of models have been put forward including a number mediated by secondary structures (Wang and Vasquez 2006). Mechanistic evidence at the enzyme level has been aided by the observation of stalling of DNA polymerase *in vitro* (Mirkin and Mirkin 2007). Single molecule assays observing the operation of individual enzymes and complexes and quantifying the influence of structure and environment may help further discriminate between the models.

Lambda exonuclease, a 24kDa highly processive enzyme which hydrolyses one strand of double-stranded DNA (dsDNA) in the 5' to 3' direction, has been studied previously in single molecule experiments using pseudo-random DNA sequences. The average digestion

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rate has been measured to be 12 nt / s (Perkins et al. 2003), 1520 nt / s (Dapprich 1999), and 32 nt / s (van Oijen et al. 2003) with an instantaneous rate varying between 0 – 100 nt / s. Sequence dependence digestion rates (van Oijen et al. 2003) and pausing of up to 12 seconds correlating with DNA motifs (Perkins et al. 2003) have also been observed. These traits have also been observed with other exonucleases (Kropp et al. 1995). Exonuclease activity is important within the repeats because of the observation that flap-specific endonuclease FEN-1 is necessary for the resolution of secondary structures in some of the triplet repeat expansion models (Singh et al. 2007).

In this report the characteristics of individual enzymes processing repetitive DNA sequences is characterized, specifically the measurement of lambda exonuclease digestion of 120 CGG triplet repeats in an elongated FMR1 gene. Repetitive DNA sequences are important because of their association with a number of functions such as regulation and reorganization of the genome (Richards and Sutherland 1996). Repeating motifs can also form stable hairpins which have been studied at the single molecule level (Woodside et al. 2006), however the interaction of DNA-modifying enzymes with these motifs is not well understood at the single molecule level. Using magnetic tweezers we combine the advantages of optical tweezers (Perkins et al. 2003) and flow-stretching experiments (van Oijen et al. 2003), enabling multiple enzymes to be studied in parallel without heating concerns and with freedom to adjust flow rates independently of the applied force.

We hypothesized that the 120 repeats of the GC-rich triplet CGG in the elongated region are similar to the GGCG motifs previously reported to correlate with pausing (Perkins et al. 2003) and require more energy to separate (Woodside et al. 2006) and therefore may provide a significant barrier to lambda exonuclease activity. Lower average and instantaneous digest rates and a higher frequency of pausing were measured for the elongated regions, supporting this hypothesis.

Materials and Methods

An elongated FMR1 gene containing 120 CGG repeats was ligated into a pGL3-basic vector (Promega Inc., Madison, WI) using the MluI and BglII restriction sites and replicated in *E. coli* under antibiotic selection. This plasmid is subsequently referred to as pGL-CGG120. Six colonies were isolated from a streaked plate and used to inoculate 5-mL tubes of liquid LB broth containing ampicillin. Care was taken to limit growth in a shaking incubator to a maximum of ten hours at 37°C to preserve replication fidelity. Plasmids were isolated and purified using a miniprep kit (QIAprep, Qiagen Inc., Valencia, CA) and ethanol precipitation and quantified by absorption at 260 nm (A_{260}) and 280 nm (A_{280}).

The length of the elongated triplet repeat region was checked by double digest of the plasmid DNA with MluI / BglII, and compared on a 1% agarose gel to a GC-rich optimized PCR amplification (Roche Applied Science, Indianapolis, IN) of the FMR1 gene insert and a pair of standard DNA ladders (O'Generuler, Fermentas Inc., Glen Burnie, MD). Well defined bands at approximately 550 base pairs (bp) were observed from four of the isolated colonies and these samples were used in the subsequent experiments.

Two commercially available double-stranded DNA sequences, the linear 48.5 kilobase pair (kbp) lambda phage and the circular phiX174 phage sequences (New England Biolabs Inc., Ipswich, MA) were used as pseudo-random DNA references. DNA samples were stored at 4°C for up to 4 days, or at -80°C for up to twelve months. The concentration of stock DNA was between 200 – 1000 $\mu\text{g} / \text{mL}$ with an A_{260}/A_{280} ratio between 1.8 and 2.2.

Absorption measurements were made in disposable ultraviolet grade cuvettes (UVettes, Eppendorf, Westbury, NY) containing 0.95 mL of buffer allowed to come to equilibrium for

a background measurement before 5 μ g of DNA was added. Both the pGL-CGG120 plasmid and phiX174 phage sequence were linearized with their unique XhoI restriction sites to provide comparable digestion substrates. Stock lambda phage DNA was placed in a 55°C water bath for fifteen minutes and rapidly cooled on ice to minimize recircularization and concatenation of the cos ends. Subsequently lambda exonuclease (New England Biolabs Inc., Ipswich, MA) was added, a baseline acquired and then the digestion was initiated with an infusion of Mg^{2+} . The rate was determined from curve fitting of the time course of measurements at 260 nm and 280 nm which were recorded at 15 second intervals. The temperature controlled stage of the spectrometer (Spectramax 384+, Molecular Devices Inc., Sunnyvale, CA) was set at 25°C, except for the temperature-dependent measurements with lambda phage DNA at 20°C, 30°C and 37°C. Fitting to extract the digestion rate and analysis of the curves was carried out in Origin (OriginLab Inc., Northampton, MA). Statistical uncertainties are described by the standard deviation and the significance level was set at $P < 0.05$.

Measurement of the digestion rate using fluorescence spectroscopy was carried out using a fluorescent spectrometer (Jobin Yvon Fluorolog FL3-22, Edison, NY). A 1:20,000 dilution of OliGreen (Invitrogen Corp., Carlsbad, CA), which has been reported to have a linear response for both dsDNA and ssDNA concentration (BioTek 2001) was used to label 100 ng samples of dsDNA. Using a time course of excitation at 490 nm and measurement of emission at 520 nm at 1 second intervals, the resulting fluorescence decay curves were analyzed. Measurement rate was increased to 0.5 second intervals for the elongated FMR1 fluorescent digest, which was prepared from the replicated plasmid by a double digest (MluI/BglII) followed by purification on a 1.5% agarose gel (Qiaex II, Qiagen, Valencia, CA).

A schematic of the setup used for the single molecule experiments is shown in Figure 1a). Following an approach similar to one previously described (Danilowicz et al. 2004), double digestion of the pGL-CGG120 plasmid with NcoI / XhoI provided two distinct overhangs onto which two modified oligos were ligated. One oligo included a digoxin label for attachment to the inner surface of a 1×1×10 mm glass capillary (Vitrocells Inc., Mountain Lakes, NJ) previously incubated with anti-digoxin. The other oligo included a biotin label for attachment to a streptavidin labeled 2.8- μ m diameter magnetic bead (Invitrogen Corp., Carlsbad, CA) on the opposite end of the same DNA strand, making the other strand available for complete digestion by an exonuclease.

After diluting and washing 1- μ L of the stock beads three times in a Mg^{2+} -free buffer suggested by the enzyme supplier, 10 attomoles of the modified linearized plasmid was incubated with the beads and lambda exonuclease enzyme for fifteen minutes in a slow rotator. After magnetic separation and resuspension, approximately 100- μ L of the suspension was loaded into the capillary using surface tension.

The experimental procedure for the single molecule experiments is described in Figure 1b). After ten minutes of incubation, a stack of five 5-mm diameter neodymium, iron, boron permanent magnets (Master Magnetics Inc., Castle Rock, CO) were used to apply an average force of 10 pN to the beads and detach any non-specifically bound beads. Then the DNA-bound beads were stretched in the opposite direction using a complementary set of magnets and the force-extension curve recorded, before a final recorded stretch in the first direction to determine the point of attachment and the number of DNA tethers. Screening for beads with a force-extension curve characteristic of a single tether and symmetric stretching, the force was reduced to a force of 3 pN and a video sequence recorded using an IX-71 microscope (Olympus, Center Valley, PA) with a 40x objective and a 1.4 million pixel, 12-bit ORCA-ER (Hamamatsu, Bridgewater, NJ) monochromatic camera using brightfield

whitelight. The bound exonucleases were activated using an infusion of MgCl_2 into one end of the capillary and a video recorded for thirty minutes. To convert the video sequence to position data, a 5×5 Laplacian of a Gaussian filter with kernel for $\sigma = 5$ pixels was applied and an ImageJ plugin used to track the center-of-mass of the beads (Sage et al. 2005). A correction was applied to this position data before the rates were determined because of the change in angle of the DNA with respect to the surface as the end-to-end length of the DNA changed. The standard deviation in the position of a fixed bead measured in this way over a ten minute period, comparable to the duration of the experiments, was 15 nm.

Results and Discussion

To provide an ensemble-averaged measurement of the digestion rate of lambda exonuclease for comparison with the single molecule experiments, the digestion of the DNA sequences were examined using absorption and fluorescent spectroscopy. Figure 2a) illustrates the digestion rate measured for each of the three unmodified substrates at 25°C. No significant difference was measured between the digestion rates of the two shorter substrates, which are both similar in length to the average processivity of lambda exonuclease, however the rate is significantly lower for the lambda phage substrate which is nine times longer and significantly longer than the average processivity of a single enzyme. For the 48502 bp long lambda phage sequence with 50% GC content the rate was 10.8 ± 1.4 nucleotides per second (nt / s), for the 5386-bp long phiX174 sequence with 44% GC content the rate was 28.7 ± 8.8 nt / s and for the 5373-bp long pGL-CGG120 sequence, with 52% GC content, the rate was 27.2 ± 9.9 nt / s. The rates measured using OliGreen as a fluorescent indicator, and illustrated in Figure 2b) were not significantly lower at 24.6 ± 4.6 nt / s and 24.3 ± 5.9 nt / s respectively. For the elongated FMR1 sequence, with 91% GC content, the rate measured by fluorescence was 15.1 ± 2.0 nt/s and significantly different to the overall pGL-CGG120 plasmid. Although DNA sensitive dyes have been observed to inhibit exonuclease activity previously (Subramanian et al. 2003; Yakovleva et al. 2006), the related PicoGreen dye was found to cause the least perturbation when used to measured DNAase activity (Tolun and Myers 2003).

The temperature dependence of the digest rate of the lambda phage sequence was measured by absorption and the results shown in Figure 2c). The average rate measured by absorption at 260 nm for the 48502-bp long lambda phage sequence was 10.8 ± 1.4 nt / s at 25°C. This rate increased to 14.7 nt / s at 30°C and 20.0 nt / s at 37°C. These rates are consistent with previous measurements (Carter and Radding 1971; Perkins et al. 2003) and indicate that small temperature fluctuations do not significantly impact digestion rate at room temperature. At more extreme temperatures, the underlying temperature-force phase diagram for DNA is more complicated (Danilowicz et al. 2004) and may contribute to more significant variations. At 37°C the rate is higher than that previously reported for fluorescently labeled DNA (Subramanian et al. 2003) although no account was taken here in the analysis for the possibility of increased ssDNA activity of lambda exonuclease at higher temperatures (Little 1981).

With biotin and digoxin-labeled oligos ligated onto both ends of all three sequences, the digestion rate of the lambda phage sequence measured by absorption decreased to 5.2 ± 3.1 nt / s, 12.4 ± 5.6 nt / s for the pGL-CGG120 sequence and 12.7 ± 4.8 nt / s for the phiX174 sequence. These results are consistent with the expectation that only one enzyme is active on the substrate, working on only one of the two strands.

Although a significant difference in the digestion rate was measured for the GC-rich elongated FMR1 gene, the data was collected from ensemble averaging of at least one

billion digestions and does not indicate whether the decrease is due to pausing or slower procession through the GC-rich sequence.

Complementing the ensemble measurements, magnetic tweezers were used to stretch individual pGL-CGG120 DNA sequences in the image plane of a microscope and the decrease in persistence length between dsDNA and ssDNA used as readout of the digestion rate (Dapprich 1999; Matsuura et al. 2001; Perkins et al. 2003; van Oijen et al. 2003). In a single field-of-view, the maximum number of digestions observed to go to completion was limited to three. This reflected the need to keep the density of DNA labeled beads low to avoid aggregation, the low DNA concentration used to give one tether per bead and that less than 10% of the beads observed with force-extension curves characteristic of a single tether went to completion, primarily because of bead-DNA-surface interactions characterized by a significant change in the motion of the bead. Figure 3a) illustrates the partial trajectories of three digestions which went to completion. Previously, it was observed that the digestion rate of lambda exonuclease is sequence specific because of the energy landscape associated with the Gibb's free energy calculated from the energy required to melt the hydrogen bonds between base pairs and the stacking energy of the helix (van Oijen et al. 2003). Figure 3b) is a prediction of the digestion rate based on the Gibb's free energy of the pGL-CGG120 sequence based on an average rate of 12.4 nt / s measured by absorption spectroscopy. Figure 3c) is a plot of the experimentally measured rate from a single digestion, with a 10 second moving average used to smooth the data. For the complete pGL-CGG120 sequence, the Pearson product-moment correlation coefficient of individual complete digestions with the predicted rate was $r^2 = 0.184 \pm 0.088$ ($n = 10$), indicating a weak correlation between the digestion rate and the Gibb's free energy landscape derived from the sequence information. Within the region of the triplet repeats near the beginning of the digestions the correlation coefficient was $r^2 = 0.61 \pm 0.12$. When the digestion sequences were averaged first and then the correlation coefficient calculated, $r^2 = 0.24$ for the complete sequence and $r^2 = 0.68$ for the region of the triplet repeats indicating the smoothing of noise and variation in enzyme activity increases correlation. It should be noted that the model used to correct for angle of the DNA in these experiments does not remove all trends as can be seen from Figure 3c) and the sequences not optimally aligned which may also lead to an underestimation of the correlation.

The rate statistics from the ten complete digestions are plotted in Figure 4a) smoothed using a three second moving average, to illustrate the spread in "instantaneous" enzyme digestion rate. From a Gaussian fit of all the data from the ten digests ($R^2 = 0.975$) displayed in Figure 4a) this smoothed "instantaneous" rate of an individual enzyme in a one second time interval was 14.7 ± 26.3 nt / s. This result is within the statistical error of the ensemble absorption measurements under the same conditions and is in good agreement with previous measurements of mean rate and distribution. From the ten digests, the "instantaneous" digestion rate in the region of the triplet repeats is illustrated in Figure 4b) with a fitted average of 8.6 ± 13.5 nt / s, and is statistically significantly different from a random sample of the same size taken from the pooled population data, but consistent with the expectation for half of the rate measured for the fluorescent digest of the elongated FMR1 sequence. The lower average and narrower distribution of "instantaneous" digestion rates within the triplet repeat sequence supports our hypothesis of the GC-rich region providing a significant barrier for lambda exonuclease activity. A definition was used to define pause points, where the "instantaneous" digestion rate was consecutively below 10 nt / s for at least two seconds, and an independent two sample t-test of the rates within the pause point was not statistically significantly different from 0 nt / s. The distribution of the length of these pauses within the triplet repeat region is illustrated in Figure 4c). Although the distribution of pause times is similar to those previously reported to correlate with motifs containing GGCG (Perkins et al. 2003) and observed both within the triplet repeats and in the surrounding vector sequence,

no common pause points could be defined. The frequency of pausing in the region of the triplet repeats was 8.3 kbp^{-1} while the frequency of pausing in the rest of the sequence was 1.2 kbp^{-1} although there was no significant difference in distribution of pause times between the two regions. This result supports the hypothesis that GC-rich repetitive sequences provide a significant barrier to lambda exonuclease activity by increasing the probability of pausing in addition to decreasing the average digestion rate.

Conclusions

In conclusion we have measured a sequence-dependent rate and pausing of individual lambda exonuclease enzymes during the digestion of a DNA sequence containing 120 CGG repeats. A decrease in digestion rate was resolved using fluorescence spectroscopy and confirmed by the observation of individual enzymes which showed the decrease was due to a combination of a narrower distribution of “instantaneous” rates within the elongated repeating sequence and an increase in pause frequency.

It should be noted that these results were biased by the requirements for the force-extension of the tether to be characteristic of a single B-conformation dsDNA molecule and for the digestion to have gone to completion, limiting analysis to only temporary pauses in characteristic single dsDNA tethers. Results from absorption spectroscopy indicated that at least 75% of double-stranded base pairs were converted to single-stranded base pairs at an average rate within the experimental error of the single molecule experiments, indicating that these assumptions did not significantly bias the average of the single molecule results.

Magnetic tweezers have the advantages of not dissipating energy, parallel data collection and not requiring continuous flow, providing a strong platform for further study of enzyme interaction with repetitive DNA structures. Work is on-going to observe the recreation of the complementary strand by DNA polymerase, following digestion by lambda exonuclease, with the goal of characterizing replication fork stalling of individual complexes. A comparison of sequences containing repeats considered to be within normal range with pre and full mutation sequences taking into account epigenetic differences would give a more fine-grained analysis of the mechanisms which give rise to expansion. These techniques can also be applied to other trinucleotide repeat disorders, such as Friedreich's ataxia, which do not have the same GC-rich characteristics as Fragile X syndrome.

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References

- Bacolla A, Gellibolian R, Shimizu M, Amirhaeri S, Kang S, Ohshima K, Larson JE, Harvey SC, Stollar BD, Wells RD. Flexible DNA: Genetically unstable CTG center dot CAG and CGG center dot CCG from human hereditary neuromuscular disease genes. *Journal Of Biological Chemistry*. 1997; 272:16783–16792. [PubMed: 9201983]
- BioTek. Quantitation of ssDNA using OliGreen Fluorescent Stain. In: Inc. B., editor. Application Notes. Winooski, VT: BioTek Inc.; 2001.
- Carter DM, Radding CM. The role of exonuclease and beta protein of phage lambda in genetic recombination. II. Substrate specificity and the mode of action of lambda exonuclease. *J Biol Chem*. 1971; 246:2502–2512. [PubMed: 4928646]

- Danilowicz C, Kafri Y, Conroy RS, Coljee VW, Weeks J, Prentiss M. Measurement of the phase diagram of DNA unzipping in the temperature-force plane. *Phys Rev Lett*. 2004; 93:078101. [PubMed: 15324279]
- Dapprich J. Single-molecule DNA digestion by lambda-exonuclease. *Cytometry*. 1999; 36:163–168. [PubMed: 10404963]
- Gatchel JR, Zoghbi HY. Diseases of unstable repeat expansion: Mechanisms and common principles. *Nature Reviews Genetics*. 2005; 6:743–755.
- Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Warren ST, Schlessinger D, Sutherland GR, Richards RI. Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)_n. *Science*. 1991; 252:1711–1714. [PubMed: 1675488]
- Kropp B, Leoni L, Sampaiolese B, Savino M. Influence of DNA superstructural features and histone amino-terminal domains on nucleosome positioning. *FEBS Lett*. 1995; 364:17–22. [PubMed: 7750535]
- Little JW. Lambda exonuclease. *Gene Amplif Anal*. 1981; 2:135–145. [PubMed: 6242844]
- Matsuura S, Komatsu J, Hirano K, Yasuda H, Takashima K, Katsura S, Mizuno A. Real-time observation of a single DNA digestion by lambda exonuclease under a fluorescence microscope field. *Nucleic Acids Res*. 2001; 29:E79. [PubMed: 11504887]
- Mirkin EV, Mirkin SM. Replication fork stalling at natural impediments. *Microbiol Mol Biol Rev*. 2007; 71:13–35. [PubMed: 17347517]
- Perkins TT, Dalal RV, Mitsis PG, Block SM. Sequence-dependent pausing of single lambda exonuclease molecules. *Science*. 2003; 301:1914–1918. [PubMed: 12947034]
- Richards RI, Sutherland GR. Repeat offenders: simple repeat sequences and complex genetic problems. *Hum Mutat*. 1996; 8:1–7. [PubMed: 8807329]
- Sage D, Neumann FR, Hediger F, Gasser SM, Unser M. Automatic tracking of individual fluorescence particles: application to the study of chromosome dynamics. *IEEE Trans Image Process*. 2005; 14:1372–1383. [PubMed: 16190472]
- Singh P, Zheng L, Chavez V, Qiu JZ, Shen BH. Concerted action of exonuclease and gap-dependent endonuclease activities of FEN-1 contributes to the resolution of triplet repeat sequences (CTG)_n- and (GAA)_n-derived secondary structures formed during maturation of Okazaki fragments. *Journal Of Biological Chemistry*. 2007; 282:3465–3477. [PubMed: 17138563]
- Subramanian K, Rutvisuttinunt W, Scott W, Myers RS. The enzymatic basis of processivity in lambda exonuclease. *Nucleic Acids Res*. 2003; 31:1585–1596. [PubMed: 12626699]
- Tolun G, Myers RS. A real-time DNase assay (ReDA) based on PicoGreen fluorescence. *Nucleic Acids Res*. 2003; 31:e111. [PubMed: 12954787]
- Usdin K, Grabczyk E. DNA repeat expansions and human disease. *Cellular and Molecular Life Sciences*. 2000; 57:914–931. [PubMed: 10950307]
- van Oijen AM, Blainey PC, Crampton DJ, Richardson CC, Ellenberger T, Xie XS. Single-molecule kinetics of lambda exonuclease reveal base dependence and dynamic disorder. *Science*. 2003; 301:1235–1238. [PubMed: 12947199]
- Wang GL, Vasquez KM. Non-B DNA structure-induced genetic instability. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*. 2006; 598:103–119. [PubMed: 16516932]
- Woodside MT, Behnke-Parks WM, Larizadeh K, Travers K, Herschlag D, Block SM. Nanomechanical measurements of the sequence-dependent folding landscapes of single nucleic acid hairpins. *Proc Natl Acad Sci U S A*. 2006; 103:6190–6195. [PubMed: 16606839]
- Yakovleva L, Handy CJ, Yagi H, Sayer JM, Jerina DM, Shuman S. Intercalating polycyclic aromatic hydrocarbon-DNA adducts poison DNA religation by Vaccinia topoisomerase and act as roadblocks to digestion by exonuclease III. *Biochemistry*. 2006; 45:7644–7653. [PubMed: 16768460]

Figure 1.

a) Schematic of the experimental setup used for the single molecule experiments. One end of the DNA sequence is attached to the inner surface of a glass capillary by an antibody-antigen reaction and is stretched using a magnetic bead attached to the other end via a biotin-streptavidin reaction (not to scale). b) After stretching the DNA in opposite directions with 10 pN to determine point of attachment and a single tether, the force was reduced to 3 pN and Mg^{2+} added to one end of the capillary. As the exonuclease digests one of the two strands, the persistence length and end-to-end distance decreases.

Figure 2.

Measurement of the lambda exonuclease digestion rate using spectroscopy. a) Digestion rates at 25°C for the 48502 bp lambda phage, 5386 bp PhiX174 phage and the 5373 bp pGL-CGG120 sequences measured by absorption. b) Comparison of rates for the shorter sequences measured by absorption and fluorescence spectroscopy at 25°C. c) Temperature dependence of the digestion rate for the lambda phage sequence.

Figure 3.

Data from individual lambda exonuclease digests of the pGL-CGG120 sequence. a) Partial traces of template position versus time for three independent digests (offset by 50s intervals, smoothed with a 10 s moving average and truncated at 1200 bp for clarity). The area between the dotted lines represents the region of the CGG repeats in the template. b) Simulation of the digestion rate based on bond energies for the complete pGL-CGG120 sequence using a 100bp moving average. c) Digestion rate calculated from the center trace of a) smoothed with a 10 s moving average.

Figure 4.

Digestion rate statistics from a set ($n=10$) of smoothed (3s moving average) one second “instantaneous” rates of individual lambda exonuclease digestions of the pGL-CGG120 sequence. Distribution of rates for a) the complete pGL-CGG120 sequence and b) the CGG repeat region. c) Within the triplet repeat region the distribution of pause times, for which the measured digestion rate was not significantly different from 0 nt/s for more than 2 s.