Stability of Synthetic Single-Strand DNA as a Criterion for the Usage as Marking System

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Abstract: DNA is a carrier of genetic information and can be used as an invisible barcode. For its successful application, stability is one important criterion. This report describes the investigation of synthetic single-strand DNA (ssDNA) damage under several environmental conditions with Real-time PCR. We measured the amplification ratio of amplifiable ssDNA against time and calculated the half-life. Comparing the half-life of all DNA degradations, ultraviolet irradiation leads to the fastest ssDNA decay, followed by ssDNA treatment at 80°C in the presence of water and acidic ssDNA incubation. However, ssDNA is protected against high temperatures by complexation to hydroxyapatite. This study shows for the first time half-life values for ssDNA, which are important for technical applications such as the usage as a marking system. Also this study is of crucial importance for all other applications, in which DNA damage can lead to misinterpretations in PCR results.

Keywords: DNA stability, DNA damage, amplification ratio, rate constant, half-life, Real-time PCR.

INTRODUCTION

Bio-based products for human use such as food, wood, leather, glues or paper are often exposed to extreme conditions when they are processed or, later, during their use. Today, DNA may be used as marking and identification system for such products either by measuring their original DNA or after addition of synthetic DNA. However, environmental influences such as heat, moisture, extreme pH or ultraviolet radiation (UV) lead to DNA damages e.g. modified bases, abasic sites, single and double strand breaks or dimmers [1, 2]. All these natural occurring DNA lesions can be summarized as total DNA damage. A common feature of DNA lesions is their ability to hinder [3, 4] or block [5] polymerase enzymes during amplification. For this reason the quantitative real-time polymerase-chain-reaction (qPCR) is a reliable method to detect the total DNA damage. To date there are two different qPCR methods which characterize total DNA-damage of DNA samples: (1) determination of the amplification ratio of damaged DNA and (2) the determination of the degradation ratio of damaged DNA. The amplification ratio of DNA samples is calculated by quantifying damaged DNA and normalisation to an undamaged reference DNA of the same concentration [5]. In contrast, the degradation ratio is measured by amplifying a short and a long amplicon from the same DNA sample and calculating the ratio from both quantities [6]. The degradation ratio is based on the fact that the measured DNA quantity in PCR depends on the length of the chosen amplicon [7-9] and it is used for measurements where no reference is available e.g. during forensic analysis. With both of these two methods information about the quality and the amplifiability [7] of DNA are obtained, because both methods only detect intact DNA molecules without damages.

Most studies in this field deal with the effects on polymerase progression of either specific artificial DNA lesions [3, 4, 10-15] or of natural lesions in ancient DNA [6, 8, 9, 16, 17]. Also, the formation of specific DNA lesions such as abasic sites is entirely described by the determination of rate constants and activation energies for these specific lesions (for review see Lindhal [1]). Furthermore, DNA decay is described as a natural decay for double stranded DNA (dsDNA) which was exposed to ultraviolet radiation or for hydrogen peroxide-induced mitochondrial DNA damage [5, 18, 19].

However, studies about total DNA damage at technically used environmental conditions rarely exist, such as at elevated temperatures or at acidic pH. Therefore, we established a method to visualise the time-dependent concentration decay of amplifiable DNA under such conditions. The concentration of amplifiable DNA reflects the total DNA damage, independent of its exact chemical nature. We investigated the time-dependent total DNA damage of ssDNA by qPCR, measuring the concentration of amplifiable DNA at different points of time under several environmental effects and calculating the amplification ratio, the rate constant and half-life. The

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E-ISSN: 2311-1755/14

time-dependent amplification ratio strongly depends on the amount of amplifiable DNA. Because the total DNA damage accumulates over time, the time-dependent amplification ratio decreases and the rate constant and half-life is calculated from that decrease. Therefore, the measured rate-constant and the calculated half-life are apparent constants, which represent the accumulation velocity of non-amplifiable DNA damage.

Within this study, we investigated the total DNA damage and therefore the change of amplifiable DNA to non-amplifiable DNA under the following extreme conditions: 80°C in dry and wet environment, pH 2.0 (Theorell-Stenhagen-buffer, (TSB)), UV-light (254 nm). We chose these three conditions, because elevated temperature, acidic pH and UV are the main initiators for DNA damage. Also these three conditions appear during leather production, and our main aim is to establish a leather marking system based on ssDNA. Furthermore, we tried to enhance the stability of amplifiable DNA at 80°C in wet environment with DNA/ hydroxyapatite.

An invisible marking system based on ssDNA is outstandingly interesting for products, which are difficult in their marking and traceability. Such products are, for example, bulk materials, liquids or laminar materials which are upgraded by subsequent steps as cutting in smaller pieces, sawing or lining. But also the results of this study are of great interest to researchers using DNA for technical applications e.g. bar code systems or working with ancient or damaged DNA in qPCR.

MATERIALS AND METHODS

Synthetic single-strand oligonucleotides (ssDNA; 1/50, 1/60, 1/70) and the associated primers for PCR analysis were designed manually and were obtained from Life Technologies GmbH (Darmstadt, Germany). For sequences and other characteristics of the ssDNA see Table **1**.

Stability Assay pH 2.0

Aqueous DNA (1/50) was aliquoted to 10 pg and dried in a speed vac (Eppendorf, Hamburg, Germany). Three aliquoted DNA pellets were resolved in 50 µl TSB (pH 2.0; 33 mM citric acid, 33 mM phosphoric acid, 57 mM boric acid, 34.3 mM sodium hydroxide; pH was adjusted with 0,1 M HCl) and were incubated at room temperature in the dark for time periods of 0, 1, 2, 3, 4 days, respectively. The acidic samples were subsequently neutralised with a solution containing 33 mM citric acid, 33 mM phosphoric acid, 57 mM boric acid, 34.3 mM sodium hydroxide. Neutralised samples had a DNA concentration of 0.05 pg/µl and were analysed in qPCR for total damage. For abasic site detection, samples were treated in the same way as above at pH 2.0 and were afterwards incubated for time periods of 0, 6, 12, 18, 24, and 30 hours, respectively. Neutralisation was followed by a digestion assay with endonuclease V, *t. maritima* (fermentas, St. Leon-Rot, Germany). The digestion assay was done as followes: 1 µl sample, 1 U enzyme, 65°C, 30 min in 1x appropriate reaction buffer. Digested samples were analysed for total damage in qPCR.

Stability Assay 80°C

Aqueous DNA (3/70) was aliquoted to 50 pg and was covered with oil to compensate volume loss. Three aliquoted ssDNA samples were incubated at 80°C for time periods of 0, 1, 2, 3, 4, 24 hours, respectively. After treatment, all samples were allowed to cool to room temperature. Oil was removed from all samples and the aqueous DNA was analysed in qPCR. In a second approach aqueous DNA (1/50) was aliquoted to 50 ng and was dried in the speed vac. Three dried ssDNA samples were incubated at 80°C for time periods of 0, 1, 4, 7, 11, 14, 18, 20, 25 and 27 days, respectively. After the treatment all samples were allowed to cool to room temperature. The DNA pellet was recovered by incubation in 10 mM Tris-HCl, pH 9.0 (peQlab; Erlangen, Germany) for 30 min. The obtained

Table 1: Characteristics of Used Synthetic ssDNA

Name	Sequence (5' to 3')	% GC	Purity	Primer Position
1/50	CGA GAA GCA CTG TGG ATG AAC GCG TTA GAG TAC CTG CTC ACT GTC CTC CT	54	desalted	Forward 1-20 Reverse 31-50
2/60	TCG AAC GTA TTG CGA CAA GCA TAG TGT CGT CCT CGC GGG TGT TGC TAG AGC CCA AAA CAC	53	desalted	Forward 1-20 Reverse 41-60
3/70	ACC AGC TAG ACG ACC CAG TGA CGA CAG CTA TGA CGA CCC AAC TGC TAG CGT GGG CTG CTC AGT CGA GAA T	57	desalted	Forward 1-20 Reverse 51-70

DNA solution was analysed in qPCR. Aliquotes were produced as followed for experiments with precipitated hydroxyapatite (Merck, Darmstadt, Germany): 20 mg of hydroxyapatite were vortexed with 0.6 µg aqueous ssDNA (2/60) for 3 min to form hydroxyapatite -DNA-complexes in water. Three aliquoted samples were incubated at 80°C for time periods of 0, 3, 18, 24, 48 and 72 hours, respectively. After heat treatment, all samples were cooled to room temperature. Water was removed and 300 mM phosphate buffer (pH 7.4) was added [20] followed by vortexing for 5 min. The phosphate buffer containing the damaged DNA and 3 ng of 3/70-ssDNA as a reference were purified with the CyclePure Kit (peQlab) according to the manufacturer's instructions. The purified DNA was analysed in qPCR.

Stability Assays UV-Light (254 nm)

100 ng aqueous ssDNA (1/50) were attached to a sterile filter paper. Water was allowed to evaporate before the filter papers were fixed in a PCR workstation from peQlab (3.4 J/m^2 per sec, personal request). Three aliquoted ssDNA samples on filter paper were irradiated with UV light (254 nm) for time periods of 0, 1, 1.5, 2, 3, 5, 6 hours. A lightproof covered control was simultaneously incubated for 6 hours. The control was used as blank value for A_0 (for details see analysis of qPCR-data). By dissolving the filter paper in ddH_2O, the DNA was recovered and analysed in qPCR.

Quantitative Real-Time PCR

The total DNA damage was detected by amplifying the treated and non-treated samples with qPCR based on SYBR Green I dye detection. QPCR was performed in low-profile, 96-well, clear PCR plates sealed with Microseal® 'B' Film on the CFX96™ Real-Time-System (all from Bio-Rad, München, Germany). Each qPCR-reaction (20 µl) contained 1x PCR buffer S, 2 mM $MgCl_2$, 100 nM dNTPs, 0.25 U Hot *taq*-DNA-polymerase (all from peqlab), 33 pmol forward and 33 pmol reverse primer (Life Technologies GmbH), and 1x SYBR® Green I (Sigma-Aldrich, Hamburg, Germany). The PCR parameters consisted of an initial denaturation step at 95°C for 3 min, followed by 29 cycles of 95°C denaturation for 10 sec, 55°C annealing for 15 sec and a 72°C elongation for 15 sec, followed by a melt curve from 55°C to 90°C in 0.5 increments.

Analysis of qPCR-Data/Statistics

Data analysis of the described real-time assay was carried out with the basic analysis steps from Bustin and Nolan [21]. Checked data were used for relative quantification based on a Standard Curve Method in separate tubes (instructions from user bulletin no. 2, PE Applied Biosystems) in duplets. Due to the lack of internal reference DNA, normalisation was done externally over sample size and over comparing treated samples to untreated samples by defining the relative amplification ratio (R_A) [5]. R_A is described by the ratio of the measurable starting quantity of treated samples (A_D) and the measurable DNA starting quantity in an untreated sample (A_0) ($R_A=A_D/A_0$). Non-treated controls were handled in the same way as the treated samples (with t= 0 d). Statistic analysis for identification of outliers for the three aliquoted samples was carried out with Dixons Q-test (n= 3; α= 0.05). Standard deviations were calculated by error propagation and formulas from Muller *et al*. [22]. The time-dependent amplification ratio behaviour of a damaged template matched a natural decay with the formula of the first reaction rate $R_A= R_0$ exp (-kt), where R_0 is the amplification ratio of non-damaged template and k is the rate constant. Plotting the negative natural logarithm of the amplification ratio (-ln A_D/A_0) against time results in a straight line, whose slope corresponds with k. The rate constant was calculated by linear regression using SigmaPlot 11.0. The half-life $t_{1/2}$ was determined with k= ln 2/$t_{1/2}$.

2.6 Inhibition Assays

The influences of neutralised TSB and digestion solution with endonuclease V on qPCR were checked with ssDNA of known concentrations (100 pg/µl, 10 pg/µl, 1 pg/µl, 10 fg/µl, 1 fg/µl, 0.1 fg/µl). Intra and inter assay variation were checked by generating two standard curves with duplets. The measured DNA concentration in neutralised TSB and in the digestion solution was compared to a standard curve in water (10 pg/µl, 10 fg/µl, 1 fg/µl, 100 ag/µl, 10 ag/µl).

RESULTS

The formation of total DNA damage in ssDNA under different environmental conditions was detected by qPCR. This method is based on the assumption that several strand lesions can block or at least obstruct PCR amplification with DNA polymerases [5]. Time-dependent DNA degradation curves based on the amplification ratio were measured and the rate constant and the half-life of ssDNA were calculated. Acidic treatment, temperature treatment as well as exposure to UV light led to a natural decay of the investigated ssDNA. Amplification ratio curves of equal

shape were also detected for double stranded DNA which was exposed to ultraviolet radiation [5, 18] or for hydrogen peroxide-induced mitochondrial DNA damage [19].

pH 2.0

Because the inhibition assay for neutralised TSB-mixture showed no intra-assay variation [unpublished results], the samples were directly analysed by qPCR after neutralisation. Figure **1a** shows the detected amplification ratio behaviour of ssDNA at pH 2.0 against time. The time-dependent behaviour of an exponential decay only depends on the rate constant, whose determination is demonstrated in Figure **1b**. The rate constant for the decay of aqueous ssDNA at pH = 2.0 is 0.58 ± 0.10 d^{-1}, the half-life is 1.20 ± 0.42 d (Table **2**). We investigated the influence of abasic sites on qPCR by repeating the pH-experiment followed by a digestion step. After neutralization, the samples were digested with endonuclease V, which transforms abasic sites to strand breaks. DNA purification after the digestion assay was unnecessary because the inhibition assay for DNA standards containing endonuclease V in qPCR resulted in a standard curve with a coefficient of determination of 0.999 [data not shown]. The rate constant for ssDNA decay in pH = 2.0 with digestion is 1.74 ± 0.21 d^{-1} (Figure **2a**; Table **2**). The corresponding half-life is 0.40 ± 0.10 d (Table **2**).

80°C

The rate constant for the decay of aqueous ssDNA at 80°C was measured to be 9.07 ± 0.73 d^{-1} (Table **2**, Figure **2b**). The half-life for this reaction is 0.08 ± 0.01 d. This means after 1.92 ± 0.24 h half of the initial concentration of amplifiable DNA is damaged in aqueous solution at 80°C. The same experiment was

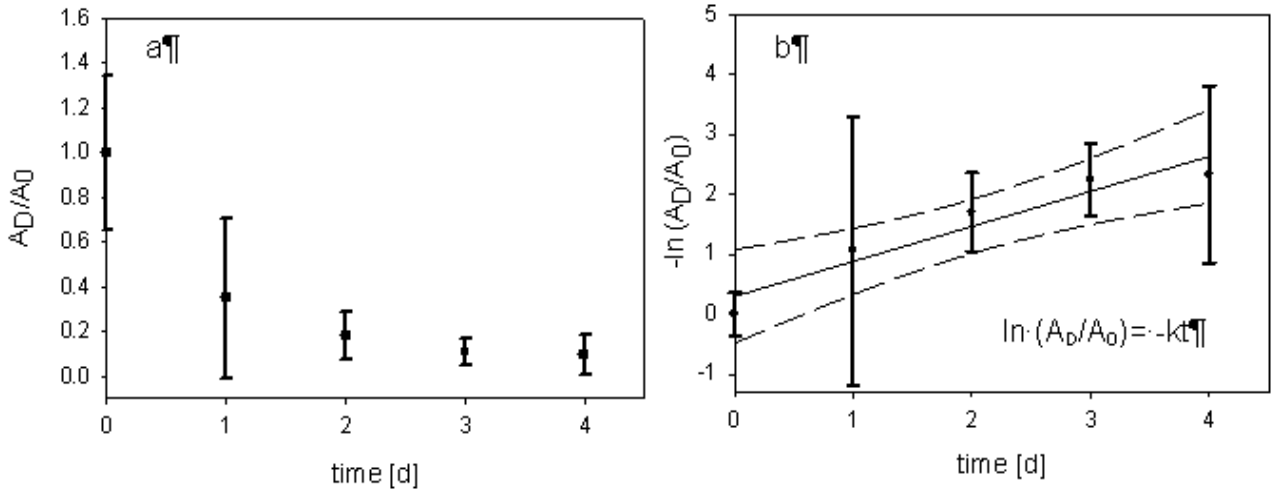

Figure 1: (**a**) Time dependent amplification ratio (A_D/A_0) behaviour for single stranded DNA in acidic solution (pH 2.0). Each point represents the mean value of three samples, measured in duplets, respectively. (**b**) Determination of the rate constant k for DNA decay under pH 2.0. The negative natural logarithm of the amplification ratio (-ln A_D/A_0) is plotted against time, the slope of the resulting curve is the rate constant k for the decay of single stranded DNA in acidic solution. The solid line is the linear regression; the dashed lines are the 95 % confidence band.

Table 2: Overview of the Rate Constants (k) and Half-Lives ($t_{1/2}$) of ssDNA Decay under Different Environmental Influences

Stability Assay	$R^{2\,a}$	k [d^{-1}]	$t_{1/2}$ [d]
80°C (wet)	0.97	9.07 ± 0.73	0.08 ± 0.01
80°C (dry)	0.87	0.06 ± 0.01	11.6 ± 1.98
80°C (hydroxyapatite)	0.96	3.42 ± 0.37	0.20 ± 0.02
pH2 (wet)	0.92	0.58 ± 0.10	1.20 ± 0.42
pH2 (wet, digestion[b])	0.93	1.74 ± 0.21	0.40 ± 0.10
UV (λ=254 nm)	0.87	14.35 ± 2.49	0.05 ± 0.01

[a]coefficient of determination.
[b]The stability assay in Theorell-Stenhagen-buffer (pH 2.0) was followed by digestion with Endonuclease V.

performed with dry ssDNA at 80°C and the amplification ratio shows likewise a time-dependent logarithmical decay (Figure **2c**) with a half-life of 11.6 ± 1.98 d (Table **2**). Compared to aqueous ssDNA, the half-life is raised 145-fold for dry ssDNA at 80°C. DNA-hydroxyapatite-complexes in water were prepared to enhance the stability of aqueous ssDNA (2/60) at 80°C and ssDNA decay was determined (Figure **2d**). In fact, hydroxyapatite increased the resulting half-life for aqueous ssDNA to 0.20 ± 0.02 d at 80°C (Table **2**), which is 2.5-fold higher than for aqueous ssDNA at 80°C without the stabilisation by hydroxyapatite.

UV Light

Also, the time-dependent total DNA damage induced by UV radiation (λ = 254 nm) was checked for ssDNA. The plotted negative logarithm of the amplification ratio for UV-induced DNA damage (Figure **2e**) is a linear graph with a rate constant of 14.35 ±

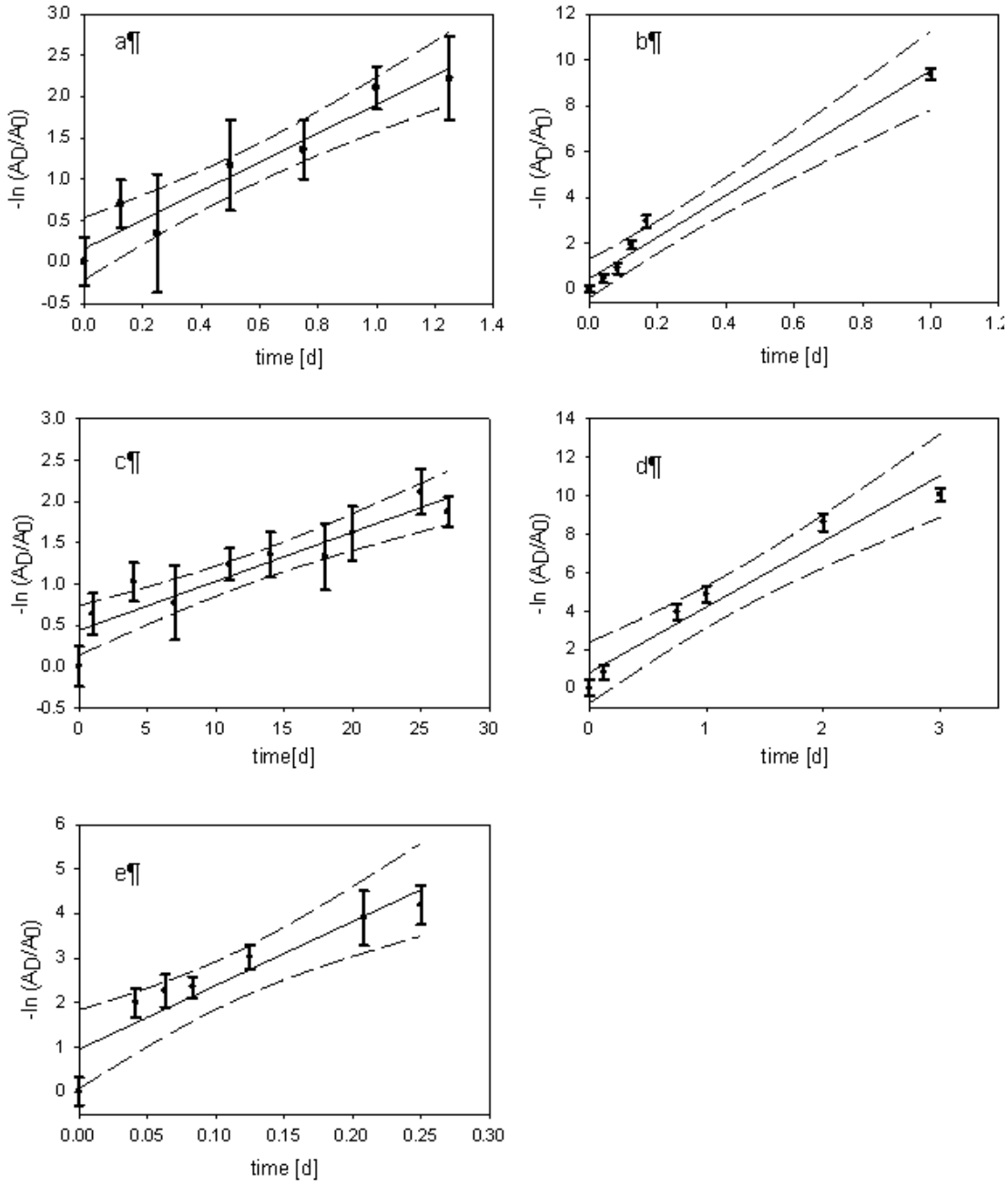

Figure 2: Determination of the rate constant for DNA decay under the following conditions: (**a**), aqueous ssDNA at pH2, followed by endonuclease V digestion, (**b**) aqueous ssDNA at 80°C, (**c**) dry ssDNA at 80°C, (**d**) ssDNA- hydroxyapatite-complexes in water at 80°C, (**e**) dry DNA under UV light (254 nm). Each point represents the mean value of three samples, measured in duplets, respectively. The solid line is the linear regression; the dashed lines are the 95 % confidence band.

2.49 d^{-1} (Table **2**). The associated half-life for the ssDNA decay induced by UV radiation (3.4 J/m^2 per sec) is 0.05 ± 0.01 d^{-1}. Thereby the stability of ssDNA under UV light of a common laboratory-type UV lamp is significant lower than the stability of ssDNA at 80°C in a wet environment.

DISCUSSION

pH 2.0

Acidic treatment of DNA leads to an incorporation of a labile positive charge by protonation of the organic base and to hydrolysis of the N-glycosylic bond (depurination) [23] and therefore to the formation of abasic sites. The rate of depurination increases with decreasing pH in a range of 4.5 to 6.0 at 70°C [24] and with increasing temperature [25]. Such acid-induced abasic sites involve strand breakage [1]. The average lifetime of an abasic site is 190h under *in vivo* conditions at 37°C [25] and 2000h in a neutral phosphate buffer at 37°C [26]. Whereas strand breaks act as absolute blocks during qPCR for the polymerase, abasic sites are known to retard strand elongation [3, 11-14]. Abasic sites block mainly strand elongation, but with a minor feasibility the polymerase shows a significant readthrough [3] for templates with abasic sites. This hindrance of the polymerase mainly concerns the initial cycles during qPCR, because only the synthesis of the first copy of the DNA strand with an abasic site is hindered. The result is a less “early cycle reaction efficiency” [15]. During such a translesional synthesis preferentially dATP is inserted opposite an abasic site, but also 1- or 2-base deletions are possible [3]. The efficiency of a translesional synthesis is dramatically reduced by polymerases with proofreading activity [3, 11]. Because the used *taq*-polymerase had no 3’,5’- exonuclease function, the abasic sites will either block the amplification or lead to translesional synthesis during qPCR. Therefore the amount of amplified DNA consists of (1) DNA strands amplified exponentially from undamaged templates, (2) DNA strands amplified with less efficiency in the initial cycles from templates with lesions e.g. abasic sites (mutagenesis) [14] and (3) unfinished DNA strands amplified linearly from templates with strand breaks or abasic sites. This incalculable influence of abasic sites on the progression of polymerases leads to high standard deviations of the mean amplification ratio in Figure **1a**. We investigated the influence of abasic sites on qPCR by repeating the pH-experiment followed by a digestion step. The half-life is three times faster for the decay with digestion than without digestion. During a pure acid-based ssDNA decay, various strands with abasic sites occur. Without digestion, these labile strands serve as amplicons for the *taq*-polymerase, which elongates them with low efficiency during the first cycles. Accordingly, only the amplification ratio curve based on a subsequent digestion step illustrates the true behaviour of DNA decay under acidic conditions [5]. But DNA lesions not only block or hinder the used polymerase during amplification. It must also be considered that primer binding is the more inefficient, the more lesions occur within the primer binding site and the DNA strand is possibly not available during PCR. And not only abasic sites occurred under acidic treatment of ssDNA, also e.g. hydrolytic deamination leads to modified bases, which may lead to mutations in an adjacent qPCR [1, 3, 14]. However, the results imply that *taq*-polymerase is adequate to investigate DNA damage by qPCR.

80°C, Wet

Purines and pyrimidines are released by heating the DNA in neutral aqueous solution [27]. During further progress of heating, the accumulated abasic sites result in strand breakages [25, 28]. Lindhal and Andersson [25] calculated an activation energy of 24 ± 1.5 kcal/mol for chain breakage at abasic sites in dsDNA in a buffer mimicing *in vivo* conditions (pH 7.4). Therefore, the formation of strand breakage at an abasic site depends strongly on temperature. In contrast to ssDNA at pH=2.0, at elevated temperatures abasic sites are more quickly transformed to strand breaks. Due to this fact, the time-dependent amplification ratio under elevated temperatures mostly depends on strand breakage, which is also reflected by the lower standard deviations (Figure **2b**). Interestingly, the amplifiable DNA at 80°C shows a more rapid decay than a first-order reaction (see Figure **2b**). A straight-line fit for a time period lower than 0.2 d results in a rate constant of 17.69 d^{-1} ($t_{1/2}$=0.04 d; R^2=0.96), which means a two time higher apparent half-life in contrast to the overall straight fit in Figure **2b**. The low coefficient of determination indicates that a straight-line fit might not be suitable. Such a more rapid decay could depend on different, not considered base modifications or DNA lesions, which influences the amplification efficiency of the first-copy strand during the initial cycles of qPCR.

During dry heat treatment (Figure **2c**), the water molecules are largely absent, and only ambient air moisture is available for hydrolytic formation of abasic sites and strand breaks. Thereby, the DNA decay at

80°C is explicitly retarded by decreasing the water content of the environment [1].

Lindhal [1] stated that hydroxyapatite can reduce the rate of depurination [1] and therefore, an adsorption of ssDNA to hydroxyapatite should reduce the total DNA damage under elevated temperatures. The measured half-life for ssDNA, which was attached to hydroxyapatite, indicates an enhancement of ssDNA stability at 80°C by complexation to hydroxyapatite. The phosphate groups of the DNA interact with the Ca^{2+}- ions on the mineral surface to form a reversible complex based on charge interaction [20]. A complexation to hydroxyapatite leads to a 2.5-fold higher half-life in contrast to the half-life of ssDNA at 80°C without the stabilisation by hydroxyapatite. The interaction of DNA with hydroxyapatite shields the points of attack of the DNA against hydrolysis, resulting in less DNA lesions and strand breaks. This is also discussed as reason for the persistence of DNA in ancient samples from Neandertals [29] or mammoths [30], where DNA is attached to the bone material hydroxyapatite [1].

UV Light

Yamauchi *et al*. [31] have shown that the DNA damage induced by irradiation and measured by PCR is a mononuclear reaction, because the measured level of strand breakage is independent from the starting amount of plasmid DNA. Therefore the calculated apparent half-life depends not on the initial DNA concentration. UV treatment of ssDNA basically lead to two forms of mutagenic DNA lesions: (1) cyclobutan-pyrimidine dimers (CPDs), formed by two adjacent pyrimidines, and (2) 6-4 photoproducts [2]. Both forms of DNA lesions abort the progression of *taq*-polymerase. Sikorsky *et al*. [14] showed that a thymine dimer reduces the efficiency of a real-time PCR amplification by 99.1 %. The *taq*-polymerase amplifies DNA molecules with photoproducts only linearly because the elongation of the DNA strand is aborted at such particular lesions [5]. UV irradiation leads to the fastest ssDNA decay, followed by ssDNA treatment at 80°C in the presence of water and acidic ssDNA incubation.

CONCLUSION

Acidic conditions of pH 2.0, elevated temperatures of 80°C at wet and dry conditions as well as ultraviolet radiation of a wavelength of 254 nm lead to a natural decay for single stranded DNA. The apparent half-life of all DNA degradations indicate that irradiation with ultraviolet radiation leads to the fastest ssDNA decay, followed by ssDNA treatment at 80°C in the presence of water. We can confirm the statement of Lindhal [1] that DNA damage induced by elevated temperatures is reduced by dry storage or by adsorption to hydroxyapatite. SsDNA is also damaged under acidic conditions. However, the measurement of total DNA damage induced by acidic conditions is only reasonable if a digestion step is included. The results show that unprotected ssDNA is not suitable for a leather marking system, unless extreme high DNA concentrations would be used to mark products. But this practice would lead to extreme high costs for such a marking system. The best choice for this marking system is to incorporate small amounts of DNA and to protect that DNA (e.g. hydroxyapatite) against damaging effects. Further investigations concerning the encapsulation of ssDNA and the embedding of ssDNA in a stabilising matrix are currently under way.

QPCR is a reliable method for measurements of total DNA damage. And knowledge about the formation of amplifiable DNA to non-amplifiable DNA is important for almost any DNA study based on qPCR. The calculation of the half-life is of particular importance not only in technical applications, where DNA is used as marking system, but also in forensic analytics, in research on living cells and cancer research. Because DNA lesions can reduce efficiency of qPCR [14], DNA damage can lead to misinterpretations also in diagnostics or during simple laboratory measurements.

Also the results are completely transferable to natural dsDNA. We assume the same qPCR behaviour for ssDNA and dsDNA, because during the initial melting step also dsDNA is melted to ssDNA for amplification. For this reason no ssDNA or dsDNA specific qPCR effects should appear during amplification in PCR. Also for dsDNA the same natural decay curves were produced in prior works [5, 18, 19] (without determining the rate constant). Thus our single stranded laboratory model can be adopted to the whole double stranded reality. Certainly, these findings are of great interest to all molecular biologists, who work with ancient or damaged DNA in PCR or qPCR, because determining the half-life leads to information about the quality and the amplifiability of DNA in PCR. Also the accumulation of total DNA damage under special environmental effects can be anticipated by knowing the particular half-life of DNA.

ACKNOWLEDGEMENTS

We thank Prof. Dr. rer. nat. Schlömann for scientific discussion and Dr. Martin Strangfeld for statistics.

Results have been gained within the IGF-project 16443BR of the Research Association Leather and Plastic Sheeting was funded *via* the AiF on behalf of the Bundesministerium für Wirtschaft und Technologie (Federal Ministry of Economics and Technology) based on a resolution of Deutscher Bundestag. We thank for the support granted.

ABBREVIATIONS

DNA = deoxyribonucleic acid

ds = double strand DNA

PCR = polymerase-chain-reaction

qPCR = real-time polymerase-chain-reaction

ss = single strand DNA

TSB = Theorell-Stenhagen-buffer

UV = ultraviolet

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Received on 04-02-2014 Accepted on 03-04-2014 Published on 11-04-2014

DOI: http://dx.doi.org/10.12970/2311-1755.2014.02.01.5