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PH- and salt-dependent molecular combing of DNA: experiments and phenomenological model

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Abstract
λ-DNA as well as plasmids can be successfully deposited by molecular combing on hydrophobic surfaces, for pH values ranging from 4 to 10. On polydimethylsiloxane (PDMS) substrates, the deposited DNA molecules are overstretched by about 60–100%. There is a significant influence of sodium ions (NaCl) on the surface density of the deposited DNA, with a maximum near to 100 mM NaCl for a DNA solution (28 ng μl⁻¹) at pH 8. The combing process can be described by a micromechanical model including: (i) the adsorption of free moving coiled DNA at the substrate; (ii) the stretching of the coiled DNA by the preceding meniscus; (iii) the relaxation of the deposited DNA to the final length. The sticky ends of λ-DNA cause an adhesion force in the range of about 400 pN which allows a stable overstretching of the DNA by the preceding meniscus. The exposing of hidden hydrophobic bonds of the overstretched DNA leads to a stable deposition on the hydrophobic substrate. The pH-dependent density of deposited DNA as well as the observed influence of sodium ions can be explained by their screening of the negatively charged DNA backbone and sticky ends, respectively. The final DNA length can be derived from a balance of the stored elastic energy of the overstretched molecules and the energy of adhesion.

1. Introduction

Molecular combing, first published in 1994 by Bensimon [1], is an efficient method for stretching, arranging and immobilizing long DNA molecules on various surfaces in a simple way. It has become the basis for a well defined handling of DNA in biophysics as well as bionanotechnology, for instance in the investigation of DNA replication of yeast chromosomes [2], manipulating single DNA molecules for studying DNA–protein interactions [3], site-specific deposition of DNA onto micro-patterned aminopolymer films [4], stretching DNA between lithographically patterned polystyrene lines on a substrate [5], assembling of two-dimensional DNA networks [6], DNA templated fabrication of metallic nanowire arrays [7, 8], and transfer printing of DNA [9]. Figure 1 demonstrates as an example a transferred DNA network on a glass substrate.

The principle of the molecular combing technique with an inclined substrate is described in figure 2. DNA molecules are deposited on a solid surface from a solution. In the process, the molecules change from the coiled conformation in the solution to the stretched conformation on the surface. They are oriented parallel to the direction of motion of a liquid front. The adsorption begins with the stochastic contact of one end of the molecule with the substrate. It is controlled by the experimental conditions, such as the pH value of the DNA solution [10, 11], the presence of additional cations in the solution [11], and the degree of hydrophobicity of the surface [5, 12]. In the following, the partially adsorbed molecules are stretched by the receding meniscus and are adsorbed in the stretched conformation.

The adsorption of DNA on a planar or curved surface has been studied extensively in the past. From the theoretical point of view, essential features of that highly complex problem can be considered as a particular case of the fundamental process of adsorption of a polyelectrolyte at a charged or neutral substrate. The large number of relevant system
parameters, such as the length and stiffness of the polymer, its linear charge distribution, the long-range Coulomb interaction, the influence of a salt concentration in the solvent, means that any modelling has to be restricted to some important aspects of a given experimental situation. Thus it is not surprising that a complete understanding of the problem is still missing. However, for a large class of processes with dominating electrostatic interaction there are already well elaborated models which facilitate the explanation of critical phenomena such as the adsorption–desorption transition and its dependence on the substrate curvature as well the Debye–Hückel screening length [13, 14].

In the case of molecular combing of DNA, additionally to the electrostatic interaction between coiled or stretched DNA molecules the hydrophobic interaction with the substrate plays a major role. Therefore the mentioned models can only help us to understand one part of the whole problem. The explanation of one key point of the experiment, the formation of a stable adhesive binding of the end of the DNA at the hydrophobic substrate, needs another approach. Experimentally, it has been shown that the DNA can be stretched to more than its full natural length [15, 16]. That means there should be a high adhesion force fixing the end of the molecule at the substrate. As has been pointed out by Allemand et al [10], for the adsorption of double stranded DNA molecules on hydrophobic surfaces a change of the equilibrium structure of the molecules in aqueous solution has to occur. Hidden hydrophobic regions should be exposed to the substrate by some external force. Alternatively, structural defects such as ‘sticky ends’ or denatured basepairs could offer such hydrophobic bonding sites. The stability of the double helix can be controlled by the pH value of the buffer. Outside of the physiological pH range (between 5 and 9) DNA bases are protonated or deprotonated, so that the hydrogen bonds are broken and the double strand is partially melted. Within the physiological pH range there is no protonation or deprotonation, so that only accidentally molten basepairs are possible. Additional cations in the solvent increase the stability of the double stranded molecules as they diminish the Coulomb interaction of the negatively charged backbone.

With an increasing salt content melting of DNA is hindered and the melting temperature increases [17]. This stabilization mechanism would result in a reduction of adsorbed molecules. In contrast, experimentally we observe an increasing of adsorption under the influence of added salt in a certain concentration range. Therefore it would be of interest to elucidate the underlying mechanism. It could be connected with the increasing screening of the negatively charged coiled DNA in solution. Positive ions in the solvent lead to a screening of the negative charges due to the formation of a diffusion controlled Debye layer as well as a short-range aggregation (Stern layer). The repulsion of the coils is diminished and the adsorption of the molecules can occur with growing density. However, such a mechanism cannot completely explain the behaviour because for high salt concentration the adsorption tendency is diminished.

Previous DNA adsorption experiments by Allemand et al demonstrated a high adsorption rate at hydrophobic surfaces (glass coated with vinyl silane, polystyrene) only in a small pH range near about 5.5, whereas on PMMA optimum combing has been observed between pH 6.5 and 7.5. The length of the DNA was larger at hydrophobic surfaces which confirmed the previous observation of Bensimon [12] that the final length

![Figure 1](image1.png)  
**Figure 1.** Fluorescence image (a) and atomic force microscopy (AFM) image (b) of a DNA network of λ-DNA aligned by the molecular combing technique on a polydimethylsiloxane (PDMS) stamp and transferred onto a glass surface.

![Figure 2](image2.png)  
**Figure 2.** Principle of molecular combing with an inclined substrate: a DNA molecule binds with one end to the substrate and is stretched by the receding meniscus.
of the stretched DNA is linked to the hydrophobicity of the substrate. It would be of major interest to find an option for efficient molecular combing of DNA in a wide range of processing conditions.

In the following we will report experiments of molecular combing of DNA on PDMS substrates. PDMS is an interesting material for transfer printing of DNA arrays to other substrates. The pH-dependent adsorption on PDMS surfaces has not been analysed previously. For intended technical applications of the transfer printing of DNA molecules, for instance in the transfer of bound nanoojcts, it is essential to vary the pH value of the buffer solution.

Here we show that a high adsorption rate of stretched DNA molecules can be achieved in a wide pH range (between 4 and 10) on the hydrophobic surface of PDMS. The ratio of adsorbed DNA additionally strongly depends on the concentration of sodium chloride in the solution. These experimental results can be explained by a phenomenological model.

2. Experimental details

2.1. Materials and other chemicals

Block-shaped substrates were made from PDMS (Sylgard 184 Silicone Elastomer Kit, Dow Corning). In order to obtain identical surfaces for all of the adsorption experiments, surfaces were used which were exposed to air during cross linking of the polymer. The DNA solution contained λ-DNA (48,502 basepairs, New England BioLabs) with a concentration of 28 ng μl⁻¹ and a staining rate of YOYO-1-iodide of 0.05 (one molecule YOYO-1-iodide per 20 basepairs). For different pH ranges the following buffers were used: pH 2.0–4.0: citrate buffer according to Sörensen; pH 4.5–6.2: 50 mM MES buffer; pH 6.9–9.0: 10 mM Tris/EDTA buffer; pH 9.7–10: 10 mM AMPSO buffer. To investigate the dependence of the number of adsorbed molecules on the concentration of sodium chloride dissolved in the Tris/EDTA buffer (10 mM/1 mM, pH 8), we used 1; 10; 100 and 1000 mM NaCl. For the adsorption experiments with plasmids the plasmid pHW 1014/2c (7219 basepairs, contour length of 1000 mM NaCl. For the adsorption experiments with plasmids which have not been cut in various concentrations (1; 10; 100 and 1000 mM NaCl). The addition of the salt causes an increase in the fraction of adsorbed molecules starting at a concentration of 10 mM NaCl. At 100 mM NaCl, the number of adsorbed molecules reaches a maximum, whereas at 1000 mM NaCl it was observed to decrease again. DNA molecules are stretched and overstretched independently of the concentration of sodium ions.

2.2. Spectroscopy

The analysis of the dependence of the extent of denaturation of the double strands on the pH value of the DNA solution was carried out with an ultraviolet/visible (UV/vis) spectrophotometer (Cary 50 BIO, Varian Deutschland GmbH, Darmstadt).

2.3. Molecular combing

Molecular combing was executed by the ‘method of a rolling drop’: PDMS substrates were tilted approximately 80° with respect to the horizontal position and one drop of DNA solution (25 μl) was deposited at the upper edge of the substrate. As a result of gravitational force, the drop rolled down the inclined plane and dripped into a cap.

2.4. Microscopy

The dry PDMS substrates with DNA molecules were put on cleaned coverslips and were analysed using an inverse fluorescence microscope (Axiovert 200 M, ZEISS) with 100 × 1.4 oil objective.

3. Results and discussion

3.1. Experimental results

3.1.1. pH-dependent DNA adsorption. Figure 3 shows the results of the molecular combing experiments. In contrast to published results [10], which report a high adsorption rate at hydrophobic surfaces only near a pH value of 5.5, it is demonstrated that optimal conditions for strong and simultaneous specific adsorption of DNA molecules are given in a wide range of the pH value (from 4 to 10).

After adsorption in a highly acidic buffer at pH 2 the molecules can be seen as bright spots. Because of the strong and unspecific adhesion, no stretching is possible and the molecules adsorb in coiled conformation. At pH 3 the direction of the meniscus propagation is visible. However, the adhesion is also strong so that the molecules cannot be stretched. At pH 4 the shape of the adsorbed molecules is changed: there is a significantly high portion of stretched molecules. Identical adhesion behaviour is detected in the pH range from 5 to 10. There is a strong end-specific adhesion and molecules are stretched and overstretched, some by 100% of their contour length. At pH 10 the proportion of adsorbed molecules decreases.

3.1.2. Plasmid adsorption. Plasmids which have not been cut form closed DNA loops without any positions of preferential denaturation, like sticky or blunt ends. Therefore, only randomly denatured basepairs are possible. As a typical result, figure 4 shows that the molecular combing of plasmids on PDMS surfaces is practicable. Most of the molecules (here 86%) adsorb as closed loops, and they are seen as short lines with high fluorescent intensity. Only a few molecules adsorb in linearized conformation and appear as longer lines with less fluorescent intensity (see: picture detail). Obviously, the molecules were broken before molecular combing took place which leads to end-specific binding.

3.1.3. The dependence of the DNA adsorption on ionic effects. Figure 5 shows the effect of sodium chloride on molecular combing of λ-DNA on PDMS. Sodium chloride has been dissolved in the Tris/EDTA buffers (10 mM/1 mM, pH 8) in various concentrations (1; 10; 100 and 1000 mM NaCl). The addition of the salt causes an increase in the fraction of adsorbed molecules starting at a concentration of 10 mM NaCl. At 100 mM NaCl, the number of adsorbed molecules reaches a maximum, whereas at 1000 mM NaCl it was observed to decrease again. DNA molecules are stretched and overstretched independently of the concentration of sodium ions.
3.2. Modelling

With the following model we would like to develop a phenomenological description of the main mechanisms which control the force acting on the DNA during adsorption on a hydrophobic surface and stretching under the influence of the moving liquid meniscus. In figure 6, the three process steps during molecular combing are shown: (i) the adsorption of free moving coiled DNA on the substrate, (ii) the stretching of the coiled DNA by the preceding meniscus, (iii) the relaxation of the deposited DNA to the final length \( l_f \). We consider the characteristic situation when the DNA is fixed with one end segment of length \( l \) at the substrate by an adhesion force \( F_{adh} \), whereas the main part of the molecule is still moving free. The preceding liquid acts with a meniscus force \( F_m \) on the DNA which causes a transition from a coiled conformation of the DNA in the solution to a stretched shape. The shape of the stretched DNA will be approximated by a straight cylinder with diameter \( D \cong 2\text{nm} \).

For a successfully working molecular combing process, the following relationship between the maximum adhesion force \( F_{adh,max} \), the meniscus force \( F_m \) and the entropic force for perpetuation of the coiled conformation \( F_c \) has to be fulfilled

\[
F_{adh,max} > F_m > F_c. \tag{1}
\]

3.2.1. Adhesion force \( F_{adh} \). As a good approximation we can assume that the force between the PDMS surface and the DNA during molecular combing is transferred by shear stress along the adhesion length \( l \). For a conservative estimate of the maximum adhesive force we assume that only half of the molecule surface is involved in the bonding. Then we get for the maximum adhesion force

\[
F_{adh,max} = \tau_{adh} \frac{\pi D}{2} \tag{2}
\]

where \( \tau_{adh} \) is the shear strength of the interface. Favoured sites for the adhesion on a hydrophobic surface are the sticky ends of the DNA. Under this assumption the shear strength can be estimated as follows (\( a = 0.34 \text{nm}, \) distance of two basepairs)

\[
\tau_{adh} \cong \frac{G_{adh}}{a} \tag{3}
\]

where \( G_{adh} \) is the specific adhesion energy at the PDMS–DNA interface. Experimentally we found that the 12 base sticky ends of the \( \lambda \)-DNA are the favoured adsorption sites. With a characteristic value for hydrophobic surfaces (PDMS: \( G_{adh} \approx 2 \times 10^{-2} \text{Nm}^{-1} \)) we get for the shear strength \( \tau_{adh} \) of an adsorbed DNA molecule on a PDMS surface the estimate

\[
\tau_{adh} = 0.6 \times 10^8 \text{N m}^{-2} = 60 \text{MPa}. \tag{4}
\]

Figure 5. Influence of the concentration of sodium chloride in the TRIS/EDTA buffer (pH 8) on the adsorption of λ-DNA molecules on PDMS ((a) without NaCl, (b) 1 mM, (c) 10 mM, (d) 100 mM, (e) 1000 mM NaCl). From a concentration of 10 mM NaCl upwards, the fraction of adsorbed molecules increases and passes a maximum value at 100 mM NaCl.

Figure 6. Sequence of process steps during molecular combing.

Due to the lower cross section of the sticky ends in the contact region, a lower force is transferred to the single strand. Thus in equation (2) the contact surface amounts to about $\pi l_{\text{sticky end}} D$ for sticky ends. This gives for the critical adhesion force of a sticky end

$$F_{\text{adh}}^{\text{sticky end}} \approx \tau_{\text{adh}} l_{\text{sticky end}} \frac{\pi D}{4} \approx 385 \text{ pN}.$$  \hspace{1cm} (5)

This value is significantly larger than the entropic force $F_c$ for stretching the DNA to a nearly straight molecule (a few piconewton). Therefore, any adsorbed λ-DNA will be stretched under the influence of the preceding meniscus. As we can conclude from equation (5), that will also be fulfilled for a minimum transfer length of one base distance $a$. It also explains the observation that very short DNA as plasmids can be oriented and stretched by molecular combing, as shown in figure 4.

We can assume that with increasing load $F_m$ the double stranded DNA undergoes a large conformational change. As it has been recently shown by molecular dynamics simulation, such overstretching can be connected with an unzipping transition [18] which leads to additional hydrophobic regions presented for the substrate surface.

In order to adsorb the DNA in a straight shape the length $l$ always has to be lower than the persistence length of the DNA ($\approx 50$ nm or 150 basepairs). However, with increasing hydrophilicity of the substrate resulting in a larger critical transfer length, the adsorption of randomly oriented DNA segments may be expected. As an alternative to hydrophobic interaction, single covalent binding sites at the substrate can also be used for stable loading. Already a single covalent binding site can transmit forces of some nanonewton [1] which would be enough for stable adsorption of the DNA.

3.2.2. Density of adsorbed DNA. The experiments have shown that there is a significant influence of the pH value on the density of combed DNA adsorbed on the hydrophobic PDMS surface. When we keep in mind that the basic mechanism of DNA adsorption is the directed motion of coiled DNA caused by the moving meniscus, then it is obvious that the distance of the immobilized DNA is governed by the minimum spacing of...
the coiled DNA near the edge of the meniscus (see figure 6). In the entire pH range from 4 to 10 we observe that a large number of molecules are adsorbed and stretched; however, there is a maximum of adsorbed molecules at pH 4. We observe that at pH 4 the minimum distance of adsorbed DNA is in the range of the diameter of a DNA coil which can be estimated as \( \sqrt{\frac{\text{DNA}}{\text{electrostatic force}}} \approx 0.9 \mu m \). That means that at this pH there is no additional Coulomb repulsion between the coils. At pH values smaller than four, the adhesion of coiled DNA is too strong to allow stretching of the molecules. There are at least two mechanisms which could be responsible for such behaviour. First, as noted already by Allemand et al [10], at low pH DNA bases undergo protonation. That protonation causes a destabilization of the DNA which is connected with exposing the hydrophobic core of the DNA, thus improving the sticking probability at the PDMS surface. The adsorption of coiled and denatured DNA at very low pH \( \text{pH} < 3.5 \) can be interpreted by such a mechanism, which can be supported by UV/vis spectroscopy (see appendix A.1). A second mechanism seems to be relevant at higher pH. Here we also observe combed DNA, but with lower surface density. This could be explained by an enhanced repulsion of the DNA coils in the solution. Outside of the physiological pH range in alkaline buffer DNA bases undergo deporation so that every time an additional negative charge is created [19]. Therefore the coils are more negatively charged at this pH range. The repulsion of charged molecules increases and the result is a lower density of adsorbed DNA.

Similarly, the influence of the addition of a salt can be explained. As we have seen in the experiment, the change of the concentration of sodium chloride in the TRIS/EDTA buffer (pH 8) is connected with a pronounced nonlinearity of the density of molecules adsorbed on PDMS. From a concentration of 10 mM NaCl upwards the fraction of adsorbed molecules increases and passes a maximum value at 100 mM NaCl. The increase of the adsorption with increasing salt concentration could be connected with the increasing screening of the negatively charged coiled DNA. But at higher concentration the sticky ends of the DNA will be completely covered with Na\(^+\) ions causing a decreased adhesion probability at the hydrophobic substrate. When we assume that the sticky end is \( n \) bases long, then the probability \( p_{\text{Na}^+} \) that all sites are occupied with one Na\(^+\) ion can be estimated as

\[
p_{\text{Na}^+} \cong \left( \frac{N_{\text{Na}^+}}{\pi a D^2} \right)^n \\
\cong \left( \frac{6.025 \times 10^{23}}{M} c_{\text{Na}^+} \exp \left( \frac{\Delta G_{\text{ads}}}{k_B T} \right) \frac{\pi a D^2}{4} \right)^n \tag{6}
\]

\[
p_{\text{Na}^+} \cong (6.99 c_{\text{Na}^+}/1 \text{ M})^n.
\]

The number \( N_{\text{Na}^+} \) of adsorbed Na\(^+\) ions per phosphate-ion site along the backbone (with the characteristic volume \( \pi a D^2/4 \)) is proportional to the bulk concentration of Na\(^+\) ions \( c_{\text{Na}^+} \) weighted with a factor describing the decrease of free enthalpy \( \Delta G_{\text{ads}} \) per Na\(^+\) ion adsorbed at the negatively charged backbone. A rough estimate of \( \Delta G_{\text{ads}} \) can be given by the Coulomb interaction between a phosphate ion and a sodium ion in a distance \( R \) with \( \Delta G_{\text{ads}} = \frac{e^2}{4\pi \varepsilon_0 R} \). Assuming for \( R \approx 0.3 \text{ nm} \) we get \( \Delta G_{\text{ads}}/k_B T \approx 2.3 \).

Depending on the number of bases \( n \) forming the sticky end we find a sharp increase of the probability between 0.08 M < \( c_{\text{Na}^+} < 0.14 \text{ M} \) which could explain the sharp decrease of the adhesion probability for \( c_{\text{Na}^+} > 100 \text{ mM} \) (see also figure 7).

### 3.2.3. Overstretching of the adsorbed DNA.

After stable binding of the end point of the DNA to the substrate, the preceding meniscus can exert a force \( F_m \) on the aligned DNA which scales with the surface tension of the water–air interface \( \gamma \equiv 7 \times 10^{-2} \text{ N m}^{-1} \) [1]. Thus the maximum force of the preceding meniscus is in the range of \( F_m \cong \gamma \pi D \cong 440 \text{ pN} \). Under such a force the DNA will be highly overstretched. Therefore, conformational transitions have to be assumed. As shown in single molecule experiments with piconewton force resolution of the force sensors, two transitions have been observed. In the range of 60 pN a highly cooperative transition of the natural B-DNA into the overstretched S-DNA occurs [12, 16, 20]. The transition to a ladder-like arrangement of the basepairs is connected with an extension of the natural contour length by about 70%. As shown by Rief et al [21] and Clausen-Schaumann et al [22] with further load increase the double helix melts into single strands which can be stressed without fracture up to 800 pN. With subsequent load relaxation a reannealing into the double helix can be observed. Whereas the cooperative B–S transition is not rate dependent, the melting and reannealing show a loading rate dependence with a characteristic transition rate of about 10–20 kb s\(^{-1}\). In the molecular combing process the velocity of the liquid meniscus is in the range of about 10 mm s\(^{-1}\); thus the single \( \lambda \)-DNA is influenced by the propagating meniscus for about 2 \times 10^{-3} \text{ s}. Therefore, the second melting transition seems to be not very likely for larger DNA sequences. Also, any hysteresis effect due to the rate dependent melting transition can be excluded.

![Figure 7. Probability \( p_{\text{Na}^+} \) that all sites of a sticky end are occupied with Na\(^+\) ions depending on the molar concentration \( c_{\text{Na}^+} \) in the solution. The sticky end is \( n \) bases long with \( n = 4, 8 \) and 12.](image-url)
for low adhesion energy a linear increase of the final relaxed length of the overstretched molecule equals the adhesion energy per unit length. Thus with the contour length of the stress-free straight DNA, whereas for a more hydrophilic substrate such as PDMS with $G_{\text{adh}} \approx 3 \times 10^{-2}$ N m$^{-1}$ the overstretched molecule relaxes to a final length of about $l_f \approx 0.95l_0$. As seen in figures 3 and 5 the experimentally observed length distribution of the combed molecules can be explained with an adhesive energy in the range between $1 \times 10^{-2}$ and $3 \times 10^{-2}$ N m$^{-1}$. In contrast, figure 9 demonstrates the fewer stretches and the lower molecule length on a hydrophilic substrate. Before the molecular combing process was performed, the PDMS surface was treated with plasma (air) so that the contact angle was about $30^\circ$. The molecules were not overstretched but stretched to a length of maximal $l_f \approx l_0$.

4. Conclusion

$\lambda$-DNA as well as plasmids can be successfully deposited by molecular combing at the hydrophobic surface of PDMS for a wide range of the pH value (from 4 to 10). The variation of the pH allows the control of the surface density of the deposited DNA. Maximum density is observed at pH 4 which can be explained by minimum Coulomb repulsion of the coiled DNA in the solution. In the range between pH 5.5 and pH 8 the change of surface density shows a plateau-like behaviour. The exposing of hidden hydrophobic bonds of the overstretched DNA is the fundamental process leading to a stable deposition on the hydrophobic substrate. Alternatively, the presence of single covalent bonds could also fulfill the necessary condition for a stable combing process. Adding monovalent cations (sodium) to the solution can be used as an additional mechanism to control the surface density of deposited DNA. This mechanism is caused by screening of the negatively charged DNA backbone and sticky ends, respectively. As the final length of the overstretched molecules is governed by the balance of stored elastic energy in the DNA and the work of adhesion it should be possible to control the final length by tailoring the specific adhesion energy $G_{\text{adh}}$.

Acknowledgments

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of glycosidic linkages between bases and sugar moieties [24].

In comparison to the absorption in physiological range (pH 8), the typical absorption maximum at 260 nm increases at pH 4 and 10 due to protonation or deprotonation of the bases (see figure A.1). At very low pH values 1 and 2 absorption decreases clearly, and expanded maxima are shifted to higher wavelengths. This effect shows a disappearance of the bases as a result of protonations, which cause dissociations of glycosidic linkages between bases and sugar moieties [24].

Appendix A

A.1. UV/vis spectroscopy of pH-dependent denaturation of λ-DNA

In comparison to the absorption in physiological range (pH 8), the typical absorption maximum at 260 nm increases at pH 4 and 10 due to protonation or deprotonation of the bases (see figure A.1). At very low pH values 1 and 2 absorption decreases clearly, and expanded maxima are shifted to higher wavelengths. This effect shows a disappearance of the bases as a result of protonations, which cause dissociations of glycosidic linkages between bases and sugar moieties [24].

Figure A.1. Absorption of λ-DNA molecules at various pH values shows partial melting of the double strands at pH 4 and pH 10 and disappearance of the bases at very low pH values.

Appendix B

B.1. Approximate calculation of the relaxed final DNA length

For the evaluation of equation (7) the nonlinear dependence of stretching force \( F_{DNA}(l) \) on the helix length is approximated by a step-wise linear dependence neglecting the very small contribution of the entropic force (see figure B.1).

Following experimental data as given in [25], we make a simplifying ansatz

\[
F_{DNA}(l) = \frac{340}{3} pN(l/l_0 - 14/17)(\Phi(l/l_0 - 14/17) - \Phi(l/l_0 - 1) + (20 + 500(l/l_0 - 1)) pN \\
\times (\Phi(l/l_0 - 1) - \Phi(l/l_0 - 1.1)) + 70 pN \\
\times (\Phi(l/l_0 - 1.1) - \Phi(l/l_0 - 1.7)) + (70 + 1600 \\
\times (l/l_0 - 1.7)) pN\Phi(l/l_0 - 1.7).
\]  

(B.1)

Here \( l_0 \) is the length of the stress-free straight helix (\( l_0 = 16.2 \mu m \)), and \( \Phi(x) \) is the Heaviside step function. With this approximation the integral in equation (7) can be solved analytically. From that we get an explicit solution for the relaxed final length depending on the adhesion energy \( \gamma = f(G_{adh}) \), as presented in figure 8.

Figure B.1. Approximate force–displacement diagram for DNA under tensile load.

References