Determination of the Thermodynamic Parameters of DNA Double Helix Unwinding with the Help of Mechanical Methods

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Supporting Information

ABSTRACT: For the first time, rupture event scanning (REVS) procedure based on quartz crystal microbalance (QCM) and involving only mechanical action was used to determine the height of the energy barrier for dsDNA unwinding. Melting point was determined with the help of this procedure. To determine the thermodynamic parameters including enthalpy, DNA denaturation was represented as a unimolecular process. This allowed us to recover the energy profiles from the experimental data obtained by force measurements at different scanning times (reaction times) for different temperatures. The thus obtained results were compared with the data obtained with the help of another mechanical method, namely, atomic force microscopy. The mechanism of DNA unwinding in QCM-based experiments through the unzipping mode, as proposed by us in previous works, was confirmed. Thus, we demonstrated that REVS procedure may be used to assess the thermodynamic parameters of dsDNA unwinding.

INTRODUCTION

Intermolecular forces holding the DNA helices are of key importance for biological processes. For example, they provide the response of signaling system or immune reaction. The bonds underlying these processes not only play decisive part in binding but also form a stable natural molecular structure. The intramolecular stability is a compulsory condition for functioning, so there should be a substantial energy barrier separating the natural and denaturated states. How high that barrier should be? Only measurements can give an exact answer. To carry out the measurements of this kind, one needs rather sensitive method that would not introduce distortions. DNA denaturation through helix unwinding (a transition from dsDNA to ssDNA) may be carried out either by rising temperature or using mechanical methods (apart from numerous chemical methods involving the addition of various reagents, which we will not consider in the present work).

Mechanical separation of DNA double helices allows obtaining the information on how DNA replication in vivo takes place. DNA replication in living organisms is a very complicated process that involves specialized proteins. At present, several methods are mainly used for mechanical unwinding; these methods allowing one to determine the forces of noncovalent bonding are optical or magnetic tweezers or atomic force microscopy (AFM).1–9 Biomembrane force probing is also used for this purpose.10

For instance, it was demonstrated with the help of AFM that thermal oscillations contribute to the separation process; the probability for the system to stay in the bound state decreases with temperature rise.11 Because of this, bond lifetime depends on the temperature; under mechanical action (stretching), the dependence on the time of force action is also observed. With an increase in stretching rate, the contribution from thermal oscillations decreases, and definite force values are to be obtained. Theoretical works describing DNA unwinding under the action of a constant force imply the occurrence of thermal equilibrium and predict helix unwinding to be a phase transition of the first kind, for which a minimal external force is necessary.12–16 This force decreases with an increase in temperature and is equal to zero in the thermal point of denaturation.

In the present work, we continue to develop the procedure proposed by us previously.17–19 It was demonstrated that unwinding of the DNA double helix may be carried out with the help of quartz crystal microbalance (QCM). In particular, temperature dependence was studied previously.20 In the present work, we made an attempt to determine the height of the energy barrier. To compare our data with the data available from literature and to verify our approach, we carried out parallel AFM-based measurements with our oligonucleotides. This comparison confirmed once more that unwinding proceeds according to the mechanism proposed by us in our previous work.19

The schemes of measurement for both AFM- and QCM-based procedures are shown in Figure 1. One can see that the QCM-based procedure is much easier in arrangement; in particular, the measurement of melting point is much easier due to the availability of the additional heater. Moreover, it is known that the QCM-based method can be applied for the measurement of the interaction between the sample and the immobilized molecule, which is not possible for experiments the AFM. For example, recent work demonstrated that QCM allows for the determination of the association constant between a single-stranded DNA probe and a single-stranded DNA target.21
surface. A more detailed description and comparison of the methods can be found in the review.\textsuperscript{18}

It should be kept in mind that AFM-based procedure allows measurements in two modes (shear and unzipping), whereas the QCM-based one works only in the unzipping mode.

In our work, to study the temperature dependence of double helix unwinding, we used both methods (AFM- and QCM-based), and compared the data obtained. Unlike for AFM, the QCM-based procedure allows one to obtain a result averaged over an ensemble of particles in a single measurement, so it is not necessary to carry out several hundred measurements. Another essential advantage is that in QCM-based procedure, the degrees of freedom are not limited, unlike for the case of AFM when a cantilever is fixed to a molecule.

In the present work, we used QCM for the first time to determine the height of the energy barrier for dsDNA unwinding. A comparison with the AFM data shows that the QCM-based approach may be used to assess the thermodynamic data.

\section{EXPERIMENTAL SECTION}

Model oligonucleotides specially synthesized with the help of the automatic solid-phase method from commercially available precursors were used in the work. The formulas of the nucleotides are shown below.\textsuperscript{19}

M2: 5’-NH$_2$-(CH$_2$)$_6$-GATCATAGCTTCGAAAGATC-3’
N2-1: 5’-GATCTTTTCGAAGCTATGATC-3’
N2-3: 5’-GACCTTTCGAAGCTATGATC-3’
N2-4: 5’-GATCTTTTCGAAGCTATGATC-3’

M2 was fixed on the QCM surface, N2-1 is fully complementary to oligonucleotide M2, whereas N2-3 and N2-4 have mismatches (marked). The concentration of oligonucleotides fixed on the surface (M2) was chosen to eliminate the mutual effect of the oligonucleotides on each other. Relying on the size of the molecules, we determined that the proper concentration of M2 on the surface should not exceed 1%. Oligonucleotides were deposited using the procedure described elsewhere.\textsuperscript{19}

\textbf{Measurements with the Help of QCM.} The AT-cut quartz plates with the resonance frequency of 14.3 MHz were used in the work.

The scheme of the setup is shown in Figure 1. Harmonic voltage $U_0$ is smoothly increased from 0 to 7 V; the maximal voltage (7 V) was chosen on the basis of the results of previous experiments.\textsuperscript{19} Only helix unwinding occurs within this voltage range. The time of voltage scanning was varied from 1 to 300 s.

With an increase in voltage, the amplitude of shear oscillations of the QCM surface increases, and unwinding of the double helix occurs. At the moment of unwinding, a characteristic signal appears, which is taken from the filter adjusted to the third harmonic within a narrow band of 5 kHz. The signal is then passed through an additional amplifier to the input of a device playing the role of spectrum analyzer, connected to a PC through the USB port. The value of this signal gives the information about the number of broken bonds, whereas the position over the voltage gives the value of detachment force, or unwinding force in the case under consideration (for more details, see the Supporting Information).

\textbf{Measurements by Means of Atomic Force Spectroscopy.} The force curves were recorded with the help of atomic force microscope MultiMode 8 AFM (Bruker). All of the measurements were carried out in the aqueous medium in the presence of phosphate-buffered saline solution (10 mM, pH 7.4) as the buffer agent. Data were analyzed using the Force Reader program.

The force of bond rupture was measured with the help of AFM for two modes: shear and unzipping (see Figure 2). The force value determined with the help of QCM corresponds to the unzipping mode, which is in good agreement with the model proposed previously.\textsuperscript{19}

\section{RESULTS AND DISCUSSION}

In our experiments, temperature was varied from 15 to 50 °C. Temperature was not risen above this value because it was impossible to determine the moment of rupture at a higher temperature. A more detailed description and comparison of the methods can be found in the review.\textsuperscript{18}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schemes of measurements by means of AFM (a) and QCM (b). The directions of force application for the unzipping mode (c) and the shear mode (d) are shown. Schematic of helix unwinding in the case of QCM is shown (e).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Distribution of probabilities plotted on the basis of informative force curves selected from 1000 cycles of cantilever advance retract. The data were approximated with Gaussian function. Curves for two modes are shown: ○, shear mode; □, unzipping. Force value obtained for unzipping corresponds to the value determined with the help of QCM. Measurements were carried out in a buffer solution at $T = 30$ °C. Loading rate: 500 nm s$^{-1}$.}
\end{figure}
temperature. The dependencies of rupture forces on temperature for two methods, AFM and QCM based, are shown in Figure 3. Melting temperature was determined by extrapolation to the point where the rupture force is equal to zero. Thus, the obtained value for a completely complementary pair of oligonucleotides is 57°C. The melting temperature ($T_m$) determined with the help of UV melting was 58.4 ± 0.1°C. In the case when mismatches were present (M2/N2-3, M2/N2-4), the melting temperature was 53 and 51°C, respectively, whereas the values determined with the help of UV melting were 53.8 ± 0.1 and 52.8 ± 0.1°C, respectively.

The rupture force depends on the scanning rate. The reciprocal value to scanning rate in the QCM-based procedure is an equivalent of the loading rate in the AFM-based procedure. In our case, scanning time is the time interval within which the amplitude of voltage supplied to the QCM increases from 0 to 7 V. For long time intervals, saturation occurs, and the probability of rupture tends to a definite value depending on temperature, that is, rupture occurs practically at the same voltage. So, we see two regions: for short scanning time, the contribution from unwinding due to thermal oscillations decreases, and unwinding occurs at a larger amplitude of surface oscillations. With an increase in temperature, the role of thermal oscillations increases. The dependence of the rupture force on the loading rate and scanning rate is shown in Figure 4. The load value for the fixed rate may be obtained as a product of the rate and the cantilever stiffness coefficient. The range of loading rates in which force measurements are usually carried out is 1−100 nN/s. For QCM-based studies, we proposed to use the scanning rate (the rate of an increase in the amplitude of alternating voltage supplied to the QCM during measurement). It was demonstrated experimentally that the rupture force depends on scanning time. Scanning time is the time interval during which the amplitude of voltage supplied to the QCM changes from 0 to the maximal value. For scanning time shorter than 15 s, we observe a decrease in rupture force with an increase in scanning time. The rupture force becomes constant for scanning time longer than 15 s. The linear fitting for the curves shown in Figure 4a is presented in the Supporting Information as Figure S1. All of the data obtained with the help of QCM were processed using MatLab software. The data presented in Figure 4b relating to two temperature points are shown as an example explaining how calculations were carried out. Actually, six temperature points were involved, each point included 7−10 measurements.

Below we will consider the denaturation process, or unwinding of the DNA double helix, as a unimolecular reaction (see Figure 5):


The reaction proceeds in the presence of an external force. In the general case, reaction rate is equal to

\[ w = k_o \exp(\Delta E/k_B T) \]

where \( k_o \) is the pre-exponential factor, \( \Delta E \) is the activation energy (depending on the applied force as shown below), \( k_B \) is Boltzmann constant, and \( T \) is the temperature.

So, if we know the geometry of the molecule and the rupture force at a given temperature, it is possible to determine the dissociation energy \( \Delta E_{0} \).

Considering this unimolecular reaction, we will adhere to the idea developed for AFM. Bell was the first to postulate a linear decrease in dissociation energy \( \Delta E_{0} \) under the action of the exerted force \( F \)

\[ \Delta E = \Delta E_{0} - Fx \]  

where \( x \) is displacement. A linear increase in the force of bond rupture depending on the logarithm of loading rate was reported. A similar behavior was demonstrated for protein unwinding. This is a direct consequence of the exponential decrease in the lifetime of a bond to which the force is applied.

Bond lifetime is

\[ \tau(F) = \tau_o \exp(\Delta E/k_B T) \]

where \( \tau_o \) is the frequency pre-exponential factor and \( k_B T \) is the thermal energy.

Bond length \( x \) depicts the reaction coordinate. This value is interpreted as a distance between the ground state and the energy barrier along the reaction route. The reaction will follow the route corresponding to the lowest energy barrier. The rate constant for the thermal contribution into dissociation \( K_{\text{off}}(F) \) for the bond to which the external force is applied may be easily estimated from eqs 1 and 2

\[ K_{\text{off}}(F) = K_{\text{off}} \exp(F/F^o), \quad F^o = k_B T/x \]

Bond lifetime is an essential parameter to describe the kinetics of bond rupture. The value directly measured in the experiment, both with AFM and with QCM, is the rupture force. Because rupture is a random process, we cannot expect to measure only one specific rupture force. We observe a value averaged over many bonds. As shown in Figure 4, bond rupture depends on the scanning time. Lifetime \( (t) \) for a given temperature \( (T) \) can be determined by scaling at the zero rupture force from the dependence of rupture force on the reciprocal scanning time (see Figure 4b), from which we obtain the dependence \( \ln t \) on \( 1/T \).

The enthalpy of activation for dissociation can be determined from the slope of \( \ln t \) versus \( 1/T \), which gives 90 ± 15 kcal/mol. Although only an estimated value, it is in good agreement with the data obtained by other authors using AFM- and NMR-based procedures.

**CONCLUSIONS**

Temperature stability and determination of thermodynamic characteristics of oligonucleotides were assessed through the direct measurement of the force of unwinding of a complementary pair of oligonucleotides. For the direct measurement of rupture force, AFM and QCM in the active mode were used. Smoothly increasing the alternating voltage at the QCM, we thus increase the amplitude of its surface oscillations and detect the amplitude at which the rupture occurs. The sensitivity of these procedures allows their reliable application to study DNA stability.

The QCM-based procedure has several advantages over the AFM-based procedure, which is widely used for these measurements. First, a QCM measurement is carried out not over a single object but over many objects at once, and we obtain an averaged result in one measurement. Second, sample preparation is much simpler for QCM than for AFM because immobilization is to be carried out only at the substrate in the QCM-based procedure, whereas for AFM, it is necessary to carry out immobilization on the cantilever and search for a complementary oligonucleotide on the substrate.

Within the QCM-based procedure, the melting point of oligonucleotides is determined from the temperature dependence; this value is in good agreement with the data obtained by means of UV melting.

The enthalpy of activation is determined from the dependence of the force on scanning time and is in good agreement with the values obtained using other methods.

The presence of mismatches causes destabilization of the complex, and unwinding force decreases. The most substantial decrease in the force is observed when mismatches are present at the outer end of oligonucleotide, which serves as a good confirmation of the fact that unwinding starts from the outer end (unzipping). The numerical value of unwinding force determined with the help of QCM-based procedure is 30–40 pN, which is in agreement with the data obtained by means of AFM for unzipping mode.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01815.

QCM-based method of rupture force measurement; determination of rupture force in a liquid medium; linear fitting of experimental data (PDF)

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The authors declare no competing financial interest.

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