Dynamics of FEL-Light-Induced Changes in Thin DNA Films Observed by Brewster Angle Microscopy

M. SCZEPAN, G. FURLINSKI, D. WOHLFARTH, W. SEIDEL, K. FAHMY

Abstract

The function of biopolymers is determined by their structural dynamics. Investigation of the structural dynamics is thus important for understanding the processes and superstructures involved in the function of biomolecules. A large number of superstructures is particularly important for DNA function. Our experiments aim at the induction and kinetic analysis of the IR-induced transitions between such states.

In order to observe changes induced in thin DNA films by FEL irradiation, Brewster angle microscopy, which is a proven technique for the evaluation of thin organic films [1] has been employed. If a sample is observed under Brewster angle conditions, small changes in the refractive index of the surface can be detected and thus thin layers on the surface of a substrate or liquid can be made visible. Small changes in the refractive index and thickness of a thin film as a result of structural transitions will induce changes in the reflectivity of the film.

Results and Discussion

Experiments have been performed using thin layers of DNA (pUC21 plasmid DNA, PlasmidFactory) on ZnSe and CaF_2 substrates. The thickness of the layers has been estimated to be 50-800 nm by interference fringe counting in combination with absorbance measurements. These samples were irradiated with FEL light at different wavelengths. Fast changes of reflectivity - following the temporal structure of the FEL light intensity which are followed by slow relaxation processes ($\approx$ 30 ms) were observed (see Fig. 1). Experiments to determine the influence of the micropulse energy (20-100 nJ), film thickness, macro bunch length (200-1200 μs) and humidity have been performed and revealed a strong influence of these parameters on amplitudes and time constants of the reflectivity variations.

Analysis of the data for films of different thicknesses - especially for films with thicknesses between 50 nm and 150 nm - reveal that changes in the reflectivity on the longer time scale are the result of a decrease in the refractive index of the sample while the fast ($< 30\,\mu$s) changes can be attributed to variations (increase followed by decrease) of film thickness due to heating. A model for the heat transport in the sample has been established, allowing the estimation of the temperature reached in the sample as a function of film thickness and pump power. Estimated temperature jumps were found to be in the 50-500 K range with the equilibrium temperature established within less than 50 μs. For high film thicknesses and pump intensities resulting in high film temperatures, a more complex behaviour of the reflectivity was observed. In this case, thickness changes - probably as a result of variations in sample hydration - seem to contribute to the slow changes in reflectivity. The changes in the refractive index of the sample can be the result of structural changes (base pair stacking, strand opening [2]) as well as of variations in the hydration state of the DNA [3]. In summary, we have shown that FEL-pulses can induce rapid DNA melting, allowing to observe base pair separation and annealing with a time resolution not accessible by conventional experiments on DNA-structural transitions.

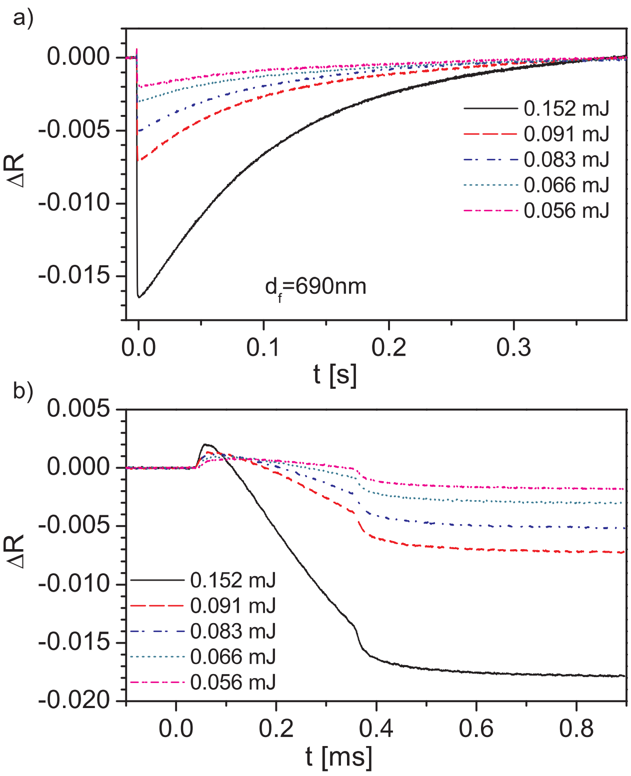

Fig. 1: *Change of reflectivity of a DNA film (thickness 690 nm) during and past FEL irradiation in long (a) and short (b) time scale as a function of light intensity/macro bunch energy.*

[1] S. Hénon, J. Meunier, Rev. Sci. Instr. 62 (1991) 936

[2] S. Elhadj, G. Singh, R. Saraf, Langmuir 20 (2004) 5539

[3] S. Weidlich, S.M. Lindsay, A. Rupprecht, Biopolymers 26 (1987) 439