Determination of the number of proteins bound non-specifically to DNA

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***Short Abstract* —We have determined the change in the number of proteins bound non-specifically to DNA as a function of applied force on tethered DNA. Using magnetic tweezers, single molecules of λ DNA were stretched in the absence and presence of λ repressor protein (CI). The force versus extension data was analyzed using a model for quantitative determination of protein binding. The results indicate that the non-specific binding of CI changes the force-extension relation significantly in comparison to that of naked DNA. We demonstrate that the force-extension relation provides an effective approach for estimating the number of proteins bound non-specifically to DNA.**

***Keywords* — non-specific binding, CI protein, repressor, DNA.**

Protein binding to DNA plays a fundamental role in many cellular and viral functions, including gene expression, physical chromosome organization, chromosome replication, and genetic recombination [1, 2, 3]. Therefore, quantitative approaches with which to elucidate the physics of protein-DNA binding could provide for a deeper understanding of cellular function. Many transcriptional regulators are known to bind to DNA both specifically and non-specifically, thus non-specific binding may play a biological role. While specific DNA binding is easily characterized with a variety of established biochemical techniques, such as gel electrophoresis, which use short DNA sequences, non-specific binding is difficult to characterize.

Recently, Zhang and Marko [4] proposed a thermodynamic approach, which may be used to estimate the number of non-specifically bound proteins from extension versus force measurements. In a recently published work [5], we have determined the change in the number of λ repressor proteins bound to a 16 kbp-long fragment of λ DNA by applying the Zhang-Marko approach [4] to our experimental data. Using magnetic tweezers, single molecules of λ DNA were repeatedly stretched and relaxed in the absence and presence of 170 nM λ repressor protein (CI). CI binds to six specific sites of λ DNA with nanomolar affinity and also binds non-specifically with micromolar affinity . The results indicate that the non-specific binding of CI changes the force-extension relation significantly in comparison to that of naked DNA. The DNA tether used in our experiment would have about 640 bound repressors, if it was completely saturated with bound proteins. We find that as the pulling force on DNA is reduced from 4.81 pN to 0.13 pN, approximately 138 proteins bind to DNA, which is about 22% of the length of the tethered DNA. Our results show that 0.13 pN is not low enough to cause saturation of DNA by repressor and 4.81 pN is also not high enough to eliminate all the repressors bound to DNA. This demonstrates that the force-extension relation provides an effective approach for estimating the number of proteins bound non-specifically to a DNA molecule.

A more detailed study is in progress using different protein concentrations which will allow determination of the protein binding affinity, of the absolute number of proteins bound. Indeed, a detailed and quantitative characterization of non-specific protein binding to DNA is essential to the understanding of this extremely important physiochemical phenomenon which is involved in many (in time, we may find all) DNA transactions and may be a significant part of most regulatory mechanisms based on long-range protein-protein interactions on the genomic scaffold.

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