Characterization of a Protein - Nucleosome Interaction

Application Note NT017

The Decondensation factor 31 binds to mono-nucleosomes

Dr. Thomas Schubert

Lab of Prof. Gernot Längst, University of Regensburg in cooperation with **2bind GmbH**, Germany

Abstract

Chromatin, the packaging form of the genome possesses two fundamental functions. On the one hand it compacts the DNA to fit into the nucleus and on the other hand it allows access to the underlying sequences for essential DNA dependent processes. Nucleosomes represent the basic structural component of chromatin. Here we characterize the specific interaction of the Decondensation factor 31 (Df31) with mono-nucleosomes. Df31 was recently shown to form a complex with snoRNAs and chromatin, to generate accessible higher order structures of chromatin.

Introduction

Chromatin is the combination of DNA and proteins that make up the contents of the nucleus of a eukaryotic cell. Chromatin compacts the DNA, so it fits into the cell nucleus, helps to prevent DNA damage, and to control gene expression and other DNA dependent processes. The primary protein components of chromatin are histones that associate with DNA in 1.65 helical turns, forming an octamer of the histone proteins H2A, H2B, H3 and H4.

The resulting structure, the nucleosome is the basic structural component of chromatin (van-Holde, 1989). Although the nucleosome is a very stable protein-DNA complex, it is not static and has been shown to undergo a number of different structural re-arrangements including nucleosome sliding and DNA site exposure (Langst and Becker, 2001).

In addition the nucleosome represents a binding partner for various proteins and serves as scaffold to establish multi-protein complexes at chromatin.

In a recent study the *Drosophila* Decondensation factor 31 (Df31) was shown to be involved in regulating chromatin compaction and to be tethered to chromatin (Schubert *et al*., 2012). To identify the interaction partner of Df31 within the nucleosome, MicroScale Thermophoresis analyses were performed.

Results

In this application note, the binding behavior of Df31 to salt assembled mono-nucleosomes was evaluated, employing MicroScale Thermophoresis.

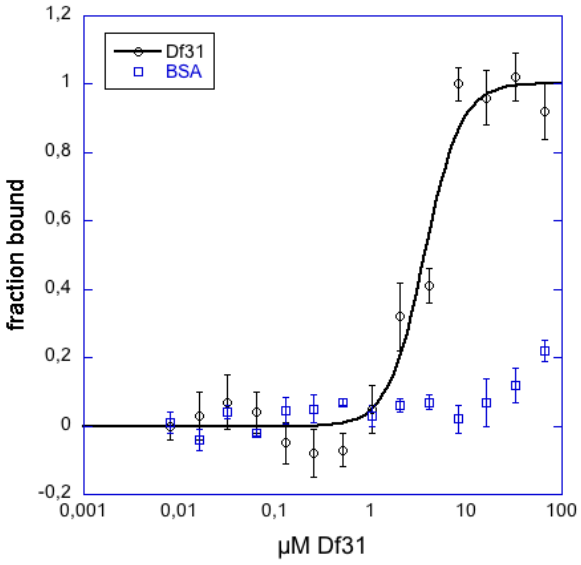

Fig. 1 Measurement of Df31 binding to Cy5-labeled mono-nucleosomes. MST values were normalized to fraction bound. 3 independent measurements where performed to obtain the depicted binding curve. The K_d of the Df31-mono-nucleosome interaction was 3.72 ± 0.56 µM.

Mono-nucleosomes were assembled on the Cy5-labeled 601 positioning sequence according to Rhodes and Laskey, and used at a concentration of 0.13 µM in the MST experiments (Rhodes and Laskey, 1989).

To this a serial dilution of the Df31 protein was added with starting concentration at 65 µM. The samples were filled into standard capillaries and incubated at 27 °C for 15 min, prior to the MST measurement.
For the assay three independent measurements were performed. A clear binding curve for the Df31 protein with mono-nucleosomes could be detected. However, BSA as control protein showed no binding to mono-nucleosomes.

The calculated K_d from the measurements of the Df31 binding to mono-nucleosome was 3.72 ± 0.56 µM. As control we also measured the interaction of BSA with Cy5-labeled mono-nucleosomes. The control experiment was performed as described for the Df31. No interaction could be detected.

Conclusion

The study provides an example that MicroScale Thermophoresis is capable of measuring and detecting specific interactions between nucleosomes and their interacting proteins. Straightforward control experiments proof the specificity of the interaction. Experiments are easily setup and affinities can be determined in a timely manner.

Material and Methods

Assay conditions

For the experiment Cy5-labeled mono-nucleosomes were used at the concentration of 0.13 µM.
Unlabeled Df31 and BSA proteins were added in 1:1 dilutions beginning at 65 µM. Samples were prepared in a buffer containing 20 mM Tris-HCl pH 7.4, 1.5 mM $MgCl_2$, 0.5 mM EGTA, 200 mM KCl, 10 % Glycerol and 0.1 (v/v) % NP-40. For the measurement the samples were filled into standard capillaries.

Instrumentation

The measurements were performed on a NanoTemper Monolith NT.115 instrument.
The measurement was performed in standard capillaries at 30 % LED and 50 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec.

References

Langst, G., and Becker, P. B. (2001). ISWI induces nucleosome sliding on nicked DNA. Mol Cell *8*, 1085-1092.

Rhodes, D., and Laskey, R. A. (1989). Assembly of nucleosomes and chromatin in vitro. Methods Enzymol *170*, 575-585.

Schubert, T., Pusch, M. C., Diermeier, S., Benes, V., Kremmer, E., Imhof, A., and Langst, G. (2012). Df31 Protein and snoRNAs Maintain Accessible Higher-Order Structures of Chromatin. Mol Cell.

van-Holde, K. E. (1989). Chromatin. Springer Verlag, 497.