Characterization of a Protein - Histone Interaction

Application Note NT-MO-015

The Decondensation factor 31 specifically interacts with histones H3 and H4 but not H2A and H2B

Dr. Thomas Schubert

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

Abstract

Histone proteins are associated with DNA and form a nucleoprotein structure termed chromatin. Eukaryotic genomes are packed into chromatin for storage and transport of the DNA. To regulate access to DNA for various essential processes such as transcription and translation, histone modifying, DNA modifying and chromatin binding proteins specifically interact with chromatin structures. Here we characterize the specific interaction of the Decondensation factor 31 (Df31) with core histones. 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). The histones within nucleosomes serve as docking platforms for modifying enzymes and other proteins. The modification of chromatin structure is essential for its function (Luger and Richmond, 1998; Wolffe, 1997). Chromatin modifications determine the degree of chromatin compaction and were shown to be associated with the differential regulation of the genomic target sites.

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 the recombinant core histones H2A, H2B, H3 and H4 was evaluated, using MicroScale Thermophoresis.

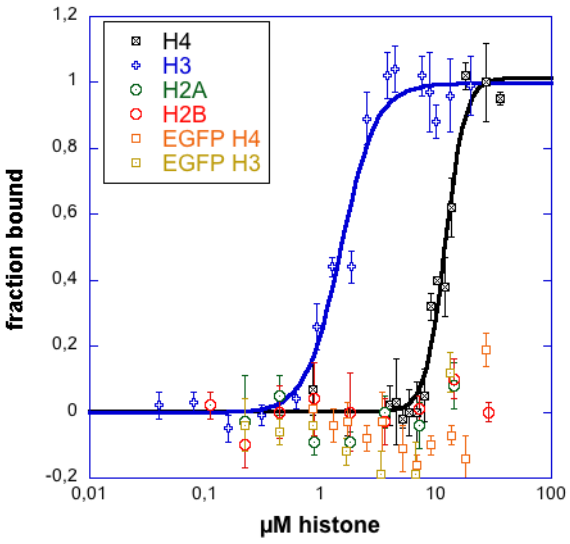

Fig. 1 Measurement of Df31-EGFP binding to core histones. MST values were normalized to fraction bound. 3 independent measurements were performed to obtain the depicted binding curve. The K_d of the Df31-H3 interaction was 1.5 ± 0.15 µM, the K_d of the Df31-H4 interaction was 12 ± 0.6 µM.

An EGFP tagged version of Df31 was used at a concentration of 1.4 µM in the experiments.
A serial dilution of the core histones was added with starting concentration between 26-36 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 interaction of the Df31 protein and the core histones H3 and H4 could be detected. However, the core histones H2A and H2B showed no binding.

The calculated K_d from the measurements of the Df31 binding to H3 was 1.5 ± 0.14 µM and H4 was 12 ± 0.6 µM.
As control we also measured the interaction of EGFP alone with our target histones H3 and H4. The experiment was performed as described for the Df31-EGFP. No interaction could be detected.

Conclusion

The study provides an example that MicroScale Thermophoresis is capable of measuring and detecting specific interactions between histones 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 EGFP tagged Df31 and EFGP control protein were used at the concentration of 1.4 µM. Unlabeled core histones H2A, H2B, H3 and H4 were added in 1:1 dilutions beginning at ranges between 26-36 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, 0.1 (v/v) % NP-40 and 200 ng/µl BSA. For the measurement the samples were filled into standard capillaries.

Instrumentation
The measurements were performed on a NanoTemper Monolith NT.115 instrument.
The measurements were performed in standard capillaries at 20 % LED and 50 % MST power with Laser-On time 30 sec and Laser-Off time 5 sec.

References

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