Protein - Peptide Interaction Analysis

Application Note NT-MO-007

Binding of Histone peptides to Chromatin assembly factor I (CAF-I) p48 subunit

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Abstract

p48, the small subunit of chromatin assembly factor 1 (CAF-1), is a member of a highly conserved subfamily of WD-repeat proteins. There are at least two members of this subfamily in human (p46 and p48). p48 copurifies with a chromatin assembly complex (CAC), which contains the three subunits of CAF-1 (p150, p60, p48) and the Histones H3 and H4, and promotes DNA replication-dependent chromatin assembly.
In this study we analyze the binding of H3 and H4 peptides to p48 using MicroScale Thermophoresis (MST). The study also highlights the high content information of the MST measurements as one important benefit of MicroScale Thermophoresis.

Introduction

Five major classes of histones exist: H1/H5, H2A, H2B, H3, and H4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1 and H5 are known as the linker histones. Two of each of the core histones assemble to form one octameric nucleosome core particle, and 147 base pairs of DNA wrap around this core particle 1.65 times in a left-handed super-helical turn. In contrast the linker histone H1 binds the nucleosome at the entry and exit sites of the DNA, thus locking the DNA into place and allowing the formation of higher order structure. Histone H5 performs the same function as histone H1, and replaces H1 in certain cells. Histone proteins also play essential structural and functional roles in the transition between active and inactive chromatin states.

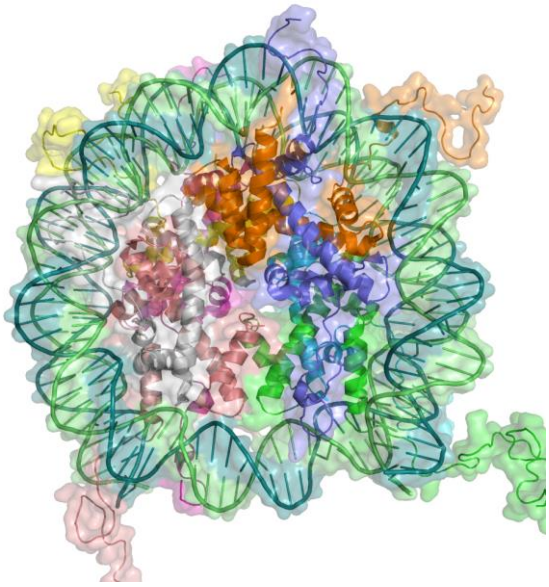

Fig. 1 Crystal structure showing the octameric core nucleosome particle consisting of two of each H2A, H2B, H3 and H4 Histones and a 147 base pairs DNA wrapped around this core particle (PDB id: 1kx5)

Chromatin Assembly Factor-1 (CAF-1) assembles newly synthesized histones H3/H4 into DNA in the first step of nucleosome assembly. Accordingly in human cells, CAF-1 is complexed to newly synthesized and acetylated histones H3 and H4. Human CAF-1 consists of three subunits: p150, p60 and p48. The small CAF-1 subunit p48 is a member of a highly conserved subfamily of WD-repeat proteins. p48 proteins are involved as histone escorts in a multistep process of cytoplasmic histone acetylation, the assembly of histones into chromatin, and the subsequent deacetylation of incorporated histones. p48 copurifies with a chromatin assembly complex (CAC), which contains the three subunits of CAF-1 (p150, p60, p48) and H3 and H4. There are at least two human homologs which are called

RbAp46 and RbAp48 (for Retinoblastoma protein associated p48). Human p48 can bind to Histone H4 in the absence of CAF-1 p150 and p60.

Results

In this study, we have investigated the binding of Histone peptides to the Chromatin assembly factor I p48 subunit using MicroScale Thermophoresis. The peptides derived from the N-terminus of Histone H3 (N-terminal long [H3NL] and N-terminal short [H3NS]) as well as of the N-terminus of Histone H4 (N-terminal long [H4NL]).
For the MST measurements the NT-647-labeled p48 and NT-647-labeled H3NL peptide were used at a constant concentration of ~ 50 nM.
We first checked for the interaction of NT-647-labeled p48 with the histone H4 peptide H4NL.

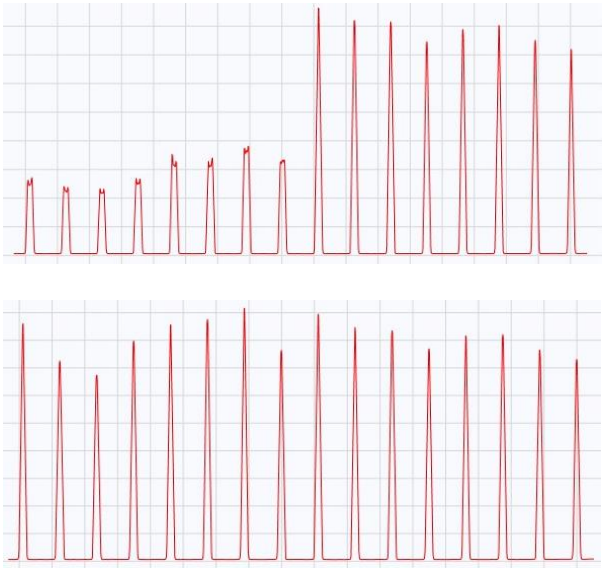

Fig. 2 NT-647-labeled p48 - H4 peptide H4NL capillary scans in standard and hydrophilic capillaries. Switching to hydrophilic capillaries prevented unspecific binding of the labeled protein to the capillary walls.

The initial capillary scan in standard treated capillaries indicated unspecific binding of labeled p48 to the capillary walls at high concentrations of the H4NL peptide which could be prevented by choosing hydrophilic treated capillaries (Fig. 2). The resulting binding curve resulted in a calculated K_d of 0.137 ± 0.062 µM (Fig.3).

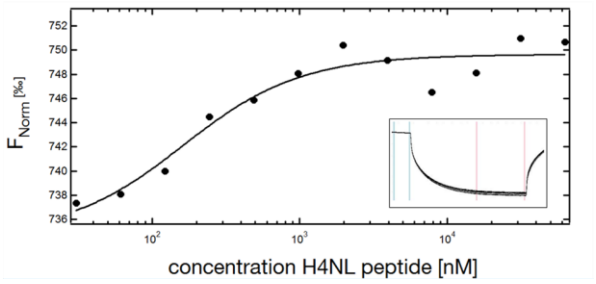

Fig. 3 Shape of the MST curves and binding curve of NT647-labeled p48 vs. H4NL peptide. The binding curve represents the data points from 1 measurement. The calculated K_d is 0.137 ± 0.062 µM.

We next checked the interaction of p48 with Histone H3 derived peptides. Fig. 4 shows the resulting binding curve for the H3NS peptide - NT-647-labeled p48 interaction with a calculated K_d of 15.76 ± 2.18 µM.

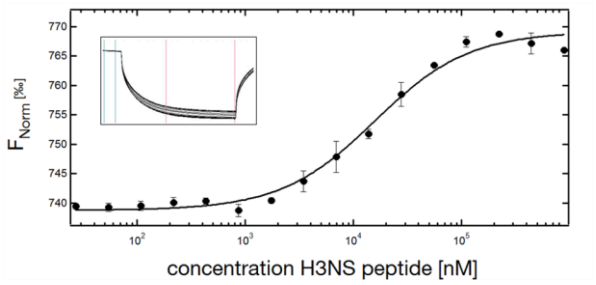

Fig. 4 Binding of H3NS peptide to NT-647-labeled p48 in standard treated capillaries: shape of the MST-curves and the resulting binding curve. The binding curve represents the data points from 3 measurements. The calculated K_d is 15.76 ± 2.18 µM.

We then measured the interaction of p48 with NT-647-labeled H3NL peptide which covers the complete N-terminus. The p48 protein stock used for this measurement was stored in a buffer containing 10% glycerol. To make sure, that no buffer effects are observed the dilution buffer for the H3NL-NT-647 labeled – p48 experiment was prepared accordingly making sure that the final glycerol concentration was 5 % in all samples.
Fig. 5 shows the resulting binding curve and the shape of the MST curves. The calculated K_d was 4.2 ± 0.93 µM, which indicated a slightly higher affinity of the H3NL peptide for p48 compared to the H3NS peptide. This K_d was in good agreement with the K_d determined for the H3NL – p48 interaction when p48 was labeled, which gave a dissociation constant of 6.95 µM (data not shown).

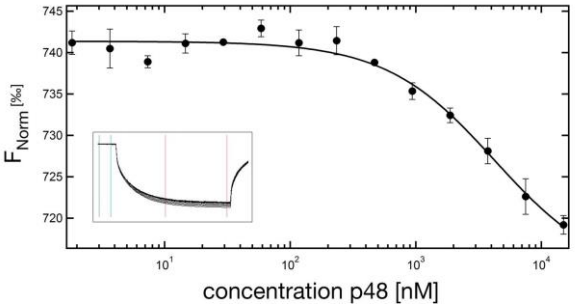

Fig. 5 Binding of NT647-labeled H3NL peptide to p48 in hydrophilic treated capillaries: shape of the MST-curves and the resulting binding curve. The binding curve represents the data points from 3 measurements. The calculated K_d is 4.2 ± 0.93 µM. This value is in good agreement with the K_d determined for the H3NL – p48 interaction when p48 was labeled, which gave a dissociation constant of 6.95 µM (data not shown).

Conclusion

The study provides an example for measurements of protein-peptide interactions with MicroScale Thermophoresis. In this study we worked with labeled protein as well as labeled peptide. It also illustrates the high content information of the measurement which allows to directly adjust and optimize the assay conditions either by changing the type of capillaries or by adjusting the buffer conditions.

Material and Methods

Assay conditions
For the experiments either p48 or the H3NL peptide were labeled with the Monolith NT™ Protein Labeling Kit RED (Cat#L001) according to the supplied labeling protocol. Labeled protein / peptide was used at a concentration of ~ 50 nM. H4NL peptide was titrated in 1:1 dilutions beginning at 250 µM, H3NS peptide was titrated in 1:1 dilutions beginning at 883 µM, and p48 was titrated in 1:1 dilutions beginning at 15 µM.
The p48-NT-647 labeled – H3NS and the p48-NT-647 labeled – H3NL (data not shown) experiments were performed in HEPES Buffer with 100 mM NaCl and measured in standard treated capillaries (Cat#K002) and hydrophilic treated capillaries (Cat#K004), respectively. Both the p48-NT-647 labeled – H4NL experiment and the H3NL-NT647 labeled – p48 experiment were performed in MST optimized buffer (50 mM Tris buffer pH 7.4 containing 150 mM NaCl, 10 mM $MgCl_2$ and 0.05 % Tween-20) and measured in hydrophilic capillaries (Cat#K004). For the H3NL-NT-647 labeled – p48 experiment the MST optimized buffer was supplemented with glycerol (final concentration 5 %) to ensure the same buffer condition for all dilutions of p48.
All binding reactions were incubated for 10 min at room temperature before they were loaded into the capillaries.

Instrumentation
The measurements were done on a NanoTemper Monolith NT.015 instrument.

All measurements were performed at 40 % LED and 40 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec.

References

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