Protein-Small Molecule Interaction Analysis

Application Note NT-MO-001

Binding of the geldanamycin derivative 17-DMAG to Hsp90

measured with fluorescence label and label-free

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Abstract

For proper folding, many proteins involved in signal-transduction pathways, cell-cycle regulation and apoptosis depend upon the ATP-dependent molecular chaperone Hsp90. Consequently Hsp90 turned out to be an attractive target for cancer therapeutics. In this study we demonstrate the binding of the geldanamycin derivative 17-DMAG to Hsp90 using MicroScale Thermophoresis (MST). The study also highlights the high content information of the MST measurements as one important benefit of MicroScale Thermophoresis.

Introduction

The cytosolic heat shock protein 90 (Hsp90) is the focus of several drug discovery programs for anti-cancer therapy. The action of Hsp90 underpins the maintenance of the transformed state through its function in the conformational maturation and activation of many client proteins involved in many of the pathways that hallmark cancer. Consequently, cancer cells are vulnerable to Hsp90 inactivation (Whitesell *et al.,* 2005).

Geldanamycin, its derivates like 17-DMAG and all the Hsp90 inhibitors in current clinical trials (Trepel *et al.,* 2010) target the ATP-binding site of Hsp90 (Fig. 1) preventing its ATPase dependent activity, which is essential for function *in vivo* (Panaretou *et al.* 1998). Inhibition of Hsp90 leads to a reduction of cellular levels of oncogenic client proteins, such as mutated p53, Akt, Bcr-Abl, and ErbB2 (McDonald *et al.* 2006). Wild-type human Hsp90 binds DMAG with a K_d of 0.35 ± 0.04 μM *in vitro*. The stoichiometry of binding was close to a 1:1 ratio of ligand to Hsp90 monomer (Onuoha *et al.* 2007).

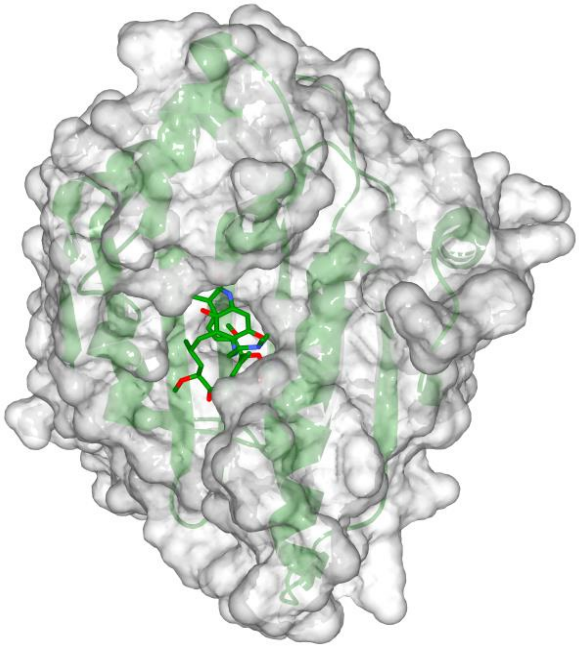

Fig. 1 Crystal structure showing 17-DMAG in complex with Hsp90.

Results

In this study, we have investigated the binding of the geldanamycin derivative 17-DMAG to wild-type human Hsp90 using MicroScale Thermophoresis.

Hsp90, fluorescently labeled with NT647, was used at a concentration of ~ 40 nM. The 17-DMAG stock was dissolved in 50 % (v/v) ethanol at a concentration of 500 µM. We used 50 µM 17-DMAG as the highest concentration for the serial dilution. At this concentration the final ethanol concentration still was 5 % (v/v). To make sure that no buffer effects are observed the dilution buffer was prepared accordingly making sure that the final ethanol concentration was ~ 5 % (v/v) in all samples. After 1 min incubation at room temperature the samples were loaded into standard treated capillaries. Fig. 2 shows the initial capillary scan, the shape of the MST curves and the resulting binding curve. The initial capillary scan already indicated slight unspecific binding of the labeled Hsp90 to the capillary walls. Subsequent analysis of the MST curves revealed high fluctuations in the data.

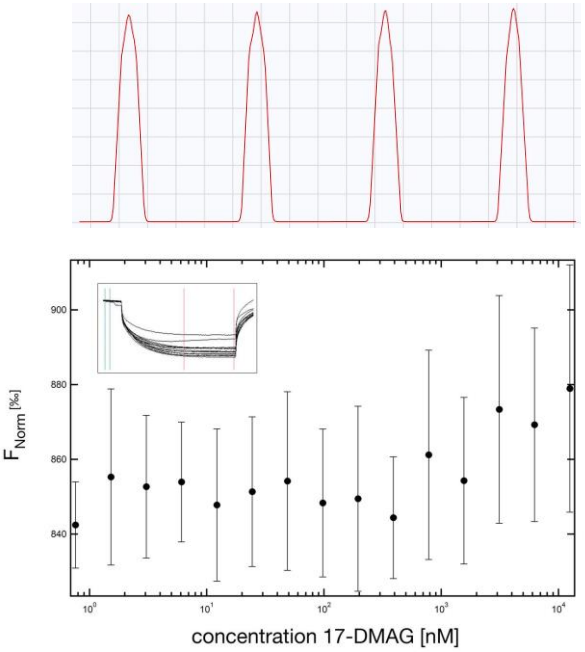

Fig. 2 Binding of 17-DMAG to Hsp90 in standard treated capillaries: initial capillary scan (upper), shape of the MST curves (inset) and the resulting data points (n = 3 independent measurements, lower). The initial capillary scan already indicated slight unspecific binding of the labeled Hsp90 to the capillary walls as does the high standard deviation of the data points.

The presence of double peaks in the capillary scan after the measurement in standard treated capillaries (Fig. 3) clearly indicated unspecific binding of the labeled Hsp90 to the capillary walls.

Fig. 3 Capillary scan after the measurement in standard treated capillaries: the double peaks clearly indicate unspecific binding of labeled Hsp90 to the capillary walls.

We therefore tested hydrophobic and hydrophilic capillaries. Fig. 4 shows the initial scan, the MST curves and the resulting binding curve for the measurement in hydrophilic capillaries.

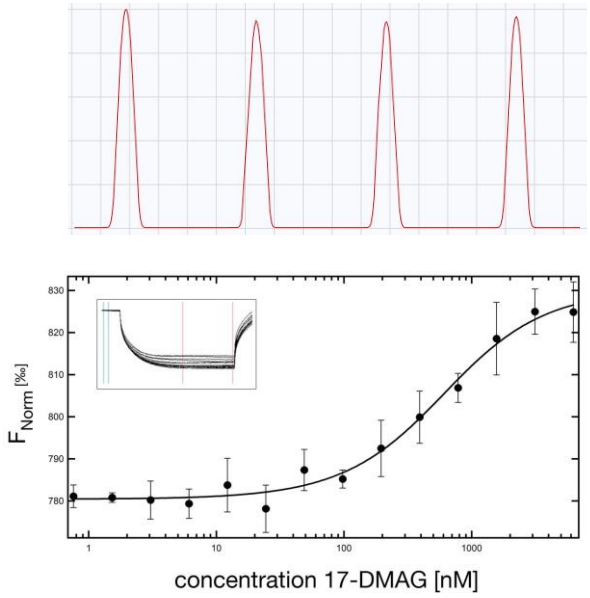

Fig. 4 Measurement of Hsp90 *vs.* 17-DMAG in hydrophilic capillaries: initial capillary scan (upper), shape of the MST curves (inset) and the resulting binding curve (n = 3 independent measurements, lower). The calculated K_d of 0.503 ± 0.099 µM is in good agreement with the published K_d of 0.35 ± 0.04 µM (Onuoha *et al.* 2007).

In comparison to the standard capillaries the standard deviations of the data were small. Also after the measurement no unspecific binding of labeled Hsp90 was observed (Fig.5).

Fig. 5 Capillary scan after the measurement in hydrophilic capillaries.

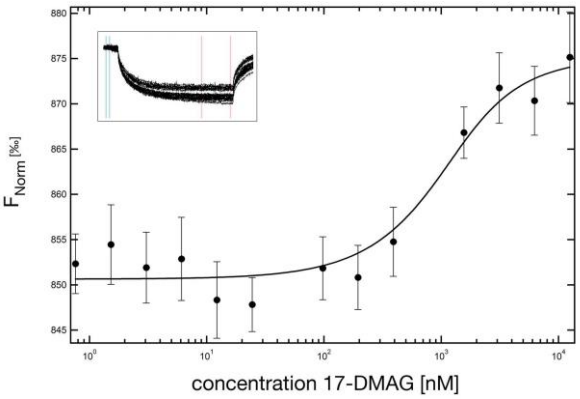

Fig. 5 Label-free measurement of Hsp90 *vs*.17-DMAG in thin wall label-free capillaries. Pre-tests indicated that the 17-DMAG itself shows no auto-fluorescence. From the resulting binding curve (n = 2 measurements) a K_d of 0.593 ± 0.387 µM was calculated.

The calculated K_ds from both measurements were 0.503 ± 0.099 µM and 0.593 ± 0.387 µM which corresponds well to the published K_d of 0.35 ± 0.04 µM (Onuoha *et al.,* 2007).

Conclusion

The study provides another example that MicroScale Thermophoresis is also capable of measuring interactions of small molecules with proteins. It also illustrates the high content information of the measurement which allows directly adjusting and optimizing the assay conditions either by changing the type of capillaries or by adjusting the buffer conditions.

Material and Methods

Assay conditions

For the experiment human Hsp90, purified as previously described (McLaughlin *et al.,* 2002*),* was labeled with the Monolith NT™ Protein Labeling Kit RED (Cat#L001) according to the supplied labeling protocol. Labeled Hsp90 was used at a concentration of ~ 40 nM. 17-DMAG (Cambridge Bioscience) was titrated in 1:1 dilutions beginning at 50 µM, which still contained 5 % (v/v) ethanol. Samples were diluted in a 50 mM Tris-HCl, pH 7.4 containing 150 mM NaCl, 10 mM $MgCl_2$ and 0.05 (v/v) % Tween-20 supplemented with ethanol at a final concentration of 5 % (v/v) to make sure that all samples contained the same ethanol concentration. For the measurement the samples were filled into hydrophilic capillaries (Cat#K004) or thin wall LF capillaries.

Instrumentation

The measurements were performed on a NanoTemper Monolith NT.115 instrument and the Monolith NT-LabelFree.

The measurement was performed at 40 % LED and 40 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec.

Label-free measurements were done on the Monolith NT-LabelFree in thin wall LF capillaries at 20 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec.

References

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