Antibody-Drug Conjugates (ADCs)

Application Note NT-MO-027

Studying the interaction of the antibody-drug conjugate SYD985 with an anti-toxin antibody

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Abstract

Antibody-drug conjugates (ADCs) have been recognized as a promising new class of therapeutic agents for the treatment of cancer (1). In the generation of ADCs, cytotoxic small molecules are covalently attached to therapeutic antibodies thereby increasing their tumor cell killing capability. We have performed MicroScale Thermophoresis experiments to determine the K_d values for the interaction between fluorescently labeled antibody-drug conjugate (SYD985) and an anti-toxin (drug) antibody in phosphate buffer as well in human plasma. The data show that MicroScale Thermophoresis is a powerful tool for the study of antibody-antigen interactions in standard buffers as well as complex bioliquids.

Introduction

SYD985 is an antibody drug conjugate composed of the monoclonal IgG1 antibody trastuzumab (SYD977) covalently bound to a linker-drug. The linker-drug contains a cleavable linker and the prodrug *seco*-duocarmycin-hydroxybenzamide-azaindole (*seco*-DUBA). The linker can be cleaved by proteases in the tumor cells at the dipeptide valine-citrulline (vc), which releases the active toxin (Figure 1).

Here, we show the use of MicroScale Thermophoresis (MST) (2) to analyze the interaction of the ADC (SYD985) with a specific mouse monoclonal anti-toxin antibody. In addition we have determined the binding affinity in 50 % human plasma as well.

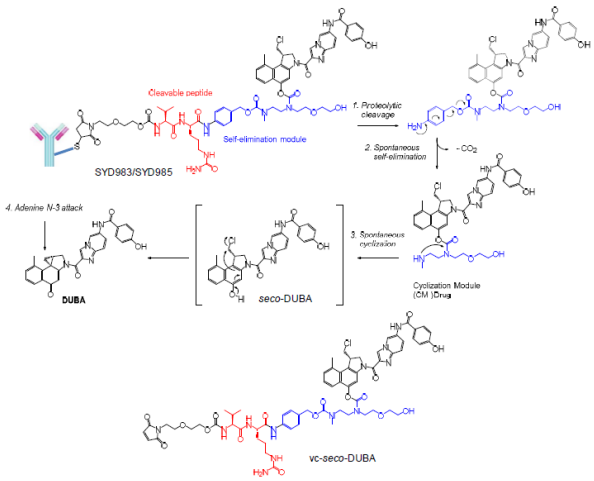

Figure 1. Mechanism of activation of SYD985 inside the tumor cell to release the toxic duocarmycin drug. When SYD985 has been taken up into the tumor cell by endocytosis the linker is cleaved in the lysosome by proteases such as cathepsin B. Subsequently, an engineered domino-reaction occurs to generate the *seco*-DUBA, which then spontaneously rearranges to form the activated duocarmycin drug. This can then bind and alkylate DNA, finally resulting in cell death.

Results

We first analyzed the binding of *seco*-DUBA, conjugated to the antibody (SYD985), to the anti-toxin monoclonal antibody. The murine anti-toxin monoclonal antibody was labeled using NanoTemper Technologies Protein Labeling Kit RED-NHS. The concentrations of labeled anti-toxin antibody were kept constant in all samples at 1.5 nM, whilst the concentration of ADC (SYD985) or of the non-conjugated antibody (SYD977) was varied. The samples were then loaded into MST Premium Coated capillaries. The MST measurements were performed at room temperature.

For the interaction of the ADC (SYD985) with the NT647-labeled anti-toxin monoclonal antibody, an (observed) K_d of 1.12 +/- 0.2 nM was found (Figure 2). As expected, no binding was observed between NT647-labeled anti-toxin monoclonal antibody and the non-conjugated antibody (SYD977), which does not contain the cytotoxic drug (Figure 2).

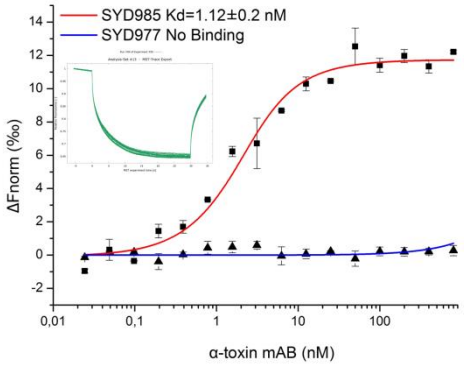

Figure 2: Binding of the NT-647 labeled anti-toxin monoclonal antibody to the ADC and the native antibody. The fluorescently labeled anti-toxin monoclonal antibody was used at a constant concentration of 1.5 nM with varying concentrations of the ADC (SYD985) or the non-conjugated antibody (SYD977). The fluorescently labeled anti-toxin antibody binds with a K_d *of* 1.12 +/- 0.2 nM to the ADC (SYD985) (squares), while no binding of the fluorescently labeled anti-toxin antibody (triangles) to the non conjugated antibody was observed. The error bars reflect standard deviation (SD) from 3 measurements.

In a second experiment, we analyzed the binding of the ADC (SYD985) to NT647-labeled anti-toxin monoclonal antibody in 50 % human plasma. As expected, SYD985 binds with a high affinity to the NT647-labeled anti-toxin monoclonal antibody (observed K_d = 1.5 +/- 0.23 nM).

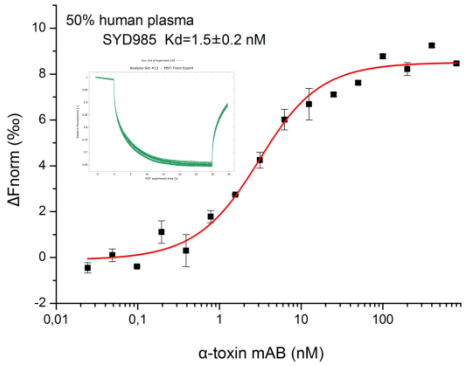

Figure 3: Binding of the NT-647 labeled anti-toxin monoclonal antibody to the ADC in human plasma. The fluorescently labeled anti-toxin monoclonal antibody was used at a constant concentration of 1.5 nM with varying concentrations of the ADC (SYD985). This experiment was performed at 50 % final concentration of human plasma. The fluorescently labeled anti-toxin antibody binds with a K_d *of* 1.5 +/- 0.2 nM to the ADC (SYD985).

Conclusions

Our experiments show that MicroScale Thermophoresis (MST) is a suitable and powerful tool for the analysis of interactions between antibodies and their antigens.

Moreover, MST is a suitable tool to investigate specificity of the anti-toxin antibody, i.e. it recognizes the drug conjugate whereas the non-conjugated antibody is not interacting at all.

Performing binding experiments in bioliquids is very challenging using orthogonal biophysical methods. Therefore, this application note underlines the advantages in applying MicroScale Thermophoresis for analyzing antibody-antigen interactions even in complex biological liquids such as human plasma.

Material and Methods

Assay conditions

Binding of labeled anti-toxin antibody to SYD977 and SYD985 in PBST buffer:

Protein Labeling: 7 µM murine IgG2a anti-toxin antibody was labeled in PBS buffer using the Protein Labeling Kit RED-NHS (L001, NanoTemper Technologies, Germany).

Sample Preparation: The concentration of the labeled anti-toxin antibody was kept constant at 1.5 nM. For the interaction analysis of SYD985 and SYD977 with the labeled anti-toxin antibody, SYD 977 or SYD985 was titrated in 1:1 dilutions. The highest concentration of SYD977 or SYD985 was 800 nM. All dilutions were performed in PBST-buffer (PBS + 0.05 % Tween-20). The samples were spun down 5 min at 4 °C, and then filled into MST Premium Coated capillaries (Cat# K005, NanoTemper Technologies, Germany).

Binding of labeled anti-toxin antibody to SYD985 in human plasma containing buffer: SYD985 was titrated in 1:1 dilutions in PBST. Then, NT647-labeled anti-toxin antibody was added into 500 µl human plasma to a final concentration of 3 nM. 10 µl of NT647-labeled anti-toxin-containing human plasma was mixed with 10 µl SYD985 dilutions. The samples were spun down 5 min at 4 °C, and then filled into MST Premium Coated capillaries (Cat# K005, NanoTemper Technologies, Germany).

Instrumentation: The measurements were conducted on a NanoTemper Monolith NT.115 instrument, at 10 % LED power and 80 % MST power.

References

1. Chari, R.V.J., Miller, M.L., Widdison, W.C. . (2014) Antibody–Drug Conjugates: An Emerging Concept in Cancer Therapy. Angew. Chem. Int. Ed. 2014, 53, 3796 – 3827
2. Jerabek-Willemsen, M., André, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P., and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. *Journal of Molecular Structure*

Please note: This Application Note replaces NT-MO-025 because it contained erroneous information.