Zurich University of Applied Sciences

Life Sciences and Facility Management

Institute of Chemistry and Biological Chemistry

Master Thesis 2011
Master of Science in Life Sciences
Chemistry for the Life Sciences

MALDI-TOF-MS Analysis of Antibody-Antigen Complexes

Master of Science Student
Miriam Iten

Lecturer
Prof. Dr. Christiane Zaborosch

Centre
Biochemistry

Industrial Partner
Cytos Biotechnology AG, Schlieren

Introduction

In the development of new drugs, it is of interest in pharmaceutical research to determine the size of antibody-antigen complexes, since it is assumed that large complexes are cleared more quickly by the body, and thus the antigen is removed faster. Antibody-antigen complexes (mAb-Ag) can be analysed using MALDI-TOF-MS. However, non-covalent complexes are difficult to detect using MALDI-TOF-MS without sample preparation, as dissociation of the complex occurs during laser irradiation and co-crystallization with the matrix. In order to prevent dissociation during the MALDI process, chemical stabilization can be carried out by means of covalent cross-linking. For cross-linking of the antibodies with the antigen the homobifunctional, amine-reactive cross-linker bis-*N*-succinimidyl-*(*pentaethylene glycol*)* ester ($BS(PEG)_5$) was employed (spacer: 21.7 Å). The crosslinker reacts with the free N-terminus and the ε-amino group of lysine side chains of the proteins. The nucleophilic N atom of the protein's primary amine reacts with the carbonyl group of the cross-linker and an amide bond is formed by nucleophilic substitution. To analyse the major cross-linked protein complexes (> 150 kDa) with MALDI-TOF-MS, an HM1 high-mass ion conversion detector from CovalX was used.

Binding Experiment with One mAb

To study the mAb-Ag complexes, a model system with IL-1β (Ag) and two anti-human IL-1β monoclonal antibodies XO01_γ2 and BS01 (mAb) was used. In the binding experiment XO01_γ2 or BS01 and IL-1β were mixed at the ratio of 1:2. The complexes formed from Ag and mAb were crosslinked through the addition of $BS(PEG)_5$ in a 56-fold molar excess.

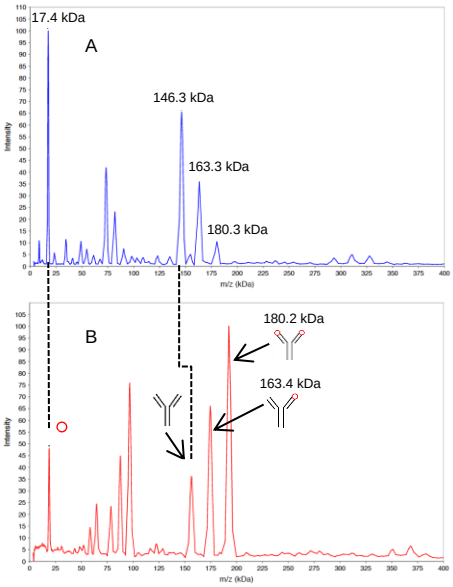

Figure 1: High-mass MALDI mass spectra of the binding experiment of IL-1β and the mAb XO01_γ2 at a ratio of 1:2 mAb:Ag. A: before addition of the crosslinker (control); B: after addition of $BS(PEG)_5$, masses after subtraction of the crosslinker mass. Matrix: sinapic acid; Detector: HM1

In the control spectrum (Fig. 1A) the peaks of the antigen IL-1β occurred at 17.4 kDa and those of the mAb XO01_γ2 at 146.3 kDa. The peaks at 163.3 kDa and 180.3 kDa corresponded to complexes without crosslinking. In the spectrum after crosslinking (Fig. 1B), two very high intensity peaks were observed at 163.4 kDa and 180.2 kDa, which could be attributed to the [XO01_γ2*__1__IL-1β] and [XO01_γ2*__2__IL-1β] complexes. The mass of the crosslinker was subtracted from the calculated masses of the complexes. BS01 was also crosslinked with IL-1β and analysed (data not shown).

Multi-Binding Experiment with Two mAbs

In the multi-binding experiment larger mAb-Ag complexes were generated and the exact masses determined using MALDI-TOF MS. The two mAbs which are directed against different epitopes were mixed with IL-1β in a ratio of 1:2:1 (mAb1:Ag:mAb2) and then crosslinked with the crosslinker $BS(PEG)_5$ in a 56-fold molar excess.

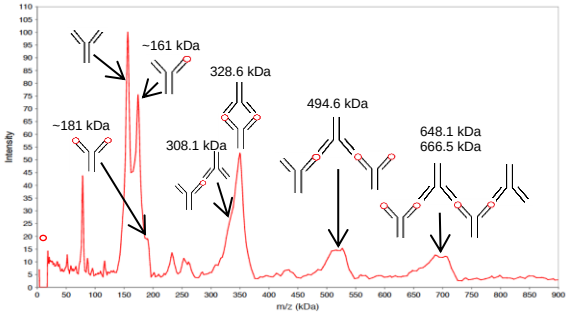

Figure 2: High-mass MALDI mass spectrum of the multi-binding experiment of IL-1β and the mAb XO01_γ2 and BS01 after crosslinking with $BS(PEG)_5$ at a ratio of 1:2:1 mAb1:Ag:mAb2. Masses after subtraction of the crosslinker mass. Matrix: sinapic acid; Detector: HM1

On the basis of the molecular masses, the peaks in the spectrum (Fig. 2) were attributed to the various mAb-Ag complexes (Tab. 1). Uncomplexed antigen was not detected; IL-1 β was therefore fully bound in complexes.

Table 1: Allocation of the mAb-Ag complexes formed

Allocation of the complexes	Expected molecular masses / kDa	Molecular masses determined / kDa
[mAb*__1__IL-1β]	164.2, 164.3	**ca. 161**
[mAb*__2__IL-1β]	181.6, 181.7	**ca. 181**
[XO01_γ2*__1__IL-1β*BS01]	311.2	**308.1**
[XO01_γ2*__2__IL-1β*BS01]	328.5	**328.6**
[__1__XO01_γ2*__3__IL-1β*__2__BS01]/ [__2__XO01_γ2*__3__IL-1β*__1__BS01]	492.8	**494.6**
[__2__XO01_γ2*__3__IL-1β*__2__BS01] or [__2__XO01_γ2*__4__IL-1β*__2__BS01]	639.7 or 657.1	**648.1**
[__2__XO01_γ2*__4__IL-1β*__2__BS01] or [__2__XO01_γ2*__5__IL-1β*__2__BS01]	657.1 or 674.5	**666.5**

Conclusion

Using the crosslinker $BS(PEG)_5$ the two mAbs XO01_γ2 and BS01 were covalently crosslinked with the antigen IL-1β and the resulting mAb-Ag-complexes were detected using MALDI-TOF-MS.