The role of CESI for functional characterization of monoclonal antibodies

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Abstract

Monoclonal antibodies (mAbs) consist of an antigen binding domain (Fab) and a crystallizable fragment (Fc). This Fc domain has several key functionalities, such as, recruitment of immune components via different Fcγ receptors (FcRs), activation of the complement system and recycling of the antibody via binding to the neonatal Fc Receptor (FcRn) which determines the half-life of antibodies. These interactions are strongly influenced by structural features of the Fc domain and, therefore, small variations in the Fc region (for example, glycosylation and oxidation) can severely impact their binding. Unfortunately, common approaches, such as SPR, provide an overall affinity response for different mAb proteoforms and assessment of their individual binding require tedious production or enrichment of specific proteoforms. In our lab, we have exploited for the first time the capabilities of capillary electrophoresis coupled with mass spectrometry (CE-MS) to study the binding affinity of antibodies and FcRs in a proteoform-resolved fashion.

We have developed different methods based on mobility-shift affinity CE-MS to study the binding of mAbs to various FcRs, namely FcRn, FcγRIIa and FcγRIIb. To this end, the FcR receptors were added to the background electrolyte whereas the mixture of antibody proteoforms was injected in the CE. As a first case, we studied the interaction towards FcRn which determine antibody half-life. By adding different amounts of FcRn to the background electrolyte, we are able to determine the relative affinity of different proteoforms based on the shifts in their mobility. We observed differences in the mobility for singly and doubly oxidized mAbs with respect to the unmodified antibodies indicating lower binding affinity. For FcγRIIa (activating) and FcγRIIb (inhibitory) receptors, glycosylation of the antibody was key for the binding. Hemiglycosylated antibodies showed a strong decrease in the binding towards both FcγRIIs. Within glycoforms differences, we also observed high mannose forms showing lower binding compared to complex type glycoforms.

The developed approach offers unique possibilities to study in solution binding of individual proteoforms and simultaneously to address their heterogeneity. Furthermore, as the receptor is free in the solution, higher-order structures can be formed reflecting the *in vivo* situation in contrast to immobilized receptors (such as, affinity LC or SPR). We believe that our approach will have a tremendous influence on the study of the interactions of mAb proteoforms with different FcRs in biopharma. Understanding these interactions is essential for developing new drugs as well as defining (and redefining) critical quality attributes of biopharmaceuticals.

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