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Short communication

Quantification and simultaneous evaluation of the bioactivity of antibody produced in CHO cell culture—The use of the ectodomain of FcγRI and surface plasmon resonance-based biosensor

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ABSTRACT

A surface plasmon resonance (SPR)-based assay has been developed in order to quantify the monoclonal antibody (Mab) Trastuzumab within the supernatant of a mammalian cell culture using the ectodomain of FcγRI (CD64) and confirm Mab bioactivity, i.e. binding to its antigen Her2, in a single biosensing experiment. Under partial mass transport limitation, we were able to quantify Mab present in unpurified samples taken throughout the cell culture. While Mab capture on the biosensor surface confirmed the ability of its Fc region to bind to FcγRI, the binding activity of its Fab region was also tested by injecting increasing concentrations of the Mab antigen (Her2). The kinetics of the interactions we recorded from 48 h post transfection until the end of the culture, were superimposable, which highly suggested that the quality attributes of the antibody were conserved throughout the process. This SPR methodology is thus of great interest for atline quality control analysis during Mab production campaign.

1. Introduction

In the past years, monoclonal antibodies (Mabs), especially IgGs, had significant success in both regulatory approval and global sales for many types of treatments against cancer as well as autoimmune, inflammatory and infectious diseases (del Val et al., 2010). The IgGs are divided in different subclasses depending on their structures (IgG1-4) (Hayes et al., 2014). Each IgG has a Y-shaped structure composed of two light chains and two heavy chains containing a Fab region that associates with the antigen and an Fc region that associates with specific receptors present on the surface of immune cells (FcγR) (Jefferis, 2012). Many expression systems exist for producing recombinant proteins at large-scale such as insect, microbial, yeast and plants but mammalian cells are the most widely used host for commercial production. Indeed, 70% of all recombinant therapeutic proteins are produced in Chinese Hamster Ovary (CHO) cells (Kim et al., 2012). During process research and development of a new therapeutic Mab, it is essential to monitor the cell culture in order to optimize protein production and evaluate product quality. In this context, the capacity to monitor online, or at least atline, the antibody integrity, from early- to late-stage process development, would be of salient interest to reduce the time and cost to market.

In the past years, surface plasmon resonance (SPR) biosensing has been shown to be a promising technique to quantify protein production and monitor protein activity of unpurified samples (Chavane et al., 2008; Pol et al., 2007). In this study, we have developed a versatile methodology using an SPR biosensor to (i) quantify monoclonal antibody production in a CHO cell culture supernatant and (ii) evaluate Mab bioactivity. This was performed in a single cycle of injections using (i) FcγRI (CD64) that displays a high affinity for IgG1,3 and (ii) the antigen of the Mab under study. For that purpose, Trastuzumab (TZM, an IgG1) and its antigen (Her2) were used as a model system.

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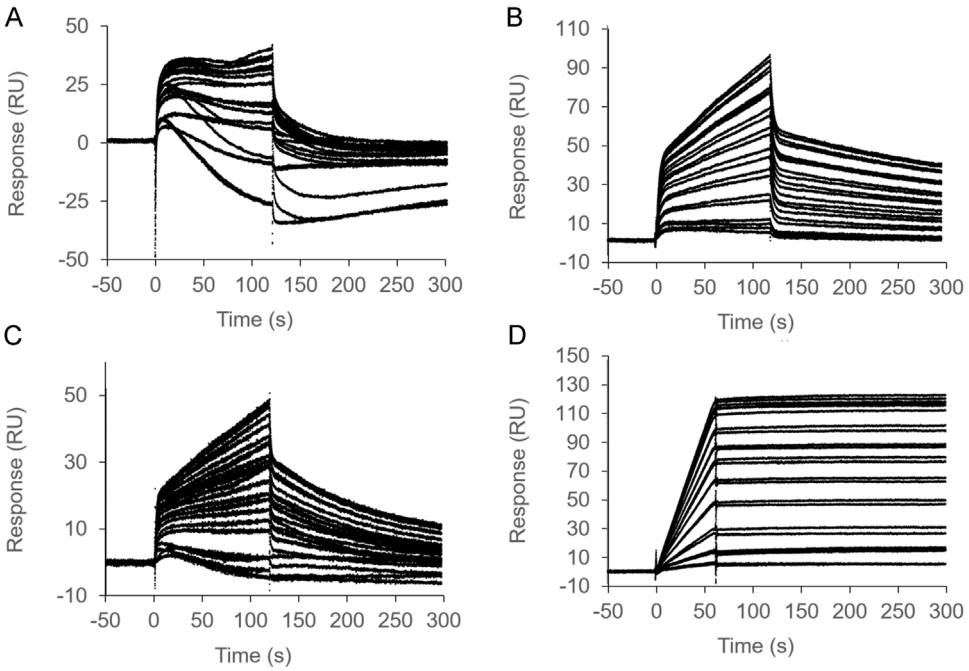

Fig. 1. Referenced sensorgrams corresponding to duplicate injections of CHO cell culture supernatants (dilution 1/50 in HBS-P 1×) over 1 600 RU of biotinylated FcγRIIIa (A), 2 000 RU of biotinylated FcγRIIa (B), 2 000 RU of biotinylated FcγRIIb (C) and 1 400 RU of biotinylated FcγRI (D) that had been captured at the biosensor surface using the Biotin CAPture kit as previously described (Dorion-Thibaudeau et al., 2016). Supernatants were collected from 24 hpt to 144 hpt (every 12 h), centrifuged (10 000 × *g*, 5 min) and filtered (0.2 μm), prior injection on the receptor and mock biosensor surfaces.

2. Results and discussion

2.1. Offline quantification of TZM using FcγRI, FcγRII and FcγRIIIa

Trastuzumab-producing CHO cells were transfected and cultured as described elsewhere (Dorion-Thibaudeau et al., 2016). Culture sampling was performed every 12 h, starting from 24 h post transfection (hpt), until cell viability was approximately 60%. Each supernatant was centrifuged and filtered prior injection on the SPR instrument surfaces (Biacore™ T100, all injections performed at 20 °C). Purified biotinylated FcγRs were captured onto the biosensor surfaces via biotin/streptavidin interactions, using the Biacore Biotin CAPture kit for all analyses (Dorion-Thibaudeau et al., 2016). High receptor densities were combined to a low analyte injection flow rate (5 μL/min) to favour mass transport limitation (MTL) (Richalet-Secordel et al., 1997); in these conditions, partial MTL was achieved.

For biotinylated low affinity receptors (FcγRIIIa, FcγRIIa and FcγRIIb), complex matrix effects were observed when injecting the diluted supernatants containing TZM. Indeed, referenced sensorgrams corresponding to immobilized FcγRIIIa and FcγRIIb (Fig. 1A and C, respectively) were below the baseline level during dissociation phase. This was due to the high amplitude and stability of the non-specific interactions occurring on the mock surface (streptavidin), which were masked when biotinylated FcγRIIIa/FcγRIIb had been captured. Furthermore, for FcγRIIa and FcγRIIb, the shape of the injection phase within each sensorgram (Fig. 1B and C, respectively) was significantly different from those recorded when injecting purified TZM over the same receptors since, for purified TZM (Dorion-Thibaudeau et al., 2016), equilibria (plateaus) were reached after less than 10 s of injection. These results highly suggested that molecular species present in the conditioned F17™ cell culture medium interacted with those surfaces and/or impacted receptor/TZM interactions (this was confirmed by injecting diluted fresh cell culture medium – data not shown). In stark contrast, referenced sensorgrams recorded with captured FcγRI when injecting diluted supernatants (Fig. 1D) were comparable to those recorded when injecting purified TZM (Dorion-Thibaudeau et al., 2016). Therefore, only biotinylated FcγRI was selected as an appropriate receptor for TZM quantification of unpurified samples by SPR.

2.2. Assay development using FcγRI

The total concentration of the TZM standard was determined by absorbance readout at 280 nm (with a molar attenuation coefficient calculated with the ExPASy ProtParam tool) while the active concentration of the stock solution was determined by performing a calibration-free concentration analysis (CFCA) on the Biacore instrument with biotinylated FcγRI as ligand by adapting the methodology described by Pol and colleagues (Pol, 2010) (TZM was injected in duplicates at 25 nM at flowrates of 5 and 100 μL/min, calculations were made with an estimated diffusion coefficient value of 3.45 10^{-11} m^2/s). The active/total TZM ratio was 60% for the TZM standard. A standard calibration curve (Fig. 2A) was then generated by injecting dilutions of a purified TZM standard on the FcγRI and mock surfaces. Linear regression between 15 and 55 s of the Mab injection over captured FcγRI (after mock signal subtraction) was performed to determine the antibody initial binding rate for total antibody concentrations ranging between 0.2 and 25 nM (n = 4). Concentrations of Mab present in unpurified supernatants were then assessed. Fig. 2B shows referenced sensorgrams corresponding to a typical experiment for quantification and simultaneous bioactivity evaluation. First, supernatants were injected at 5 μL/min for quantification (1), each Mab injection was followed by a running buffer injection to reach a stable capture of the antibody onto the receptor (2). Afterwards, increasing concentrations of the Mab antigen, Her2, were injected at 50 μL/min at concentrations of 1 nM (3), 5 nM (4), 10 nM (5) and 30 nM (6). For quantification (Fig. 2B – 1), linear regression was performed between 15 and 55 s for each supernatant (n = 4) to derive the initial reaction rate and therefore determine the active antibody concentration using the calibration curve shown in Fig. 2A.

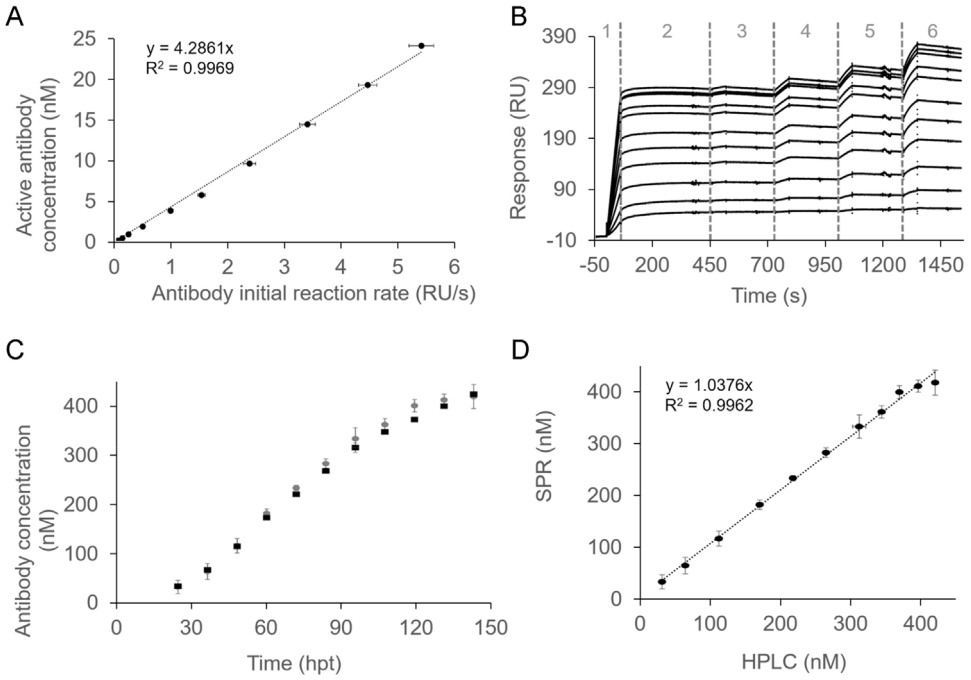

Fig. 2. Offline monitoring by SPR of a CHO cell culture to quantify TZM production and confirm TZM bioactivity. (A) Calibration curve generated by extracting the initial reaction rate of referenced sensorgrams corresponding to purified Mab injections on captured FcγRI (dilution 1/20, n = 4). (B) Referenced sensorgrams corresponding to the injection of each cell culture supernatant (1), buffer injection (2) and then injections of increasing concentrations of TZM antigen at 50 μL/min, Her2 (eBioscience, USA), at 1 nM (3), 5 nM (4), 10 nM (5) and 30 nM (6). (C) Offline quantification of active TZM within cell culture supernatants by SPR using the calibration curve shown in A (black square, n = 4), by HPLC with a protein-A column (dark gray circle, n = 3) or by SPR using the CFCA method (light gray circles, n = 3). (D) Correlation between active TZM concentrations determined by SPR with the calibration method vs. CFCA method.

To validate our quantification methodology, we also determined TZM concentration within the same supernatants by two other approaches. The first one corresponded to CFCA for each supernatant using a SPR surface on which FcγRI had been captured via biotin/streptavidin interactions (using the same protocol and the same diffusion coefficient as before while injecting 1/50 dilutions of the supernatants) (Richalet-Secordel et al., 1997). The second approach relied on quantitative HPLC using an 800-μL POROS® 20-μm Protein-A ID Cartridge (Applied BioSystems) operated according to the manufacturer's instructions (note that the protein-A HPLC method showed less than 5% inter-assay variation). Calibration was performed with injections of purified TZM. All samples were filtered prior injection to the column and eluted with 0.15 M NaCl, pH 2.0; absorbance was taken at 280 nm (n = 3).

The antibody concentrations derived from both SPR analyses (calibration curve and CFCA) and HPLC-Protein A analyses are shown in Fig. 2C. Very similar results were obtained for each time-point with the three approaches. Indeed, although protein A and FcγRI do not bind to the exact same region of the Fc portion, we observed only a 3% deviation between the HPLC approach and the SPR approach relying on a standard curve (correlation coefficient > 0.99 – data not shown). This confirmed that our methodology for quantification using FcγRI (rather than Protein A) is a valid approach. As shown in Fig. 2D, TZM concentrations determined with both SPR approaches differed only by 2% despite non-optimal conditions for CFCA measurements (since we determined QC ratios close to 0.3 for several TZM injections, which indicate that the MTL contribution to the signal was modest and thus data not optimal for concentration determination).

To further confirm the integrity and activity of the Fab regions of TZM, the signals resulting from the injections of increasing concentrations of Her2 for each time point were also processed (Fig. 2B steps 3–6). By normalizing each of these series of sensorgrams corresponding to Her2 injections, we observed that the resulting sensorgrams were superimposable from 48 hpt until the end of the culture (Fig. 3). This implies that the kinetics of interaction between TZM and Her2 did not change over this time period, in turn highly suggesting that the Mab bioactivity (i.e., the ability of TZM to bind to Her2) was not altered. The processing of the sensorgrams corresponding to the first two samples (24 and 36 hpt, shown in light gray in Fig. 3) gave curves that are not superimposed with those of the other samples, most likely due to an unfavourable signal-to-noise ratio during data normalization (too low Mab concentration in the supernatant). This limitation could be resolved by performing a longer injection of the Mab-containing supernatants corresponding to the early cell culture sampling points (in order to accumulate more Mab material on the surface prior Her2 injections).

3. Conclusion

A method for the quantification of IgG1 or IgG3, within cell culture supernatant, has been developed. The method relies on FcγRI captured at the surface of an SPR-based biosensor via streptavidin-biotin interactions. It was shown to give similar results as a quantification assay based on HPLC-protein A column, while providing more information on IgG glycosylation state (since IgG binding to FcγRI requires IgG N-glycosylation). Moreover, within a single analysis cycle, this method also enabled to confirm the integrity of the Fab region of the IgG.

Since the IgG binding to captured FcγRI is very stable and high affinity, matrix effects, as those observed with lower affinity receptors, were negligible in our model system. We believe that such will be the case for other culture media used in industry, although this point needs to be confirmed prior implantation. Altogether, this methodology is promising for the atline monitoring of the concentration of any IgG1 or IgG3, as well as the simultaneous monitoring of their critical quality attributes as long as their purified antigen is

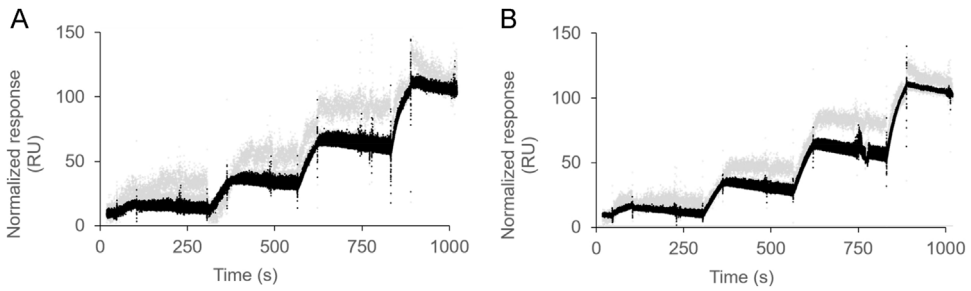

Fig. 3. Normalized sensorgrams corresponding to the interactions between Her2 and surface-captured TZM. Increasing concentrations of Her2 were injected (1, 5, 10, 30 nM, 1 min at 50 μL/min.) over captured TZM (resulting from supernatant injections at 1/50 (A) and 1/20 (B) dilutions in HBS-P buffer, followed by a 3-min buffer injection to stabilize TZM capture). All responses were normalized at the end of the buffer injection following the last Her2 injection. Supernatants corresponding to 24 and 36 hpt are shown in light gray while those corresponding to 48 up to 144 hpt are shown in black.

available. The approach is thus suitable for routine quality control in an established process or while developing biosimilars.

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