Competitive Adsorption of Monoclonal Antibodies and Nonionic Surfactants at Solid Hydrophobic Surfaces

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ABSTRACT: Two monoclonal antibodies from the IgG subclasses one and two were compared in their adsorption behavior with hydrophobic surfaces upon dilution to 10 mg/mL with 0.9% NaCl. These conditions simulate handling of the compounds at hospital pharmacies and surfaces encountered after preparation, such as infusion bags and i.v. lines. Total internal reflection fluorescence and quartz crystal microbalance with dissipation monitoring were used to follow and quantify this. Furthermore, the influence of the nonionic surfactant polysorbate 80 (PS80) on the adsorption process of these two antibodies was investigated. Despite belonging to two different IgG subclasses, both antibodies displayed comparable adsorption behavior. Both antibodies readily adsorbed in the absence of PS80, whereas adsorption was reduced in the presence of 30 mg/L surfactant. The sequence of exposure of the surfactant and protein to the surface was found to have a major influence on the extent of protein adsorption. Although only a fraction of adsorbed protein could be removed by rinsing with 30 mg/L surfactant solution, adsorption was entirely prevented when surfaces were pre-exposed to PS80. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:593–601, 2015

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INTRODUCTION

Monoclonal antibodies (mAbs) are widely used in the treatment of various diseases including inflammatory diseases and cancer, thus accounting for an expanding economic earning in the pharmaceutical field. For example, the group of cancer antibodies, which includes Panitumumab (PAN; Vectibix®) and Rituximab (MabThera®), yielded total annual sales of US$ 23.47 billion in 2012.[1] However, despite intensive research on mAbs, there still remain challenges in terms of obtaining a sufficiently stable product that can be marketed.

Antibodies highly depend on an appropriate three-dimensional fold to achieve target binding. If this ordered structure is lost, efficacy is consequently lost. Protein adsorption to solid surfaces is one process by which proteins can unfold,[2–5] and this can lead to subsequent aggregation.[6] Furthermore, protein adsorption onto particulates[7] and generation of subvisible protein aggregates because of exposure to a variety of surfaces have been associated with development of immunogenicity toward therapeutic proteins.[8–10] Nonionic surfactants have been shown to prevent or significantly reduce this detrimental physical degradation[11–15] and thus are frequently used in pharmaceutical formulations.

A range of mAbs intended for clinical use are formulated with nonionic surfactants to increase their stability, for example, Rituximab, Trastuzumab, Bevacizumab, and Cetuximab. A study performed by Garidel et al.[16] showed that the stabilizing properties of polysorbate on mAbs are not because of the formation of protein–surfactant complexes in solution. Hence, the stabilizing effect may be because of a molecular chaperone effect of polysorbate,[17] preferential hydration of the protein as described for polyethyleneglycol,[18,19] or reduced interfacial affinity of the protein because of blocking properties of the surfactant.[20,21] The latter would result in reduced protein adsorption and unfolding on the surface. As surfactants are added at very low concentration, preferential exclusion effects are expected to be minimal,[19] and thus surface–surfactant interactions represent one likely explanation for the reduced surface adsorption of proteins.[16]

The aim of the present study was to gain further insight into the blocking properties of polysorbate 80 (PS80) toward the adsorption of mAbs onto solid hydrophobic interface. Specifically, it is the intention to relate this data of model surfaces (water contact angle from 85° to 90°) to polymers such as polyethylene, polypropylene, and polyvinylchloride used in the production of infusion bags (water contact angles from 87° to 104°).[22–25] To this purpose, the adsorption kinetics of two model mAbs in the presence and absence of the commonly used surfactant PS80 were studied. Both mAbs in this study, mAb-1 and PAN, are formulated without surfactant. PAN has the advantage of being commercially available and may thus be used as a potential reference material in further studies. The use of a reference antibody allows for comparability of future studies under a variety of conditions. Two different methods were used to follow the adsorption kinetics, quartz crystal microbalance with dissipation monitoring (QCM-D) and total internal reflection fluorescence (TIRF). Whereas TIRF measurements can be made specific for a single adsorbing species by only following the specific fluorescence of that compound, in our case the mAb, QCM-D

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measurements report on the adsorption process as a whole, and yield insight into the viscoelastic properties of the adsorbed layer.[26,27]

EXPERIMENTAL

Materials and Methods

Sodium chloride, sulfuric acid (98%), hydrochloric acid (25%), ammonia solution (25%), and hydrogen peroxide (30%) were obtained from Merck (Darmstadt, Germany). N-acetyltryptophan, Tween 80® (PS80), 1-propanethiol, and (3, 3, 3-trifluoropropyl)chloromethylsilane were obtained from Sigma–Aldrich (Steinheim, Germany). All chemicals are of analytical grade and were used without further purification. Water purified to a resistivity of 18.2 MΩcm^{-1} was used in all preparations. mAb1, an IgG1 (pI 8.44) formulated with 205 mM sucrose in a 25-mM histidine buffer at pH 6, was graciously donated by Medimmune (Cambridge, UK), PAN, an IgG2, was bought as the product Vectibix® (expiry date: May 2015; Amgen, Thousand Oaks, CA). PAN, with a pI of 6.63,[28] is formulated with 100 mM sodium chloride in a 83-mM sodium acetate buffer at pH 5.8.[29] The CMC of PS80 is reported to be 14–15 mg/L in water,[30] and this value is not expected to be affected by the presence of mAbs[16] and only marginally lowered by salt.[31–33] The terminology used in this article will be relative to the CMC of PS80 in water: ½xCMC (7 mg/L), 2xCMC (30 mg/L).

Ultraviolet–visible

A nanodrop UV/Vis spectrophotometer (Thermo Scientific, Waltham, Massachusetts) was used to determine protein concentrations with an extinction coefficient of 1.4 mL mg^{-1} cm^{-1} for the antibodies. The extinction coefficient of N-acetyltryptophan was determined to be 20.5 mL mg^{-1} cm^{-1}.

Total Internal Reflection Fluorescence

Surface Modification

Silanized quartz surfaces were used for the TIRF experiments. Quartz slides from TIRF Technologies (Cary, North Carolina) were cleaned by the procedure adapted from Kern and Puotinen[34] where the surfaces are first immersed in a solution prepared from 25% NH_3, 30% H_2O_2, and H_2O (1:1:5, by volume) at 80°C for 5 min, rinsed with water, immersed in 30% HCl, 30% H_2O_2, and H_2O (1:1:5, by volume) at 80°C for 5 min, and subsequently rinsed with water and ethanol. The clean quartz surfaces are modified with (3, 3, 3-trifluoropropyl)chloromethylsilane using vapor deposition as previously described[35] under argon atmosphere.[36] To assure proper and homogenous modification, the contact angles of a sessile drop of ultrapure water on the silanized surfaces were determined. Contact angles were found to be 89 ± 1°, measured at three different sites on the surface with a Krüss G2 contact angle measuring system (Krüss GmbH, Hamburg, Germany). Each experiment was performed with a newly modified, unused surface.

Experimental Run

The quartz slide forms the central piece of the TIRF flow cell (TIRF Technologies), which is restricted by a gasket and backblock fitted with the inlet and outlet tubing. The flow cell was fitted into a Spex Fluorolog 3–22 (Jobin Yvon Horiba, Longjumeau Cedex, France). Following a modified protocol from Pinholt et al.,[36] a stable baseline was established at a constant flow rate of 4.17 μL/s. Subsequently, a constant wavelength analysis of a concentration range of nonadsorbing external standards of N-acetyl-tryptophan[37] was performed, followed by the protein sample (10 mg/mL). Each sample was loaded and oscillated with a flow rate of 4.17 μL/s, whereas the flow rate was increased to 16.67 μL/s in the dissociation phase. The latter served a double purpose: (1) faster elimination of the bulk from the cell and (2) removing loosely adsorbed protein species. Excitation and emission wavelength for the time-resolved experiments were set to 295 and 350 nm, respectively. Integration time was fixed to 0.1 s, slit widths to 5 nm. Quantification of the adsorbed amount, Γ, was performed according to the procedure by Roth and Lenhoff.[37]

Quartz Crystal Microbalance with Dissipation Monitoring

Surface Modification

Gold sensors (Biolin Scientific, Gothenburg, Sweden) were cleaned according to the manufacturer's protocol.[38] Modification was achieved by submersion into a 5-mM propanethiol solution in absolute ethanol and incubation for at least 12 h. The sensors were rinsed, kept submerged in absolute ethanol, and were used the same day. Contact angle measurements were performed prior to the QCM-D experiment. Typical values are higher than 85°.

Regeneration of the sensors was achieved by oxidation of the thiol with piranha solution.[39] Sulfates are reported to have low affinity to gold and can be removed with polar solvents.[39] All further steps were performed according to the manufacturer's protocol.[38]

Experimental Run

The sensors were mounted in a Qsense E4 (Biolin Scientific) and equilibrated at a flow rate of 100 μL/min in ultrapure water until drift of frequency was less than 1 Hz/h. The experiment was initiated by recording a stable baseline in water, then in 0.9% NaCl, followed by loading of the protein solution for 15 min under constant flow, and concluded with a 0.9% NaCl solution rinse. In some experiments, an additional rinse with PS80 at 2xCMC in 0.9% NaCl was performed. Data were analyzed in QTools 4 (Biolin Scientific). Density of the sodium chloride solution for viscoelastic modeling was estimated to be 1004 kg/m^3 by linear interpolation from reference.[40] Density of the adsorbed antibody layer was set to 1100 kg/m^3 assuming 60% water content.[41,42] Overtones 5, 7, and 9 were further fitted to the Sauerbrey approach[43] giving similar results for the total wet adsorbed amount.

RESULTS

The effect of PS80 on the adsorption behavior of the two immunoglobulins of subclass G (IgGs) in the study was performed in three distinct stages: (1) adsorption of the protein with a consecutive rinsing step with 2xCMC PS80, (2) adsorption of mixed protein and PS80 samples, and (3) adsorption of PS80 followed by protein. These are detailed in the following paragraphs.

Adsorption of Antibodies to Hydrophobic Surfaces

To investigate the influence of PS80 on the adsorption behavior of the antibodies, a reference point was established using both

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