An Investigation of the Factors Controlling the Adsorption of Protein Antigens to Anionic PLG Microparticles

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ABSTRACT: This work examines physico-chemical properties influencing protein adsorption to anionic PLG microparticles and demonstrates the ability to bind and release vaccine antigens over a range of loads, pH values, and ionic strengths. Poly(lactide-co-glycolide) microparticles were synthesized by a w/o/w emulsification method in the presence of the anionic surfactant DSS (dioctyl sodium sulfosuccinate). Ovalbumin (OVA), carbonic anhydrase (CAN), lysozyme (LYZ), lactic acid dehydrogenase, bovine serum albumin (BSA), an HIV envelope glyocoprotein, and a *Neisseria meningitidis* B protein were adsorbed to the PLG microparticles, with binding efficiency, initial release and zeta potentials measured. Protein (antigen) binding to PLG microparticles was influenced by both electrostatic interaction and other mechanisms such as van der Waals forces. The protein binding capacity was directly proportional to the available surface area and may have a practical upper limit imposed by the formation of a complete protein monolayer as suggested by AFM images. The protein affinity for the PLG surface depended strongly on the isoelectric point (pI) and electrostatic forces, but also showed contributions from nonCoulombic interactions. Protein antigens were adsorbed on anionic PLG microparticles with varying degrees of efficiency under different conditions such as pH and ionic strength. Observable changes in zeta potentials and morphology suggest the formation of a surface monolayer. Antigen binding and release occur through a combination of electrostatic and van der Waals interactions occurring at the polymer-solution interface. J Pharm Sci 94:2510–2519, 2005

Keywords: microparticles; antigen; vaccine delivery; polylactide-co-glycolide; protein adsorption; PLG; anionic particles; electrostatic; microscopy

INTRODUCTION

Next generation vaccines will be comprised mainly of recombinant protein antigens that are often poorly immunogenic. These vaccines will need optimal antigen delivery systems with or without an additional immunopotentiator to generate potent immune responses.[1,2] Emulsions, micro/nanoparticles, aluminum salt adjuvants, and iscoms have all been used as delivery systems to boost immune responses to vaccine antigens. Enhanced immune responses are induced through a variety of mechanisms, including increased persistence of antigen at the site of injection, improved targeting to antigen presenting cells, or enhanced protection of antigen linear, and conformational epitopes against degradation.[2–4]

We have previously described studies which showed that anionic poly(lactide-co-glycolide) (PLG) microparticles were effective delivery systems for adsorbed vaccine antigens, including recombinant proteins from *Neisseria meningitidis* type B (MB)[5] and human immunodeficiency virus

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(HIV).[6] The PLG polymer used in these formulations has a long history of safe use in humans.[7–9] Work with the traditional aluminum salt adjuvants has elucidated rules for predicting protein adsorption,[10] encouraging adjuvant-antigen interactions,[11] and highlighted the significance of surface electrostatic forces in antigen adsorption and release.[12] Anionic PLG microparticles[13,14] represent an alternative approach to aluminum salt adjuvants,[15] or the MF59[16] emulsion adjuvant.

To assess the strengths and limitations of anionic PLG microparticles as a delivery system for diverse antigens, we have measured the physico-chemical properties such as charge and ionic strength that have been implicated as important to the description of binding and release of a range of proteins from polymer surfaces.[17–20] In addition to vaccine relevant protein antigens, model proteins with a wide range of isoelectric points (pI) including ovalbumin (pI 4.6), carbonic anhydrase (pI 6.0), and lysozyme (pI 10.7) were evaluated, to represent proteins with diverse electrostatic properties.

Evaluation of the physicochemical properties of anionic PLG microparticles, including size, charge, and surface morphology may provide information that can be used to guide formulation strategies for diverse proteins. The complex behavior of protein on solid surfaces has been studied by various techniques[21,22] to suggest the formation of structures including multilayers. Using atomic force microscopy to image the microparticles before and after protein adsorption, we visualized the surface morphology to determine if it was consistent with our hypothesis of the formation of an adsorbed protein monolayer. Furthermore, we investigated the physico-chemical properties influencing protein adsorption to microparticles and demonstrated their ability to bind and release diverse protein antigens over a range of loads, pH, and ionic strengths.

EXPERIMENTAL

Materials

RG503 and RG502H, poly(D,L-lactide-co-glycolide) 50:50 lactide to glycolide co-polymers were obtained from Boehringer Ingelheim (Petersburg, VA). Dioctylsulfosuccinate (DSS), lysozyme (LYZ), carbonic anhydrase (CAN), bovine serum albumin (BSA), ovalbumin (OVA), lactate dehydrogenase (LADH), USP grade mannitol, sucrose, and trehalose came from Sigma-Aldrich Chemical (St. Louis, MO) Chinese Hamster Ovary derived recombinant gp120dV2 was synthesized and purified in house (Chiron Vaccine Research in Emeryville, CA) *Escherichia coli* (*E. Coli*)-derived recombinant meningococcal proteins (MB1 and MB2) were obtained from Chiron Vaccines, Siena (Siena, Italy) and were isolated and purified as previously described.[23]

Microparticle Preparation

Microparticles were prepared by a solvent evaporation technique. A 10 mL methylene chloride solution of 6% w/v polymer with 2.5 mL PBS was homogenized using a 10 mm probe, (Ultra-Turrax, T25 IKA-Labortechnik, Germany) forming water in oil emulsion which was then added to 50 mL of distilled water containing 6 μg/mL DSS and homogenized at very high speed using a homogenizer with a 20-mm probe (ES-15 Omni International, GA) for 25 min in an ice-water bath. This resulted in water in oil in water emulsion, which was stirred at 1000 rpm for 12 h at room temperature as the methylene chloride was allowed to evaporate.

Emulsion Characterization

The size distribution of the microparticles was determined using a particle size analyzer (Master sizer, Malvern Instruments, UK). The electrokinetic mobility of the PLG microparticles was measured in PBS buffer on a Malvern ZetaSizer (Malvern Instruments, UK) and the zeta potential (roughly equivalent to particle surface charge) determined. Model proteins with a range of pI were chosen to span a range of buffer adsorption conditions: lysozyme with pI 10.7, lactic acid dehydrogenase with pI 6.8, carbonic anhydrase with pI 6.0 and ovalbumin with pI 4.6. The PLG content of the suspension was measured by aliquoting 1 mL of the suspension into preweighed vials, which were lyophilized and weighed again and the average net weight change was used as PLG content/1 mL suspension.

Atomic force microscopy was employed in the noncontact mode using a Digital Instruments BioScope (Digital Instruments, Santa Barbara, CA). The polymer suspension was imaged before and after exposure to protein (1% weight protein to polymer, overnight rocking at 4°C, then sample and control lyophilized overnight). The powder