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Regular Article

Buffer formulation affects the interaction between lysozyme and polymeric nanoparticles

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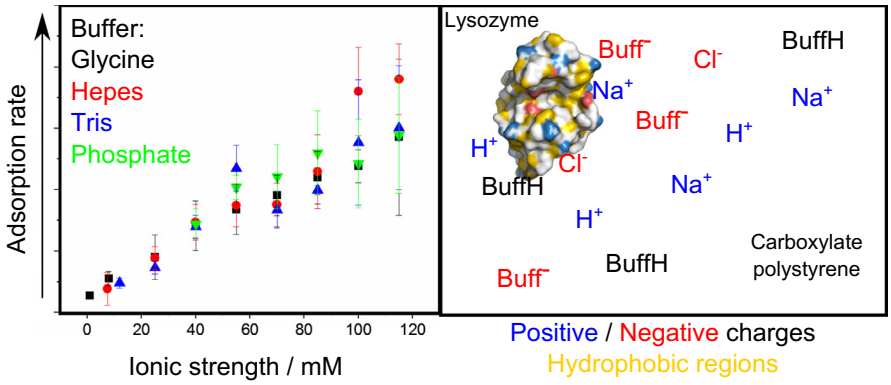
GRAPHICAL ABSTRACT



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ABSTRACT

The effect of the buffer formulation in terms of buffer identity and ionic strength on the interaction between chicken egg lysozyme and carboxyl-modified polystyrene nanoparticles has been systematically studied. The time evolution of the fluorescence of a reporter molecule shows that lysozyme interacts with the nanoparticles in all the studied conditions. The interaction results in changes in protein conformation and decrease of the colloidal stability of nanoparticles. In absence of a background salt the rate of adsorption is affected mainly by the ionic strength of the buffer solution, although, specific buffer effects may contribute to a certain extent. The identity of the different buffer components does not significantly alter the dynamics of the process in presence of salt at constant ionic strength. However, an increase of ionic strength leads to slower processes indicating that the adsorption is affected by the presence of increasing number of ions in solution.

1. Introduction

Protein adsorption on surfaces is an inevitable process in many areas such as biomedicine and biotechnology [1]. Protein adsorption depends on the nature of the surfaces, the amino acid sequence of the protein as well as the properties of the medium such as pH, temperature, and ionic strength [1,2]. Understanding the effects of simple physicochemical parameters is of most importance for applications involving protein adsorption on surfaces.

Buffers are essential for *in vitro* studies of proteins since changes in pH and ionic strength may lead to alterations on the folding landscape of proteins and the activity of enzymes. Thus, a buffer system is commonly used to ensure a stable pH and mimic

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the biological conditions of the protein under study. Besides pH of the solution and the ionic strength, ion specific effects (from background salt or buffer ions which behaviour deviate from the classical description of salt solutions) play a role for the protein structural integrity and function [3–8]. In similar way, the buffer system plays also a role for the colloidal stability of nanoparticles which can be compromised by changes in ionic strength, pH or the type of ions in solution [9–11].

Ion specific effects are often reported at high salt concentrations (>100 mM). However, such effects have been recently described in solutions at salt concentrations within the range of normal buffer concentrations in biochemical applications (10–100 mM) [11–13]. More specifically, Cugia et al. has shown that buffer ions specifically interact with protein surfaces changing its electrophoretic mobility [12]. One question that is often forgotten is the contribution of the buffer to the total ionic strength of the solution. Even though, the contribution can be generally low at pH values close to the pKa of the buffer, in some cases the buffer ions contribute significantly to the ionic strength of the solutions as in the case of phosphate buffer, an ionic buffer that dissociate into monovalent and divalent ions. The assumed small difference in ionic strength is usually equalized by addition of a background salt at a defined concentration (for example NaCl). However, for buffers that contribute strongly to the ionic strength, the contribution of buffer ions to the total ionic strength is not negligible. Therefore, buffer solutions at same pH and background salt concentration, can have a different ionic strength depending on the concentration of buffer ions. Despite the observed effects of buffers on protein adsorption, few studies have systematically investigated how different types of buffer affect the protein adsorption to nanomaterials [14–16]. Hence, the aim of this study is to explore how the formulation of a pH buffer, also when the ionic strength is adjusted by a background salt, may influence the interaction between nanoparticles and proteins.

Chicken Egg Lysozyme (Lys) and carboxyl-modified polystyrene nanoparticles (PSCOOH) have been selected as model protein and nanoparticle respectively. The interaction between Lys and PSCOOH has been extensively studied in the past [17,18]. Lys adsorbs at the particle surface and undergoes subsequent conformational changes as demonstrated by CD spectroscopy and intrinsic fluorescence [17]. Those conformational changes expose hydrophobic patches to which the reporter molecule 8-anilino-1-naphtalene sulfonic acid ammonium salt (ANS) can bind [19]. Changes in the ANS fluorescence signal with time together with the variation of the hydrodynamic diameter of the colloidal particles before and after protein addition, indicated that Lys partially unfolds at the particle surface and, upon adsorption, the colloidal stability of the system is compromised so the protein-nanoparticle complex aggregates and precipitates [18].

Here, we have studied the interaction between Lys and PSCOOH using ANS as reporter of the adsorption process. Four different buffers, Glycine/NaOH, HEPES/NaOH, Tris/HCl and Phosphate buffer, have been selected in order to study buffers with different chemical nature. The charged species that contribute to the ionic strength and may interact with the charged patches on the PSCOOH and Lys surface (see Fig. S1 [20,21]) varies in nature and charge as can be observed in Scheme 1. Each of the buffers has also been studied at different ionic strengths which were adjusted by addition of a background salt, NaCl, at a constant pH. We show that the ionic strength of buffers has a significant effect on the dynamics of the adsorption process. On the other hand, the identity of the buffer has influence on the adsorption process in absence of a background salt but this effect is overcome by the presence of a high concentration of background salt ions in solution.

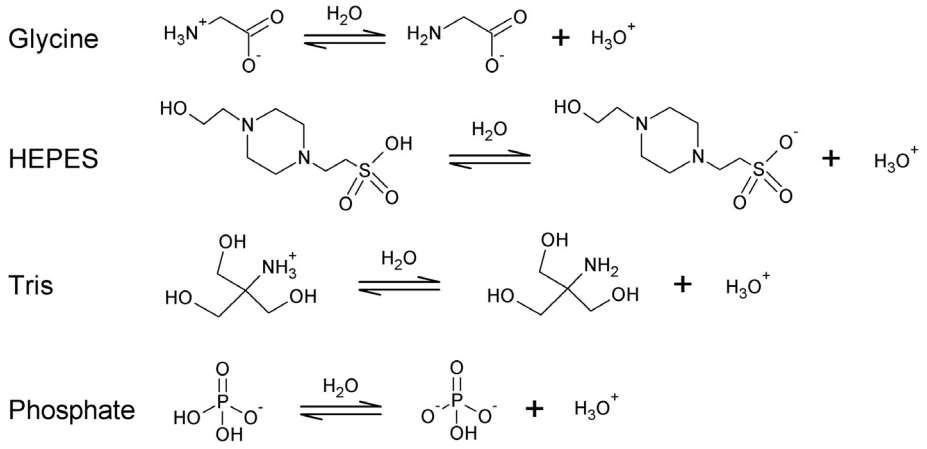
2. Materials and methods

2.1. Materials

Carboxyl-modified polystyrene nanoparticles (PSCOOH) were purchased from Polysciences Inc. The particles were dialyzed against water for a week to remove any stabilizers of the colloidal suspension and the concentration determined by weight after dialysis. The hydrodynamic radius of the nanoparticles in water was obtained by dynamic light scattering (DLS) using a Dynapro Plate reader (Wyatt Technology, Santa Barbara, CA). Chicken Egg Lysozyme (lyophilized powder, ≥90%, ≥40,000 units/mg protein) and 8-anilino-1-naphtalene sulfonic acid ammonium salt were purchased from Sigma and used without further purification. Lys and ANS were dissolved in MilliQ water and centrifuged to remove any remaining insoluble material. The concentration of Lys stock solution was calculated by UV–vis absorption spectroscopy (Agilent 8453 spectrophotometer) using a value for the extinction coefficient at 280 nm of 37,973 M^{-1} cm^{-1}.

2.2. Buffer preparation

Glycine (Glyc) (Alfa Aesar), HEPES (Saveen Werner), Trizma®BASE, $Na_2HPO_4{\cdot}2H_2O$, NaH_2PO_4 and NaCl (Sigma) were of analytical grade and used without further purification. All buffer solutions, Glyc/NaOH, HEPES/NaOH, Tris/HCl and phosphate buffer were



Scheme 1. Relevant dissociation equilibrium of the selected buffers.