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Adsorption of a therapeutic enzyme to self-assembled monolayers: effect of surface chemistry and solution pH on the amount and activity of adsorbed enzyme

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Abstract

The adsorption of a therapeutic enzyme to self-assembled monolayers (SAMs) of different functionalities (X = CH_3-, OH- and COOH-) was evaluated as a function of solution pH. Radiolabelling studies showed that the enzyme has higher affinity for hydrophobic surfaces than for hydrophilic surfaces, and that the highest adsorption was obtained at the more acidic pH values (4.5 and 5.5), despite the type of surface. IRAS and XPS measurements confirmed this tendency. Dye-binding studies and fluorescence quenching were used to investigate if a pH variation induces any conformational changes on the enzyme. Both methods suggest that lowering the pH from physiological to acidic values triggers an increased exposure of non-polar sites in the enzyme, which may modulate its adsorption behaviour to the more hydrophobic surfaces. At pH 4.5, the enzyme carries a substantial positive net charge and therefore relatively low native-state stability. As a consequence, surface binding may be favoured, irrespective of the type of surface, by providing increased conformational entropy to the enzyme. The specific activity (SA) of the adsorbed enzyme was strongly dependent on the conditions used. A decrease in SA (ca. 30% of control) was observed after adsorption on CH_3-SAMs for all the pH tested. Adsorption on gold and on the more hydrophilic SAMs (OH- and COOH-) resulted in different degrees of inactivation at the more acidic pH (4.5), and in enzyme activation (up to ca. 230% of control) at higher pH (7–8), near the isoelectric point of the enzyme.

Keywords: Adsorpotion; Enzyme; Self-assembled monolayers (SAMs); Enzyme activation

1. Introduction

Adsorption of proteins and their interaction with surfaces has been the subject of considerable research in the biomedical field. Numerous proteins, including several enzymes, have been immobilized through adsorption on different supports to be used in a wide range of therapeutic and diagnostic applications. In drug delivery, controlled protein adsorption has been used as a means to temporarily couple the protein to the vehicle [1]. Reversibility of binding and the retention of an active conformation of the protein upon immobilisation and subsequent release are essential requirements. On the other hand, the proper transport and delivery of entrapped proteins from polymeric microcarriers, for example, is also intimately linked with protein

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adsorption. In this case, however, the extent of unspecific and irreversible adsorption of the protein to the matrix should be reduced as much as possible, since a fraction of the total entrapped amount will consequently become unavailable, leading to an incomplete release profile and wastage of valuable protein [2]. The selection of the most adequate delivery matrix for a certain application could clearly benefit from previous knowledge on the adsorption behaviour of the protein of interest.

The process of protein adsorption is complex and is affected by various factors. The rate and extent of adsorption will be determined by the properties of the protein and its concentration in solution, by the characteristics of the adsorption matrix, the nature of the solvent and other properties of the medium, namely pH, ionic strength and temperature [3]. The most relevant phenomena involved in the process of protein adsorption are electrostatic and hydrophobic interactions and the intrinsic structural stability of the protein [3]. Upon adsorption, proteins generally undergo structural rearrangements depending on the type and strength of the interactions established with the matrix [4,5]. Although conformational alterations may result in protein denaturation and/or loss of functional activity [6,7], in some situations they can be beneficial rather than detrimental. For example, enzyme stability is often better retained in immobilised formulations than on native free forms [5,8] and enzymatic activity can be enhanced in the presence of an interface [9,10].

The mechanistic aspects of protein interaction with surfaces can be investigated using self-assembled monolayers (SAMs) of long-chain alkanethiols on gold ($HS(CH_2)_nX$) [11]. These highly ordered model surfaces can be prepared by exposing different functional groups (X) on the surface allowing a wide range of chemical compositions and wettabilities to be explored [12]. The objective of the present study was to investigate some fundamental aspects of the interaction of glucocerebrosidase (GCR) with surfaces using SAMs of different functionalities ($X = CH_3$, OH and COOH).

The activity and/or stability of lysosomal GCR is defective in patients with Gaucher disease, the most prevalent metabolic storage disorder, which is characterised by a number of severe disabling symptoms including bone lesions [13]. For enzyme replacement therapy (ERT), recombinant GCR is administered intravenously and is internalised by target cells by mannose receptor-mediated endocytosis [13]. Although ERT seems to be efficient in reverting most of the symptoms, the delayed and modest skeleton response remains the major issue in the overall effectiveness and management of affected patients [13,14]. This is indicative that adjuvant therapies are necessary to restore bone function more rapidly and/or to a greater extent than with ERT alone [13]. The development of a vehicle for site-specific GCR delivery to bone resident Gaucher cells is currently under investigation in our group [15], as a therapy that may be used in association with ERT.

In this study, radiolabelling was used to quantify the amount of GCR adsorbed onto SAMs as a function of solution pH. Fourier transform infrared reflection absorption spectroscopy (IRAS) and X-ray photoelectron spectroscopy (XPS) were used as alternative semi-quantitative techniques. This information was complemented with data on the catalytic activity of adsorbed GCR, with the aim of providing insight on the biological status of the adsorbed enzyme. Fluorescence studies were performed to investigate if a pH variation induces conformational changes on the enzyme.

2. Materials and methods

2.1. Preparation and characterisation of SAMs

2.1.1. Preparation of gold substrates and monolayer formation

Gold substrates were prepared as previously described [16]. Briefly, chromium (5 nm) and gold (25 nm) films were deposited by ion beam sputtering onto silicon wafers (polished/etched, crystal orientation <100>, AUREL Gmbh). The Cr layer was used to improve adhesion of gold to silicon. Wafers were coated with 1.5 μm of photoresist (PFR7790EG, JSR Electronics) to protect the film surface, and diced into pieces (0.5×0.5 and $1 \times 1\,cm^2$) using a DISCO DAD 321 automated saw.

The following alkanethiols (Aldrich): 11-mercapto-1-undecanol ($SH\text{-}(CH_2)_{11}OH$; 97%), 1-undecanethiol ($SH\text{-}(CH_2)_{10}CH_3$; 92%) and 11-mercaptoundecanoic acid ($SH\text{-}(CH_2)_{10}COOH$; 95%) were used as received. Alkanethiol solutions of 1 mM in ethanol (99.8%, Merck) were prepared under N_2 in a glove box. Immediately prior to use, gold substrates were cleaned twice in acetone, rinsed with ethanol and immersed in a "piranha" solution (7 parts of H_2SO_4 and 3 parts of 30% v/v H_2O_2) for 10 min (Caution: this solution reacts violently with many organic materials and should be handled with care). Substrates were sequentially rinsed with ethanol/distilled-deionised water/ethanol for 2 min in an ultrasonic bath, and then dried with Ar. Subsequently, they were immersed in alkanethiol solutions and incubated at room temperature for 24 h in N_2. SAMs were finally washed 3 times in ethanol in an ultrasonic bath for 2 min, dried and maintained under Ar until used.

2.1.2. Contact angle measurements

Contact angle (θ) measurements were performed by the sessile drop method (Data Physics OCA 15, equipped with video CCD-camera and SCA 20 soft-