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Bioresponsive interfaces composed of calmodulin and poly(ethylene glycol): Toggling the interfacial film thickness by protein-ligand binding

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ABSTRACT

Responsive interfaces are often realized by polymer films that change their structure and properties upon changing the pH-value, ionic strength or temperature. Here, we present a bioresponsive interfacial structure that is based on a protein, calmodulin (CaM), which undergoes a huge conformational change upon ligand binding. At first, we characterize the conformational functionality of a double Cys mutant of CaM by small-angle X-ray scattering (SAXS) and Fourier transform infrared (FTIR) spectroscopy. The CaM mutant is then used to cross-link poly(ethylene glycol) (PEG) chains, which are bound covalently to a supporting planar Si surface. These films are characterized by X-ray reflectometry (XR) in a humidity chamber providing full hydration. It is well known that Ca^{2+}-saturated holo-CaM binds trifluoperazine (TFP) and changes its conformation from an open, dumbbell-shaped to a closed, globular one in solution. At the interface, we observe an increase of the PEG-CaM film thickness, when TFP is binding and inducing the closed conformation, whereas the removal of Ca^{2+}-ions and a concomitant release of TFP is associated with a decrease of the film thickness. This toggling of the film thickness is largely reversible. In this way, a structural change of the interface is achieved via protein functionality which has the advantage of being selective for ligand molecules without changing the environmental conditions in a harsh way via physico-chemical parameters.

1. Introduction

There is a series of studies where aqueous-solid interfaces are functionalized with polymers that change their structure and properties upon changing the pH value, the ionic strength or the temperature [1–12]. Such responsive interfaces are designed to control enzyme immobilization, cell growth, drug release, and nano-particle deposition. An important type of interfacial structures is a polymer brush, where long polymer chains are densely grafted to a solid surface. For example, polymer brushes composed of poly(N-isopropylacrylamide) show a phase transition as a function of temperature from a hydrated to a collapsed polymer conformation [2,3,5–8]. This temperature response of the brush structure has a strong effect on the protein binding capacity and cell adhesion. Using polyelectrolytes, pH responsive brushes can be prepared as well, which also allow for a variable degree of enzyme immobilization [9,10]. Furthermore, surface-bound microgels offer the possibility to transfer pre-determined properties to interfaces, such as temperature-dependent swelling or pH degradation [13,14]. However, a variation of the temperature or pH value can affect the native structure and the biological activity of proteins and cells in a major way, which is limiting the use of pH- or temperature-responsive interfaces for the immobilization of proteins and the adhesion of cells. On the other hand, the protein binding capacity of a poly(acrylic acid) brush can be controlled by changing the ionic strength of the protein solution over a few 100 mM, which is more benign to maintain the native state of a protein [11,12,15].

Considering potential biomedical or biophysical applications, such as protein-based biochips and assays, a variation of the pH value, the temperature or the ionic strength is generally restricted within the boundaries given by the native state of the involved biological matter. Therefore, it would rather be advantageous to prepare bioresponsive interfacial structures that can be triggered via specific biochemical interactions without changing the environmental physico-chemical conditions. Proteins that undergo major conformational changes upon ligand binding can be the key element of these interfacial structures. For example,

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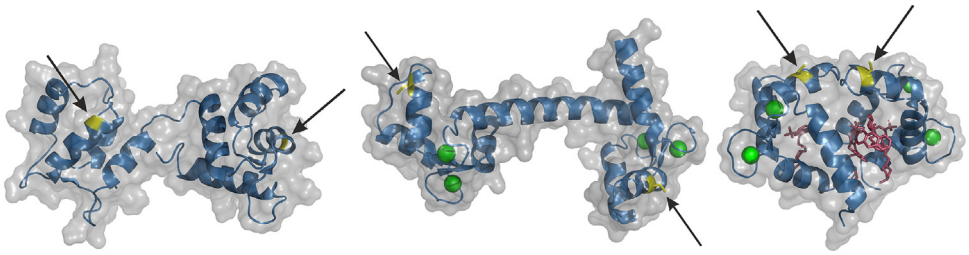

Fig. 1. Calmodulin (CaM) populates distinct conformational states upon binding Ca^{2+} ions and ligand molecules, such as trifluoperazine (TFP). From left to right, apo-CaM (T34C, T110C), holo-CaM (T34C, T110C) (Ca^{2+} shown in green), and holo-CaM (T34C, T110C) with 4 bound TFP molecules (TFP shown in red). In addition, the two threonine residues at positions 34 and 110, which are exchanged by cysteine residues in this study, are highlighted in yellow and are marked with arrows. The images were prepared with PyMOL v1.3r1 using the PDB IDs 1DMO, 3CLN, and 1LIN. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

calmodulin (CaM) reveals three distinct states free in solution (Fig. 1) [16–18]. Without ligand, Ca^{2+}-free CaM (apo-CaM) and Ca^{2+}-saturated CaM (holo-CaM) adopt an open, dumbbell-shaped conformation where two globular domains are connected by a flexible linker. Ca^{2+}-saturated CaM can be transferred into a closed, globular conformation upon binding ligands, such as peptides, proteins and small molecules. When trifluoperazine (TFP) is used as ligand, the maximum diameter and the radius of gyration of CaM are strongly reduced upon binding four TFP molecules [16].

Moreover, it has been reported that a hydrogel composed of poly(ethylene glycol) (PEG) that is cross-linked by holo-CaM shows a significant volume decrease upon CaM-TFP binding [19]. Reversely, a swelling of the gel can be induced, when the Ca^{2+} ions are removed and apo-CaM is formed [19]. Apparently, the conformational changes of a protein on the nanometer scale have been translated to macroscopic volume changes of a gel. Other examples include the hinge motion of the glucose binding protein in a poly(acrylamide) hydrogel and the ATP binding of adenylate kinase, which is cross-linking N-(2-hydroxypropyl)methacrylamide [20,21]. In all cases, it is remarkable that the structural response of the gel is induced by a specific protein-ligand binding without changing the temperature or the pH-value. In this context, it is interesting to note that bioresponsive hydrogels can also be prepared by incorporation of DNA. Changes of the hydrogel properties are induced by changes of the DNA structure due to hybridization or loop formation [22].

In view of the highly interesting properties of CaM and CaM-PEG hydrogels, we have designed interfacial structures where PEG chains are covalently bound to a solid support and are cross-linked by a double Cys-mutant of CaM. The thickness of this interfacial structure has been characterized by X-ray reflectometry (XR) in a humidity chamber (providing 100% relative humidity) after rinsing with various solutions. XR provides the scattering length density or electron density profile normal to the interface [23]. In addition, the size, shape, and secondary structure of free holo-CaM (T34C, T110C) have been characterized in solution applying small-angle X-ray scattering (SAXS) [24] and Fourier-transform infrared (FTIR) spectroscopy [25]. As we will show in this study, the CaM mutant shows a large ligand-induced conformational transition, and the prepared interfacial structures are bioresponsive, because their structure depends on the solution concentrations of TFP and Ca^{2+} ions which can both bind to CaM.

2. Materials and methods

2.1. Sample preparation

Recombinant CaM (T34C, T110C) from rat was produced using the pET-14b vector from GenScript (Piscataway, NJ, USA) and E. coli BL21-CodonPlus (DE3)-RIPL competent cells from Agilent Technologies (Santa Clara, CA, USA) as described in the literature [26]. The two mutations are located on the two lobes of the dumbbell-shaped holo-CaM and replace threonine (Thr or T) by cysteine (Cys or C) residues (Fig. 1) [19,21]. The latter are used to bind PEG covalently. The purity of the protein was checked by SDS-PAGE (Fig. S1). A strong band between 14,200 and 20,100 g mol^{-1} is observed in agreement with the calculated molar mass of 16710 g mol^{-1} of apo-CaM (T34C, T110C). D_2O, BisTris, HEPES, $CaCl_2$, trifluoperazine (TFP), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), dithiothreitol (DTT) were all purchased from Sigma-Aldrich.

Silicon wafers were kindly donated by Siltronic (Burghausen, Germany). They have a size of 1.5 cm × 2.0 cm and were cleaned in a 3:1 mixture of H_2SO_4 (95–98%) and H_2O_2 (30%) for one hour to generate free OH groups on the Si surface. These Si wafers were used for XR experiments. The clean Si wafers were immersed in a solution of 3-(trimethoxysilyl)-1-propanethiol (purchased from Sigma-Aldrich, 10% in toluene) at 50 °C for 20 min. In this reaction step, free SH groups are generated on the Si surface (Fig. 2) [27]. After reaction, the sample has been sonicated in toluene to remove unbound reagent. Using an amine-catalyzed thiol-acrylate reaction [28], four-arm PEG tetraacrylate (purchased from Creative PEG Works, 0.24 mg mL^{-1} in 20 mM HEPES buffer solution, pH = 7) is bound covalently to the surface (Fig. 2), where the HEPES serves as amine catalyst [19,29]. The reaction solution also contains 0.2 mg mL^{-1} CaM (T34C, T110C), 10 mM $CaCl_2$, 150 mM NaCl, and 1.5 mM tris(2-carboxyethyl)phosphin (TCEP). The latter reduces disulfide bonds formed by CaM (T34C, T110C). In this way, the four-arm PEG is not only reacting with the SH groups at the Si surface, but also with the SH groups of the CaM (T34C, T110C), thereby generating a PEG film with cross-linking CaM on the Si wafer (Fig. 2). The reaction time for this step was varied from 5 to 10 h. As reference, the four-arm PEG tetraacrylate has also been bound to the Si wafer in the absence of CaM (T34C, T110C). In this case, DTT has been added with a concentration of 0.5 mM to cross-link the PEG chains.

2.2. Instrumental techniques

X-ray reflectometry (XR) was carried out with the CaM-PEG modified Si wafers, which were equilibrated in a home-built humidity chamber (Fig. S2). This chamber provides 100% relative humidity and full hydration of the samples. Data were collected using the Seifert XRD 3000 TT reflectometer from GE Inspection Technologies (Ahrensburg, Germany), which was operated with the Mo-Kα wavelength (0.71 Å). Raw data were converted to reflectivity curves by normalization of the reflected X-ray intensity to the incident intensity according to $R = I/I_0$. They are scaled as a function of wavevector transfer, $Q = (4\pi/\lambda) \sin\theta$, where λ is the wavelength and θ is the angle of incidence. Reflectivity curves were analyzed