Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 794–800
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Brief Communication

Selection process generating peptide aptamers and analysis of their binding to the TiO_2 surface of a surface acoustic wave sensor

Thomas M.A. Gronewold [a], Antje Baumgartner [b], Andreas Weckmann [b], Jaqueline Knekties [b], Christian Egler [b,*]

[a] *Biosensor GmbH, Ludwig-Erhard-Allee 2, D-53175 Bonn, c/o caesar, Germany*
[b] *Caesar research center, Ludwig-Erhard-Allee 2, D-53175 Bonn, Germany*

Received 27 April 2008; received in revised form 3 September 2008; accepted 9 September 2008
Available online 4 October 2008

Abstract

The set-up presented in this article is intended for the selection of peptides which serve as specific binders to suitable materials. Additionally, the interaction of such binders with material surfaces can be characterized. Using this approach, a subset of peptides which adhere to the mineral TiO_2 was generated by means of a cell surface display library. The peptides are constrained by a thioredoxin scaffold. Selection of proteins was carried out on a silicium wafer sputtered with TiO_2 in anatase conformation. To verify binders and to analyze the binding kinetics of the diluted suspension of the purified proteins, the chip-based S-sens® K5 surface acoustic wave sensor system was used. The surface of the sensor chips was also TiO_2, resembling the material of the Si wafer selection target retaining the peptides. Several peptides were identified. The respective binding behavior differed. The data derived from real-time interaction analysis were evaluated to select for strong and specific binders. For one of these peptides, the binding kinetics was analyzed. On- and off-rate binding constants were extracted from the fitted curves. With the resulting association rate constant k_{on} and the dissociation constant k_{off}, the affinity of the peptide for the TiO_2 surface was calculated, represented by the equilibrium dissociation constant $K_D = 81$ nM.

Keywords: SAW biosensor; TiO_2; Bacterial display; Inorganic binding peptide; Peptide aptamers

1. Introduction

Research on the binding of proteins to inorganic materials has increased in recent years. The focus has been on the impact of polluting materials on the environment and the assembly of new hybrid materials [1–3]. Titanium (IV) dioxide (TiO_2) is an example of such a material. In its natural form it occurs primarily in the mineral rutile, which has a tetragonal unit cell like its polymorph anatase, both with the same degree of symmetry but with different interfacial angles. When heated above 915 °C, anatase is converted into rutile. Finely powdered TiO_2, often in the nanometer range, is a brilliant white pigment, which is heavily used in paints and plastics, and even to color food. Recent applications in suntan lotions make use of its ultraviolet-absorbing properties. Nanosized product ingredients have become abundant, but are extremely difficult to detect. They are not even visible under light microscopes. Also, little is known about the effects of such nanoparticles on the environment or after uptake in the body. Such an effect might be positive, as in nanomedicine, or negative, as in the newly developing field of nanopathology [4]. To trace such particles in products, or to trace the leakage of material during the production process into the environment, nanoparticles need to be identified specifically, selec-

* Corresponding author. Tel.: +49 228 9656 293.
E-mail address: egler@caesar.de (C. Egler).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.09.014

tively and with high sensitivity. This is done by employing proteins that can recognize molecules from a sensor array. To date, knowledge about the interactions between proteins and inorganic materials has been limited; however, this is a prerequisite for the rational design of TiO_2-binding proteins. Using a screening process, proteins can be generated that employ the properties desired.

The basis for any screening of peptide interactions is a peptide library. Two main systems are used. In the FliTrx™ system [5], peptides are displayed on bacterial flagella, while in the phage display, peptides are fused to a protein coat. The random peptide display library in the FliTrx™ has the advantage of being an *Escherichia coli*-based system that eliminates the necessity of using phages, making the screening of peptide interactions faster and easier. The peptides are constrained within a scaffold, which itself does not bind to TiO_2, to amplify the process. Peptides constrained in the protein thioredoxin are highly soluble and extremely stable. Thioredoxin is a very well characterized protein that has more than 75% of the residues involved in well-defined secondary structure motifs. This unusually high proportion explains the exceptional stability of thioredoxin. The two cysteine residues involved in forming the active center disulfide bridge form a protrusion between the middle strand of the pleated sheet and one of the helices [6]. This site is favored for the introduction of a peptide loop owing to the exposure of the expressed peptide on the outside of the resulting fusion protein. Using peptides in constrained conformation is of possible advantage for interaction screening that include, but are not limited to, (i) greater stability than unstructured peptides under biological conditions; (ii) higher binding affinities than corresponding flexible peptides; and (iii) exposure of amino acid residues that are usually buried inside flexible peptides, such as hydrophobic amino acid residues. In addition, peptides that are fused to a protein are easier to detect because they are larger than unconstrained peptides when subjected to mass-sensitive devices based on surface plasmon resonance (SPR) or surface acoustic waves (SAW).

Hundreds of peptides binding to inorganic materials have been selected from the phage and cell-surface display libraries, as reported by different groups [1,7–11]. Nevertheless, the molecular mechanisms of peptide recognition and their binding to solid materials are still poorly understood. This is mainly because there are few accessible publications on the binding kinetics and thermodynamics of these processes. By providing a technique suitable for a great variety of materials, the interaction at the interface between a protein and an inorganic material surface can be analyzed. Some groups have characterized binding using quantitative techniques such as quartz crystal microbalance (QCM), SPR and atomic force microscopy- based force–distance measurements [1,5,10,12,13]. We show that the methods presented here are a big step forward for investigations on the chemistry at the interface of proteins and engineered surfaces.

2. Materials and methods

2.1. Preparation of selection target

As the selection target, a TiO_2 layer was deposited on native 4" Si wafers in 100-orientation using reactive E-beam vaporization (3.0×10^{-2} Pa pO_2 at base pressure 3.0×10^{-4} Pa at 260 °C) to a thickness of approximately 100 nm. The resulting TiO_2 layer was in anatase conformation. The anatase structure was confirmed by X-ray diffraction (XRD) and Auger electron spectroscopy. XRD was measured using a Bruker D8 DISCOVER diffractometer with GADDS equipped with a HI-STAR area detector, using Cu K_α radiation (40 kV, 40 nA, $\lambda = 154.17$ pm). Auger measurements were performed with a Phi 690 Field Emission Scanning Auger Nanoprobe from Physical Electronics at 10 kV and 10 nA. The base pressure of the UHV chamber was 6.666×10^{-8} Pa.

2.2. Selection using cell surface display FliTrx™ (Invitrogen)

A peptide library of 1.77×10^8 primary *E. coli* clones displaying random dodecapeptides on the surface (FliTrx™ library, Invitrogen, Order No. K1125-01) was used to inoculate 50 ml of IMC broth (6.0 gNa_2HPO_4, 3.0 gKH_2PO_4, 0.5 gNaCl, 1.0 g l^{-1} NH_4Cl, pH 7.4 supplemented with 50 μg ml^{-1} ampicillin) in a sterile 250 ml shake flask. Bacteria were grown for approximately 15 h at 30 °C and 150 rpm. Aliquots corresponding to 10^{10} cells (assuming 10^9 cells ml^{-1} absorbance $unit^{-1}$ at 600 nm [14]) were transferred to 50 ml of IMC (supplemented with 100 μg ml^{-1} L-tryptophan) to induce expression of the modified flagellar protein. Bacteria were grown for 6 h at 25 °C and 150 rpm. TiO_2-covered Si wafers were positioned in the center of polystyrene culture dishes, then 20 ml of freshly prepared blocking solution (200 mg of dry milk, 600 μl of 5 M NaCl, 19.4 ml of IMC) was gently distributed by hand and incubated for 1 h at 25 °C. After decanting the liquid, 20 ml of the induced cultures (supplemented with 200 mg of dry milk, 100 μl of 10% Tween 20 and 600 μl of 5 M sterile NaCl) was added to the dish. Bacteria were exposed to the inorganic surface at 25 °C for 1 min at 50 rpm and without agitation for 1 h. At the end of the incubation period, the surface was dip-washed twice in freshly prepared wash solution (10 ml IMC) using sterile tweezers. The surface was transferred to a fresh washing bath (as described), and the wash procedure was repeated five times. To recover binders, the surface was transferred to a sterile culture dish containing 10 ml of IMC, and the plate was vortexed for 1 min to shear off the flagella. The solution was transferred to a sterile 125 ml shake flask containing 40 ml of IMC, and the cells were grown overnight at 30 °C. This completed one round of panning. The entire panning process was repeated four times to enrich for tight binders. Overnight cultures obtained after the final panning

ID	Title	Pages
2621	Selection process generating peptide aptamers and analysis of their binding to the TiO2 surface of a surface acoustic wave sensor	7