Biomaterials 76 (2016) 282–291

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Cell mechanosensory recognizes ligand compliance at biomaterial interface

Chiara Cosenza [a, b], Vincenzo Lettera [a, b], Filippo Causa [a, b, c, *], Pasqualina Liana Scognamiglio [a, b], Edmondo Battista [a, b], Paolo Antonio Netti [a, b, c]

[a] Center for Advanced Biomaterials for Health Care@CRIB Istituto Italiano di Tecnologia, Largo Barsanti e Matteucci 53 80125 Napoli, Italy
[b] Interdisciplinary Research Centre on Biomaterials (CRIB) University of Naples Federico II Piazzale Tecchio 80, 80125 Napoli, Italy
[c] Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale (DICMAPI), University "Federico II", Piazzale Tecchio 80, 80125 Naples, Italy

A R T I C L E I N F O

Article history:
Received 29 July 2015
Received in revised form
22 October 2015
Accepted 26 October 2015
Available online 27 October 2015

Keywords:
Ligand
Material bioactivation
Peptide
Mechanosensory

A B S T R A C T

Cells activate signalling through ligand-receptor bonds by sensing the mechanical properties of the surrounding extracellular matrix (ECM). Ligands, indeed, have to withstand the pulling force elicited by cell receptors through focal adhesions (FAs). On this basis, we developed functional ligands to be simply adsorbed on surfaces and constituted by a two-domain peptide: one derived from ECM proteins and available to receptors to offer biochemical cues, and another adsorbed on material to withstand the tension upon receptor engagement. Tuneable compliance of the anchoring domain of the peptide ligand was verified by single peptide analysis through molecular dynamics and adsorption measurements. We showed that the highest adsorbed peptides combined with integrin cell-binding motifs allow for the cell recognition and polarization with larger mature FA areas. On the contrary, the lowest adsorbed sequences did not provide mechanical resistance to the integrin pulling action, leading to more rounded cells with smaller FA areas. This evidence demonstrates that cell mechanosensory can discriminate ligands on surfaces and should be considered as a criterion in ligand design for material bioactivation.
© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Cell adhesion is mediated by a complex bimolecular system from both sides of the cell–matrix interface. When cells adhere to the surface, integrins are activated and bind target ligands. The bound integrins cluster together by changing their conformation and interact with cytoplasmic focal adhesion (FA) proteins, such as paxillin, to form adhesion plaques. After their maturation, FAs bind to actin filaments of the cytoskeleton to form stress fibres. This ligand-FA-cytoskeleton chain builds the mechanical integrity of the cytoskeleton and drives the behaviour of adherent cells [1,2]. Therefore, integrins -through FAs- experience mechanical forces that are exerted by the cell and counterbalanced by ligands at material interface [3]. Forces play a critical role in integrin function and activation, allowing cells to recognize and respond to specific physical features of their microenvironment [4,5]. In particular, cellular behaviour dependence upon the mechanical properties of a material surface is defined as cellular mechanosensory [6,7]. It was also demonstrated that there is a linear dependence between FA area and values of exerted forces [5,8,9]. Thus, after a specific interaction occurring between cellular receptors and ligands, cells sense the forces, activate intracellular signalling pathways and commutate the mechanical signals into biochemical ones, regulating such diverse processes as cell adhesion, polarization, migration, proliferation and differentiation [10].

Researchers have developed different approaches to promote the material surface bioactivation: physical methods, based on adsorption or self-assembly processes, and chemical methods, requiring covalent surface modification [11,12]. Physical methods have used proteins from the extracellular matrix (ECM) or their small derived bioactive peptides merely adsorbed on biomaterial surfaces dealing with unpredictable, nonspecific and potentially unstable interactions both with the cells and the material surface [13]. In this respect, we have already demonstrated that cells are capable to discriminate between covalently bound and adsorbed bioactive peptide on polymeric surfaces with preferential recognition of the conjugated one [14]. Meanwhile, in the last decade

* Corresponding author. Center for Advanced Biomaterials for Health Care@CRIB, Istituto Italiano di Tecnologia, Largo Barsanti e Matteucci 53 80125 Napoli, Italy
E-mail address: causa@unina.it (F. Causa).

http://dx.doi.org/10.1016/j.biomaterials.2015.10.068
0142-9612/© 2015 Elsevier Ltd. All rights reserved.

different amino acid sequences have been screened for specific and strong adsorption against inorganic or synthetic materials [15,16].

In this work, we overcome the disadvantages of the physical approach by designing peptide ligands, whose sequence contains two linear domains [17–19]: one, properly designed to be able to bind and be anchored to the materials surface, and the other one, containing a ligand motif present in ECM proteins, that is well known to regulate cellular behaviour, such as adhesion and spreading. The design we propose is provided in Fig. 1. We evaluated whether two-domain peptide ligands were able to create a mechanically stable interface, by preserving a sufficiently strong interaction of the anchoring part on the material after the pulling actions occurring during the cytoskeleton assembly. In particular we investigated the conformations of each peptide ligands at material interface, the compliance of the anchoring domain and the role of the relative position of the bioactive sequences on the cell recognition process. As a proof of principle, gold was selected as the material model and a gold-binding peptide as the anchoring domain, while the bioactive domains were either the IKVAV or RGD sequences.

2. Materials and methods

2.1. System construction and computational protocol

The AuΦ3 gold binding peptide used as the anchoring domain [20] and the two-domain ligand sequences are reported in Table 1. We first built random coil structures of each peptide and equilibrated the sequences in water box in the NPT ensemble at 300 K and one atmosphere for 10 ns The final conformations of the equilibration procedure were used as the starting structures for the peptide-water-gold simulations. In order to assess the rotational conformational sampling of the system, five to six independent simulations were run. Starting structures in the independent boxes were generated by rotating the peptides 45° around the head-tail axis. A gold surface of (81 × 81 × 20) Å was constructed using the Inorganic Builder module in VMD [21] from multiples of the unit cell of Au{111} surface. The gold slab was composed of five layers. Each peptide was placed with the centre of mass at 20 Å above the top layer of the gold surface and the system was solvated using equilibrated water molecules (around 9200 water molecules) through the VMD solvation package. Ions were added in order to neutralize the solution. The simulation box was constructed in a way to avoid interactions between the peptides and their nearest periodic image. We employed the CHARMM-METAL force field [22]

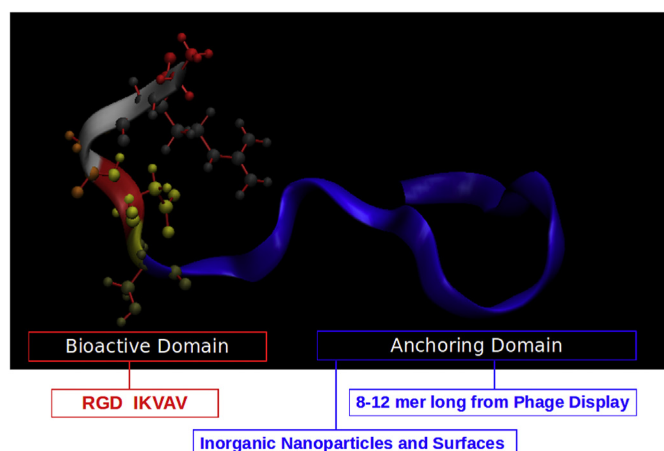

Fig. 1. Design of the two-domain peptide ligands composed of inorganic- and cell-binding motifs.

Table 1
AuΦ3 and two-domain peptide ligands. Amino acids in one letter code.

Name	Sequences
AuΦ3	TLLVIRGLPGAC
IKVAV-AuΦ3	IKVAVTLLVIRGLPGAC
AuΦ3-IKVAV	TLLVIRGLPGACIKVAV
GRGDS-AuΦ3	GRGDSTLLVIRGLPGAC
AuΦ3-GRGDS	TLLVIRGLPGACGRGDS

for the gold surface Lennard-Jones parameterization, CHARMM 22 force field [23] for the peptides and the TIP3 model for the water molecules. Gold atoms were kept fixed during the simulations. We followed the dynamics of the AuΦ3 and the bioactive sequences up to 100 ns, with 1 fs integration time step in the NVT ensemble. System coordinates were saved every 2.5 ps. We employed the NAMD package [24] to perform the calculations using periodic boundary conditions. The length and width of the periodic box were set to the size of the slab, so that a continuous infinite gold surface was constructed. During the production runs, the systems were first minimized for 800 steps, then heated at 300 K. Particle Mesh Ewald method was used with a grid spacing of 1 Å for Coulomb contributions. For van der Waals interactions the cutoff was set to 12 Å, with a smooth function between 10 and 12 Å. To keep the temperature constant the Langevin Dynamics control was employed with a damping coefficient of 2/ps.

2.2. Simulation data analysis

To assess the interaction dynamics of each amino acid during the adsorption event and the various configurations the peptides assumed, we measured alpha-carbon distances from the gold surface at several time ranges. We extracted the alpha-carbon coordinates from the trajectories and computed the averages over 1 ns of the trajectory simulation. In order to have a general picture of the adsorption parts in the whole trajectory, we also constructed adsorption maps of the system according to a given threshold. As in previous studies [25], we consider an atom adsorbed if it has displaced the second water layer from the gold surface, in particular if the alpha-carbon distance from the metal surface goes below 5 Å. Coloured parts of the maps represent the adsorption event occurrence. We also compiled a ranking of the amino acids in the chain, based on the percentage time they were adsorbed during the simulations and we compared the sequences. Data elaboration and graphical representation were performed using *ad hoc* scripting procedures in the MatLab©(MathWorks) environment. In order to assess the flexibility of the peptides in the adsorbed state, we classified the conformational ensemble, generated in the last 50 ns of trajectory, into clusters. We set a root-mean-squared deviation (RMSD) of 2 Å on backbone atoms to generate ten different clusters and classified the number of frames in a population according to previously proposed criteria [26]. The conformations were analysed adding the Clustering package to VMD (available at http://physiology.med.cornell.edu/faculty/hweinstein/vmdplugins/clustering/). We also extracted 3D images of the most representative cluster of population for each of the simulated sequences at the gold–water interface in the last 50 ns

2.3. Materials

Reagents for peptide synthesis (Fmoc-protected amino acids, resins, activation, and deprotection reagents) were purchased from Iris Biotech (Germany). Solvents for peptide synthesis and HPLC analyses were purchased from Sigma Aldrich (Saint Louis, USA). Reversed phase columns for peptide analysis were purchased from

Download English Version:

https://daneshyari.com/en/article/6485283

Download Persian Version:

https://daneshyari.com/article/6485283

Daneshyari.com