Colloids and Surfaces B: Biointerfaces 141 (2016) 558–564

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Exploring of protein – protein interactions at the solid – aqueous interface by means of contact angle measurements

I. Grabowska[a,*], W. Dehaen[b], H. Radecka[a], J. Radecki[a]

[a] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland
[b] Department of Chemistry, KU Leuven, Celestijnenlaan 200F, B-3001 Leuven, Belgium

ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form 15 January 2016
Accepted 2 February 2016
Available online 4 February 2016

Keywords:
Cu(II) or Ni(II) complexes with dipyrromethene (DPM)
Histidine-tagged VC1 domain of Receptor for Advanced Glycation End Products (RAGE)
RAGE interactions with S100B
Contact angle measurements
Acid-base interfacial properties

ABSTRACT

In this article we present the results of the studies on interactions between the VC1 domain of the Receptor for Advanced Glycation End Products (RAGE) and its ligand, the S100B protein, performed by contact angle measurements. Histidine-tagged (His_6) VC1-RAGE domain was covalently bonded to Cu(II) or Ni(II) complexes with dipyrromethene (DPM) self-assembled on gold surface. The method based on the theory of van Oss was used for the purpose of determining the Lifshitz–van der Waals (γ^{LW}) component as well as the electron acceptor—electron donor (the Lewis acid–base, γ^{+}–γ^{-}) parameters of the VC1-RAGE-S100B complex. Moreover, the surface free energies of the interactions between the VC1 domain attached to the surface and the ligand present in the aqueous phase were determined. The specificity of the VC1- RAGE interactions with the ligand studied was also proved.

1. Introduction

The immobilization of proteins on solid substrates has recently attracted much attention [1–3]. Many studies are related to the exploration of new materials for protein adsorption or methods of uniform and controlled protein immobilization. Highly efficient immobilization of biomolecules on the surface, retaining their full activity and stability, is a crucial step in biosensing technology [4–6].

Special attention has been paid to immobilization of histidine (His_6)-tagged proteins onto substrates coated with metal cations chelated by nitrilotriacetic acid (NTA), iminodiacetic acid (IDA) or pentetic acid (DPTA) [7–16]. This reaction is based on the high affinity of six histidines (called His-tag) to divalent metal cations: Ni^{2+}, Cu^{2+}, Co^{2+} or Zn^{2+} and formation of coordination bonds between the histidine imidazole ring and the metal cations. The main advantages of this strategy are controlled protein immobilization and good homogeneity of the adsorbed layer [17]. Furthermore, the His-tag could be genetically incorporated into any recombinant proteins without damage of native and biologically active structure [18].

A method for the creation of a redox-active complex based on Cu(II) ions with dipyrromethene (DPM) on the surface of gold electrodes has been recently developed [19,20]. Moreover, this new system was applied for specific immobilization of His-tagged proteins on the surface of a gold electrode [21].

The dipyrromethenes are fully conjugated flat bipyrrolic molecules, which upon deprotonation form strong chelates with metal ions, such as Ni^{2+}, Cu^{2+}, Zn^{2+} or Co^{2+}, just to name of few [22]. These complexes are neutral with regard to the electric charge [22]. Using the surface plasmon resonance technique, we have demonstrated that the binding capacity of the His-tagged Janus kinase 2 protein to the DPM-Cu(II) complex is comparable to the Biacore NTA-chip based on a NTA-modified dextran layer [11]. Thus, it has been proved that a DPM-Cu(II) complex having strong affinity towards His-tagged proteins is suitable for the creation of biofunctional surfaces.

The interactions between the Receptor of Advanced Glycation End Product (RAGE) and its specific ligands, such as amyloid-β or S100B protein were studied using bi-functional redox active layers [12,13,15,21]. RAGE is a member of immunoglobulin (Ig) superfamily, composed of a V-type immunoglobulin (Ig) domain and two C-type Ig domains (C1 and C2). This receptor is involved in cellular signalling events upon binding of variety of ligands, such as

* Corresponding author.
E-mail address: i.grabowska@pan.olsztyn.pl (I. Grabowska).

http://dx.doi.org/10.1016/j.colsurfb.2016.02.005
0927-7765/

a family of S100 proteins, amyloid-β or glycated proteins. RAGE interacts with a diverse set of ligands is associated with specific diseases, such as diabetes, Alzheimer or cancer [23,24].

The data gained by contact angles measurements associated with the van Oss approach [25–30] are frequently used for the calculation of Lifshitz–van der Waals (LW) and Lewis electron acceptor (acid)/electron donor (base) surface tension components of bacteria [31–33] or proteins adsorbed on solid substrates [34–37]. Although this approach can help to explain the mechanism of interaction of bacteria or proteins with the surface and the changes in their structure upon adsorption, there are not many reports on ligand-receptor interactions studied using this system.

Here, we have undertaken a study on the determination of the surface energy components (polar and apolar) of VC1-RAGE and its complex with S100B protein based on contact angle measurements with water, ethylene glycol and diiodomethane used as tested liquids. His-tagged VC1-RAGE was attached to Ni(II) or Cu(II) complexes with a dipyrromethene deposited on the surface of gold.

2. Materials and methods

2.1. Materials

The dipyrromethene-SH (DPM) was synthesized by Prochimia Surfaces Company (Poland). *N*-Acetylocysteamine (NAC), dichloromethane, copper(II) acetate, nickel(II) acetate, ethylene glycol, diiodomethane, NaCl, TRIS, $CaCl_2$, were obtained from Sigma-Aldrich (Poland). Methanol was purchased from POCH (Poland). All aqueous solutions were prepared with deionized and charcoal-treated water (resistivity of 18.2 MΩ cm) obtained with a Mili-Q reagent grade system (Millipore, Bedford, MA). His_6-VC1-RAGE domain and S100B protein were obtained from the Institute of Biochemistry and Biophysics of Polish Academy of Sciences (Warsaw, Poland).

2.2. The modification steps of gold substrates

The dimension of the gold substrates used in the studies was about 1 cm × 1 cm.

The gold substrates made of glass-aluminosilicate covered by 100 nm Au over a 5 nm titanium adhesion layer (Platypus Technologies, USA) were washed with Milli-Q water and subsequently with methanol followed by cleaning in a UV/ozone chamber during 15 min. After this time, the substrates were placed in a dish containing the appropriate modification solution while protecting against evaporation. The modification steps are illustrated in Scheme 1. On the other hand, the compositions of the solutions and time of modification for the subsequent steps of SAMs created are as follows:

I. **NAC/DPM SAM**: 3 h in the solution of 1 mM NAC and 0.1 mM DPM in dichloromethane, next washing with dichloromethane;

II. **NAC/DPM-Me(II) SAM**: 1 h in a solution of 1 mM metal (Ni(II) or Cu(II)) acetate in dichloromethane/methanol (V:V, 50%:50%), next washing with dichloromethane/methanol mixture and dried with nitrogen;

III. **NAC/DPM-Me(II)/His_6-VC1-RAGE SAM**: 1 h in a solution of 1 μM of His_6-VC1-RAGE domain in a buffer containing 50 mM TRIS, 450 mM NaCl, pH 7.4, next washing with the same buffer, and dried with nitrogen;

IV. **NAC/DPM-Me(II)/His_6-VC1-RAGE-S100B SAM**:

(a) 1/2 h in a solution of 10 pM of S100B protein in TRIS buffer (50 mM TRIS, pH 7.4) in the presence of 25 mM $CaCl_2$, or
(b) 1/2 h in a solution of 10 pM of S100B protein in TRIS buffer (50 mM TRIS, pH 7.4) in the absence of 25 mM $CaCl_2$.

After the creation of the above SAMs, the modified gold substrates were washed with the appropriate buffer and dried with nitrogen.

2.3. Contact angle (CA) measurements

The static contact angles (Θ) were measured with an OCA 15 instrument from Dataphysics, Germany equipped with CCD video camera. Immediately after drying with nitrogen the modified gold substrates at each step of modifications, the static contact angles were measured according to the sessile drop method at room temperature (25 °C) with 1 μl droplets of water, ethylene glycol and diiodomethane. The droplets of each liquid were placed on the modified gold substrates in such a way not to wet the same area twice. The contact angle values were determined within approximately 5 s.

3. Results and discussions

3.1. Characterization of DPM-Cu(II) and DPM-Ni(II) SAMs deposited on the surface of gold substrates and determination of the components of the surface energy

The gold substrates were cleaned using a UV/ozone chamber. Such a treatment creates a very hydrophilic gold surface. The droplet of water spreads out on it and the contact angle was almost unmeasurable. The successive modification steps caused decreasing hydrophilicity of the gold substrates. As a result, the contact angles using water, ethylene glycol and diiodomethane became possible to measure (Scheme 1).

In the first step, the procedure of deposition of a mixed SAM containing a thiol-dipyrromethene derivative on the gold substrate and an appropriate diluent was optimized. The presence of the diluent in the SAM prevents intermolecular hydrogen from bonding formation between the dipyrromethene molecules [19–21]. SAMs containing 4-mercapto-1-butanol as well as NAC used as diluent were tested. It became apparent that 4-mercapto-1-butanol was not suitable to use. Ethylene glycol and diiodomethane spread on a surface modified with a mixed SAM containing DPM and 4-mercapto-1-butanol. As a result, a contact angle was impossible to measure, probably because of the presence of OH hydrophilic groups on the surface. Finally, the solution containing 1 mM of NAC and 0.1 mM DPM in dichloromethane was selected as the best for the creation of reproducible mixed SAMs on a gold substrate.

The presence of Cu(II) and Ni(II) complexed on the surface of DPM SAM deposited on the gold support has been confirmed electrochemically [19–21,38].

In this research we studied the interactions between RAGE and S100B protein only by contact angle measurement. Histidine-tagged (His_6) VC1-RAGE domain was covalently bonded to Cu(II) or Ni(II) complexes with dipyrromethene (DPM) self-assembled on gold surface. However, the composition of self assembled monolayer containing redox active dipyrromethene-Cu(II) complex was previously electrochemically characterized [19–21].

The geometry of surface complex between dipyrromethene-Cu(II) and his-tag was described as distorted square-planar type [39–42].

Representative pictures of water droplets deposited on the surface of a gold substrate obtained after each modification step are shown in Scheme 1.

The static contact angles were recorded on solid substrates upon each step of modification, at several positions which are very close to one another. Averaging the measured values minimizes local inhomogeneities. Additionally, the use of diluent should prevent