Colloids and Surfaces B: Biointerfaces 160 (2017) 438–445

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Full Length Article

Adsorption and covalent binding of fibrinogen as a method for probing the chemical composition of poly(styrene/α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol) microsphere surfaces

Monika Gosecka[a], Mohamed M. Chehimi[b,c], Teresa Basinska[a,*], Stanislaw Slomkowski[a], Tomasz Makowski[a]

[a] *Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland*
[b] *Univ Paris Diderot, Sorbonne Paris Citeí, ITODYS, UMR CNRS 7086, 15 rue JA De Baïf, 75205 Paris, France*
[c] *Universiteí Paris Est, UMR 7182 CNRS, UPEC, 94320 Thiais, France*

ARTICLE INFO

Article history:
Received 14 May 2017
Received in revised form
21 September 2017
Accepted 24 September 2017
Available online 25 September 2017

Keywords:
Fibrinogen adsorption
Core-shell particles
Polyglycidol
Protein covalent attachment
Hydrophilic/hydrophobic surface
Polymer patches

ABSTRACT

We investigated the distribution of polyglycidol and polystyrene on the surface of poly(styrene/α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol) microspheres (random distribution or segregated into hydrophilic and hydrophobic patches), using fibrinogen (Fb) as a macromolecular probe. The fibrinogen was adsorbed or covalently attached to the surface of the poly(styrene-*co*-α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol) (P(S/PGLy)) microspheres. The P(S/PGLy) particles were prepared by emulsion copolymerization of styrene and α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol (PGLy) macromonomer initiated with potassium persulfate. The polymerizations yielded P(S/PGLy) particles with various surface fractions of polyglycidol, depending on the amount of added macromonomer and the addition process. In some syntheses, the entire macromonomer amount was added once at the beginning of the polymerization, while in others, the macromonomer was added gradually after the formation of particle seeds from pure polystyrene. XPS studies revealed that the fraction of polyglycidol in the interfacial layer of the microspheres was larger when the entire amount of macromonomer was added at the beginning of the polymerization than when it was added after formation of the polystyrene seeds. Studies of fibrinogen adsorption provided the first evidence of segregation of the hydrophobic (polystyrene) and hydrophilic (polyglycidol) components at the surface of the composite P(S/PGLy) microspheres into patches. The hydrophobic patches are composed mainly of polystyrene. However, they also contain a small amount of polyglycidol chains, making the adsorption of fibrinogen weaker than the adsorption onto the pure polystyrene. Studies of covalent immobilization of fibrinogen on the microspheres via 1,3,5-trichlorotriazine confirmed these findings.

1. Introduction

Studies of emulsion and dispersion copolymerization of comonomers that vary strongly with respect to their hydrophilicity revealed that the obtained particles have a core-shell structure with a hydrophobic core and a hydrophilic shell [1]. These observations were made for styrene-acrolein, styrene-poly(ethylene oxide) and styrene-polyglycidol macromonomer comonomer pairs [2,3,1]. The driving force for the radial separation of the hydrophobic and hydrophilic comonomer segments is due to the thermodynamics of the polymer–polymer and polymer–water interactions schematically shown in Fig. 1.

In water, the surface energy of hydrophilic polyglycidol is much lower than that of the hydrophobic polystyrene. Thus, the Gibbs free energy for polymerization yielding microspheres with hydrophilic shells (ΔG_p^I) is lower than the Gibbs free energy for polymerization yielding microspheres with an interfacial layer rich in hydrophobic polystyrene (ΔG_p^{II}).

Recent AFM observations of the granular morphology of the surface of poly(styrene-*co*-α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol) microspheres suggested that not only radial but also lateral separation of hydrophilic and hydrophobic copolymer segments may occur [4]. However, verification of the above-mentioned hypothesis is not an easy task. The resolution of the

* Corresponding author.
E-mail address: basinska@cbmm.lodz.pl (T. Basinska).

https://doi.org/10.1016/j.colsurfb.2017.09.054
0927-7765/

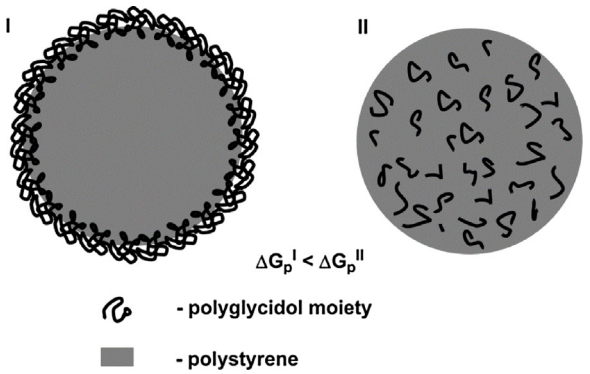

Fig. 1. Structure of microsphere with I – radial separation of hydrophobic (polystyrene) and hydrophilic (polyglycidol) components and with II – random distribution of both components.

existing experimental techniques, such as XPS, EDX and infrared microscopy, is not sufficiently high to detect the differences in chemical composition at the submicrometer level. Atomic force microscopy is primarily used for investigations of the surface morphology and hardness. While special variants of AFM have found some limited application to studies of the surface chemical composition, to the best of our knowledge, the AFM-related techniques have never been used for mapping the chemical composition of surfaces of the micrometer-scale spherical objects.

This paper describes the results of our studies for the characterization of the surface of P(S/PGLy) microspheres through the adsorption of protein molecules used as probes. Fibrinogen was selected for our studies. Fibrinogen has a tri-nodular structure. It may adopt co-linear (cylindrical or prolate spheroidal) and bend three-arm conformations [5]. The length of fibrinogen was determined by various researchers to be 48.7 and 84.7 nm, depending on whether it exists in the bent or co-linear conformations, respectively [6,7]. Thus, fibrinogen could be used for probing inhomogeneous surfaces with the domain size of the order of a few tenths of nanometers.

Our studies were motivated by the following considerations. Let us assume that there are surfaces composed of the polystyrene and polyglycidol chain segments with two limiting distributions of the respective polymer chains. In the first case, the polystyrene and polyglycidol blocks are randomly distributed, and in the second case, the polymers are segregated into patches composed almost exclusively of either polyglycidol or polystyrene, with patch dimensions exceeding the dimensions of the fibrinogen (Fb) probe (Fig. 2).

It is known that polyglycidol is an effective protein repellent that eliminates protein adsorption. However, upon activation of hydroxyl groups with 1,3,5-trichlorotriazine, polyglycidol immobilizes proteins covalently. In contrast, the surface of polystyrene is hydrophobic and facilitates protein adsorption. However, without aggressive chemical modification, polystyrene is not suitable for protein covalent immobilization. Thus, one may expect that in the case of the P(S/PGLy) microspheres with a patchy surface, the maximum surface concentration of adsorbed protein would be proportional to the polystyrene content in the interfacial layer. However, in the case when polyglycidol and polystyrene chains are distributed randomly in the interfacial layer of the adsorbing surface, the dependence of maximum surface concentration of adsorbed protein on the polystyrene fraction would be different. Namely, one may expect that for a set of microspheres with increasing polystyrene content in the interfacial layer, during the initial increase in the polystyrene fraction, the randomly distributed polyglycidol chains may still protect the entire surface from protein adsorption. At the stage when the polystyrene fraction exceeds a certain threshold value, the maximum surface concentration of adsorbed protein should rapidly increase and become stabilized at a plateau so that the concentration does not exhibit any dependence on further increases in the polystyrene fraction. By analogy, in the case of the microspheres with patchy surfaces, the maximum surface concentration of covalently immobilized protein should be proportional to the polyglycidol fraction.

Obviously, the distribution of the polystyrene and polyglycidol chains in the interfacial layer of the P(S/PGLy) microspheres may differ from the above-described cases that correspond to the limiting cases. However, any intermediate distribution of polyglycidol (or polystyrene) fractions in the particles interfacial layer should also influence the adsorption/covalent immobilization of the protein.

Fibrinogen was chosen as the probe because the dimensions of this protein (48.7×4.1 nm for the prolate spheroid approximation) are much larger than the dimensions of the polyglycidol chains. However, it should be noted that they are smaller than the diameters of the investigated microspheres (microsphere diameters ranged from 220 to 650 nm). Thus, the dimensions of fibrinogen may be close to the possible dimensions of the hydrophilic/hydrophobic patches on the particle surface (if the patches are formed).

2. Materials and methods

2.1. Reagents and other materials

Styrene (Aldrich) was purified from the stabilizer (4-*tert*-butylcatechol) by distillation at 30 °C under reduced pressure. Potassium *tert*-butoxide (Sigma-Aldrich), ethyl vinyl ether (Fluka), potassium persulfate ($K_2S_2O_8$, Fluka), methanol, $AlCl_3 \times 6H_2O$ (Sigma-Aldrich), Hyflo Super Gel (Sigma-Aldrich), and 1,3,5-trichlorotriazine (Sigma-Aldrich) were used without further purification. *p*-Chloromethylstyrene (Sigma-Aldrich) was distilled at 80 °C under reduced pressure. Triple-distilled water with pH adjusted to 6.8 by the addition of the necessary amount of K_2CO_3 was used for the synthesis of the microspheres. Deionized water (ADRONA system) was used for the incubation of particles and the preparation of phosphate buffered saline (PBS; pH = 7.4, I = 0.2 M).

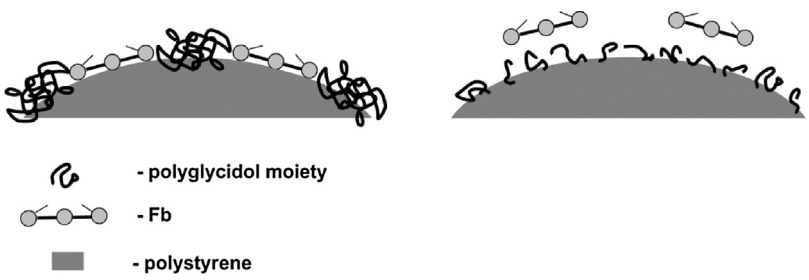

Fig. 2. Schematic illustration of fibrinogen interactions with surface of microspheres with patchy and random distributions of polyglycidol.