Colloids and Surfaces B: Biointerfaces 121 (2014) 331–339

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Branched polymer models and the mechanism of multilayer film buildup

Pradeep Waduge, Dhan B. Khadka [1], Donald T. Haynie *

Nanomedicine and Nanobiotechnology Laboratory, Center for Integrated Functional Materials, Department of Physics, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620, USA

ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 15 April 2014
Accepted 4 June 2014
Available online 12 June 2014

Keywords:
Buildup
Dendrimer
Mechanism
Multilayer
Peptide
Thermodynamics

ABSTRACT

The "in and out diffusion" hypothesis does not provide a conclusive explanation of the buildup displayed by some polyelectrolyte multilayer film systems. Here, we report initial tests of an alternative hypothesis, on which the completion of each adsorption cycle results in an increase in the number of polymer binding sites on the film surface. Polycationic dendrimeric peptides, which can potentially bind several oppositely-charged peptides each, have been designed, synthesized and utilized in comparative film buildup experiments. Material deposited, internal film structure and film surface morphology have been studied by ultraviolet spectroscopy (UVS), circular dichroism spectroscopy (CD), quartz crystal microbalance (QCM) and atomic force microscopy (AFM). Polycations tended to contribute more to film buildup than did polyanions on quartz but not on gold. Increasing the number of branches in the dendrimeric peptides from 4 to 8 reproducibly resulted in an increase in the film growth rate on quartz but not on gold. Peptide backbones tended to adopt a β-strand conformation on incorporation into a film. Thicker films had a greater surface roughness than thin films. The data are consistent with film buildup models in which the average number of polymer binding sites will increase with each successive adsorption cycle in the range where exponential growth is displayed.

1. Introduction

Polyelectrolyte multilayer films are of interest in fundamental research and technology development [1–4]. Applications of these films include optical coatings, coatings for cell pattering, drug delivery vehicle coatings and various other film technologies [3–9]. The use of polyelectrolytes to fabricate functional nanocomposite coatings has attracted considerable attention worldwide [1–4,10–12].

The method by which polyelectrolyte multilayer nanofilms are usually made is layer-by-layer assembly [1]. A thin film is formed by dipping a solid material with a charged surface, the substrate for assembly, into a dilute solution of oppositely-charged polyelectrolyte. Loosely bound polymer is removed from the film surface by rinsing. Additional layers are prepared in the same way, alternating the charge on the polyelectrolyte in successive adsorption steps. The result can be a highly uniform multilayer thin film of precisely controlled thickness, architecture and functionality. The simplest polyelectrolyte multilayer nanofilm consists of one polycation species and one polyanion. Many different polymer species are suitable for incorporation in films. A film can be fabricated on a surface of virtually any size, shape, or roughness. Nano-composites are made by substituting appropriately charged nanoparticles or other entities for polyelectrolytes [4].

There have been hundreds of experimental and theoretical studies on polyelectrolyte multilayer films by many research groups over the past two decades [1,3,5–10,12–14]. Nevertheless, significant points regarding film assembly and structure are unclear or disputed. What is known and generally accepted is that, in the typical case, the adsorbing chemical species undergo spontaneous but self-limited assembly, driven by electrostatic attraction and entropy increase under externally imposed conditions. Oppositely-charged polyelectrolytes then bind each other during the adsorption process by non-stereospecific Coulombic interactions on the film surface, releasing loosely bound counterions. The resulting entropy increase is generally greater than the entropy decrease due to the loss of translational degrees of freedom of polymers. Unknowns include details of the mechanisms of film buildup in specific cases. Explaining exponential versus linear growth in the amount of material deposited per adsorption step has

* Corresponding author. Tel.: +1 813 974 7793; fax: +1 813 974 5813.
E-mail address: dhaynie@usf.edu (D.T. Haynie).
[1] Present address: Division of Science & Environmental Policy, California State University, Monterey Bay, 100 Campus Center, Seaside, CA 93955, USA.

http://dx.doi.org/10.1016/j.colsurfb.2014.06.012
0927-7765/

been an aim of many studies [15–26]. Different models of buildup have been proposed. Roughly categorized as the "island" model [20,27], the "surface roughness" model [17,19] and the "in and out diffusion" model [18,21,28,29], these viewpoints are not mutually exclusive. Difficulties facing the third view have been discussed in recent work [26].

Here, we report results of a test of a film buildup hypothesis in which exponential growth involves the formation of dendritic structures on the film surface. On this view, the number of polymer binding sites increases in successive adsorption steps, because the number of incompletely charge-compensated polyelectrolyte molecules increases, giving an exponential rise in the amount of material deposited, at least in the range where such growth occurs; the growth mode could become linear at a later stage of the buildup process. Peptides were adopted as model polymers. Dendrimeric forms of poly(L-lysine) (PLL) were designed and synthesized, and multilayer films of the dendrimers were fabricated with poly(L-glutamic acid) (PLGA) by electrostatic layer-by-layer assembly. The dendrimeric PLL molecules were 4- or 8-branched, each with a nominal degree of polymerization (DP) of 30. UVS and CD were utilized to monitor film buildup and polymer structure in films, QCM to measure mass deposited and AFM to characterize film surface morphology and roughness. Taken together, the data from different methods provide a consistent perspective on film buildup.

2. Materials and methods

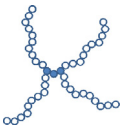
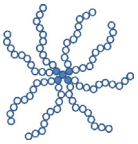
2.1. Polymers

The linear polypeptides of this study were lyophilized PLL hydrogen bromide (molecular mass 30–70 kDa, "medium"; ≤150 kDa, "large") and lyophilized PLGA sodium salt (molecular mass 3–15 kDa, "small"; 15–50 kDa, "medium"; 50–100 kDa, "large"), all from Sigma, Inc. (USA). These polymer preparations, which are polydisperse, were utilized as received. The dendrimeric polypeptides were prepared from multiple antigenic peptide (MAP) resins for solid-phase F-moc synthesis (Calbiochem, USA) at the 25-μmol scale. (For a discussion of MAP synthesis, see Ref. [30].) The usual purpose of MAPs, viz. to generate polyclonal antibodies that recognize certain linear epitopes, was irrelevant here. 4-branched and 8-branched MAPs were made. About 80 mg of product was obtained in each case. MALDI–TOF mass spectrometry analysis showed mass values that represented an integral number of branches of the predicted mass. In the 8-branched synthesis product, for example, species corresponded to the predicted mass for 8 branches of DP 30, 7 branches of DP 30, 6 branches of DP 30, and so on. Qualitatively, the same result was obtained for the 4-branched synthesis product. A more detailed analysis of the distribution of dendrimeric species was not carried out; it was assumed that if the average number of branches was not greater for the 8-branched than the 4-branched species (which seems improbable), at the least the width of the distribution must be greater. The dendrimeric peptides were also characterized by dynamic light scattering (Malvern Zetasizer Nano S, United Kingdom). The results are shown in Table 1. For comparison, the contour length of a 30-residue peptide is roughly 10 nm; the hydrodynamic radius will depend on pH and be largest when the probability of side chain ionization is highest.

2.2. Substrates

Quartz microscope slides (50 × 25 mm^2) from ChemGlass (USA) were cut into 25 × 10 mm^2 pieces for use as substrates for film analysis by UVS, CD and AFM. Each piece was prepared for film fabrication by immersion in 1% sodium dodecyl sulfate at 80 °C for 30 min, rinsing with 1% NaOH in ethanol/H_2O (50/50 v/v%) for 3 h, immersion in piranha solution (3:1 H_2SO_4 and 30% H_2O_2; considerable caution is required in the preparation and handling of this substance), rinsing with ultrapure deionized water, and drying with a stream of N_2 gas. Gold-coated QCM resonators (Q-Sense, Sweden), 14 mm in diameter and less than 1 mm thick, were exposed to UV light for 10 min, immersed in 5:1:1-mixture of deionized water, 25% ammonia and 30% hydrogen peroxide at 75 °C for 5 min, rinsed extensively with deionized water, and dried with a stream of N_2 gas.

Table 1
Polymer species of this study.

Abbreviation[a]	Description[b]	Schematic[c]	Particle radius (nm)[d]
4-K_{30}	4-Branched K_{30}		9.7 ± 0.9
8-K_{30}	8-Branched K_{30}		7.1 ± 0.6
PLL-M	Linear $K_{205–479}$		N.D.[e]
PLGA-L	Linear $E_{330–660}$		N.D.
PLGA-M	Linear $E_{99–330}$		N.D.
PLGA-S	Linear $E_{20–99}$		N.D.

[a] L = "large", M = "medium", S = "small"; see text for details.
[b] K = lysine and E = glutamic acid; X_n = n residues of X.
[c] Filled circles, lysine-based MAP core. Open circles, lysine residues added by solid-phase synthesis (dendrimeric peptides) or lysine or glutamic acid residues added by solution-phase synthesis (linear peptides).
[d] By dynamic light scattering.
[e] N.D. = not determined.