Cysteine-containing Polypeptides Allow Control over Various Properties of Biodegradable Multilayer Nanofilms

Donald T. Haynie and Yang Zhong

Bionanosystems Engineering Laboratory, Center for Applied Physics Studies, Louisiana Tech University, Ruston, LA 71272

Statement of Purpose: Study of weak polyelectrolytes in multilayer film assembly began about 1995 (Rubner, 2003). The linear charge density of a weak polyelectrolyte will depend substantially on pH. Polyelectrolyte multilayer film fabrication by layer-by-layer assembly (LBL) allows nanometer-scale control over film thickness (Decher, 1997). Polypeptides, which constitute about half of the dry mass of a living organism, are a biodegradable type of weak polyelectrolyte. The earliest work on polypeptide multilayer films involved homopolypeptides available from commercial sources (Cooper et al., 1994; Chluba et al., 2001). Since 2004 there has been increasing realization of the greater potential of designed polypeptides for multilayer nanofilm fabrication (Haynie et al., in press). Here, we present results of our exploration of the extent to which physical, chemical, and biological properties of multilayer nanofilms can be controlled by incorporation of cysteine into 32mer polypeptide chains. The results suggest that considerable control is possible in all three areas, promising for the future of biomaterials.

Methods: The polypeptides were $(KVKV)_7KVKY$ (P0), $(EVEV)_7EVEY$ (N0), $(KVKGKCKV)_3KVKGKCKY$ (P1), $(EVEGECEV)_3EVEGECEY$ (N1), $(KCKGKCKV)_3KCKGKCKY$ (P2), and $(ECEGECEV)_3ECEGECEY$ (N2). P1, N1, P2 and N2 contain cysteine, P2 and N2 twice as much as P1 and N1. All peptides were prepared by solid-phase synthesis (Li & Haynie, 2004). Films were assembled by LBL with peptides dissolved in aqueous buffer at pH 7.4 (Zheng et al., 2005) and disulfide crosslinked under mild oxidizing conditions (Li et al., in press). Film stability was assessed by immersing the films in aqueous buffer at pH 2 and monitoring the change in an observable quantity (Li & Haynie, 2004), here UV absorbance and ellipsometric thickness (Zhi & Haynie, 2004; Zhong et al., in press).

Results / Discussion: Film growth of all three pairs of peptides, namely P0-N0, P1-N1 and P2-N2, is linear by UV absorbance and by ellipsometry (Fig. 1a). The behavior of P2-N2 closely resembles that of P1-N1 but differs from that of P0-N0. Polypeptide multilayer film formation does not depend on disulfide bond formation. Differences in secondary structure of the films are evident by analysis of circular dichroism (CD) spectra (Fig. 1b); note amplitude of Cotton effects and shape of spectra. Differences in number of cysteine residues per unit length of peptide translate into differences in disassembly behavior of oxidized films (Fig. 2). In general, the more cysteine residues per unit length, the slower the disassembly kinetics and extent of disassembly after several hours.

Free thiols in adsorbed peptides could be used to tether different kinds of molecule featuring a free thiol. Because disulfide bond formation is reversible (Creighton, 1993), these molecules could then be released in response to a change in the reducing potential of the environment.

Conclusions: Differences in assembly behavior and disassembly behavior must be attributable to differences in amino acid sequence. Disulfide crosslinks stabilize polypeptide multilayer films, similar to how they stabilize native protein structure. Free thiols could also be useful for stimulated release of thiol-containing compounds in a reducing environment. Polypeptide films are biodegradable. Other biological properties, for instance immunogenicity, will depend on sequence (Zheng et al., 2005). Polypeptide multilayer films are promising for biomaterials development.

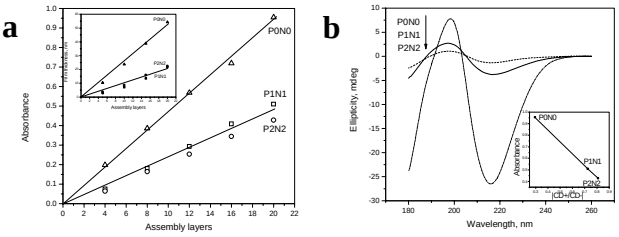

Fig. 1. Assembly of polypeptide films. (a) UV spectroscopy. Inset, ellipsometry. Agreement is good. (b) CD spectroscopy.

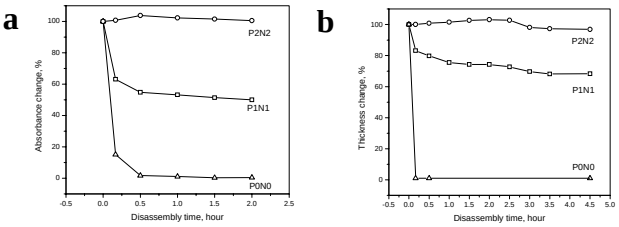

Fig. 2. Disassembly of polypeptide films. (a) UV spectroscopy. (b) Ellipsometry. Agreement is good.

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