STRUCTURE, DYNAMICS, AND PROPERTIES OF ARTIFICIAL PROTEIN HYDROGELS ASSEMBLED THROUGH COILED-COIL DOMAINS

Thesis by

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In Partial Fulfillment of the Requirements

for the degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2005

(Defended December 15, 2004)

Acknowledgements

I thank my thesis advisors, Professor David Tirrell and Professor Julia Kornfield, for their support and guidance through these years. I have been lucky to have the opportunity to work with two excellent scientists at Caltech. Their enthusiasm for science and brilliant intellectual insights have inspired me very much. I have also been deeply impressed by their dedication to assisting and supporting students in many ways, from discussing writing skills to counseling career choices. Their attitudes towards science and people will have profound influences on me during my career development.

I thank the former and current members in the Tirrell lab and the Kornfield lab. They have made my stay at Caltech enjoyable. Both labs provide me environments where I can share and discuss my thoughts on any issues about science, career, or life. And they are always willing to help. I particularly want to thank Jill Sakata, Giyoong Tae, and Michael Mackel. Jill patiently taught me the basic molecular biology techniques when I first started to work in the lab. Discussions with Giyoong were helpful for me to quickly capture the issues that needed to be addressed in my thesis project. Michael has given me tremendous help in improving my writing skills in English.

I also thank Professor Anand Asthagiri and Professor Zhen-gang Wang for their support and valuable discussions. My interactions with their students, including Niki Galownia, Nicholas Graham, Claudiu Giurumescu, and Chengzhong Zhang, also helped me make progress on my research project. I thank Professor Zhen-gang Wang for recruiting me to

Caltech in the first place. My opportunity to pursue my Ph.D. in such a wonderful institute has many constructive influences on my career.

I would like to thank the whole Caltech community for providing such a close and friendly environment. My interactions with many individuals on campus have given me a lot of pleasant memories.

I thank my parents and my sister for their love through all these years.

I want to dedicate this thesis to my husband, Ming Li. Without his love, support, and patience, I probably would not be where I am now.

Abstract

The structure-property relationships of an artificial protein hydrogel, which was constructed from a triblock protein (designated $AC_{10}A$) that contained two associative leucine-zipper endblocks and a water-soluble random coil midblock, were investigated to provide guidelines for the rational design of new generations of artificial protein hydrogels. The leucine zipper A domain is composed of six heptad repeating units designated as *abcdefg*, where the *a* and *d* positions are occupied by hydrophobic residues, and the *e* and *g* positions are mainly occupied by glutamic acid residues. In contrast to hydrogels formed from synthetic hydrophobically modified polymers, the normalized plateau storage modulus G'_{∞}/nkT of the $AC_{10}A$ gel was below 13% at all concentrations examined. This indirect evidence that $AC_{10}A$ tends to form a substantial fraction of looped configurations was supported by a fluorescence quenching experiment: significant quenching occurred in labeled d-$AC_{10}A$-a (d=tryptophan at the N-terminus, a=coumarin at the C terminus) chains mixed with a great excess of unlabelled $AC_{10}A$ chains in a solution. The strong tendency to form loops originates in large part from the compact size of the random coil midblock domain (mean $R_{H,\ C10}$~20 Å, determined from quasi-elastic light scattering of C_{10}). Despite the small aggregation number of the leucine zipper domains (tetrameric aggregates, determined from multi-angle static light scattering of AC_{10} diblock), the average center-to-center distance between aggregates in a 7% w/v $AC_{10}A$ solution is roughly 3 times the radius of gyration and 1.5 times the average end-to-end distance of the C_{10} domain. To avoid the energy penalty for stretching the C_{10} domain to form bridges, the chains tend to form loops. The importance of loops explains the nonmonotonic effect of pH on modulus and the decrease in modulus with increasing

ionic strength. It also led to the design concept of increasing the midblock length or charge density to increase storage modulus.

Dynamic properties of the $AC_{10}A$ hydrogel show correlation between network relaxation behavior and molecular exchange kinetics of the associative domain. The longest stress relaxation time changes from ca. 70 seconds at pH 8.0 to ca. 1000 seconds at pH 7.0, determined by creep measurements on 7% w/v gels. In a parallel manner, the characteristic time of the leucine zipper strand exchange varies from ca. 200 seconds at pH 8.0 to ca. 4500 seconds at pH 7.0, determined by fluorescence de-quenching after mixing a fluorescein-labeled leucine zipper solution (in which fluorescence was quenched) with a great excess of an unlabeled leucine zipper solution. Both time scales vary strongly with pH due to the associated change in charge on the *e* and *g* residues of the leucine zipper.

The observed structure-property relationships suggest that the rapid dissolution that occurs with $AC_{10}A$ hydrogels in open systems originates from the tendency of the protein to form loops, the small aggregation number of the associative domains, and the transient nature of association. For applications in which materials are surrounded by excess fluids, we demonstrated two molecular design approaches to avoid the rapid dissolution. One way to slow dissolution is to suppress loops by engineering a triblock protein with dissimilar associative endblocks, $PC_{10}A$, such that P associates only with P and A associates only with A. A $PC_{10}A$ gel erodes 500 times more slowly and exhibits a 5-fold increase in modulus compared to an $AC_{10}A$ gel at the same concentration. Alternatively, hydrogel stability in open systems can be improved by engineering a cysteine residue into each leucine zipper domain to allow covalent bond formation

following physical association of leucine zippers. Asymmetric placement of the cysteine residue in each leucine zipper domain suppresses locking-in loops and creates linked "multichains". The increased valency of the building units stabilizes the hydrogels in open systems, while the physical nature of their association retains the reversibility of gelation. The gel networks dissolve at pH 12.2, where the helicity of the leucine zipper domains is reduced by ca. 90%, and re-form upon acidification.

The ability to form robust artificial protein hydrogels in open systems opens the way to biomedical applications. Therefore, we examined their toxicity and incorporated an RGD cell-binding domain into the midblock backbone. Viability assays for mammalian 3T3 fibroblast cells cultured in the presence of the $AC_{10}A$ protein revealed no evidence of toxicity. Anchorage-dependent epithelial cells spread well on hydrogel films bearing an RGD cell-binding domain. In contrast, cells remained round on films without the cell-binding domain; significant apoptosis was induced. Encapsulated 3T3 fibroblast cells remained viable inside the hydrogel for at least 12 hours, suggesting that these materials have proper permeability for transferring oxygen, nutrients, and metabolic waste. The hydrogel containing the RGD domain was micropatterned on a PEG-modified glass surface and limited cell adhesion to the hydrogel region.

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