

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

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Abstract

The structure-property relationships of an artificial protein hydrogel, which was constructed from a triblock protein (designated AC₁₀A) 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 AC₁₀A gel was below 13% at all concentrations examined. This indirect evidence that AC₁₀A tends to form a substantial fraction of looped configurations was supported by a fluorescence quenching experiment: significant quenching occurred in labeled d-AC₁₀A-a (d=tryptophan at the N-terminus, a=coumarin at the C terminus) chains mixed with a great excess of unlabelled AC₁₀A 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 $R_{H, C_{10}} \sim 20 \text{ \AA}$, determined from quasi-elastic light scattering of C₁₀). Despite the small aggregation number of the leucine zipper domains (tetrameric aggregates, determined from multi-angle static light scattering of AC₁₀ diblock), the average center-to-center distance between aggregates in a 7% w/v AC₁₀A solution is roughly 3 times the radius of gyration and 1.5 times the average end-to-end distance of the C₁₀ domain. To avoid the energy penalty for stretching the C₁₀ 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 AC₁₀A 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 AC₁₀A 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, PC₁₀A, such that P associates only with P and A associates only with A. A PC₁₀A gel erodes 500 times more slowly and exhibits a 5-fold increase in modulus compared to an AC₁₀A 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 AC₁₀A 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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