51c Enhanced Protein Delivery from Photopolymerized Hydrogels

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Abstract summary

Undesired protein-polymer interactions within delivery matrices frequently decrease the amount of protein available for delivery. This abstract describes a method for enhancing cumulative release of protein from poly(ethylene glycol) hydrogels by using a pseudo-specific protein ligand. A model was developed to predict the release behavior of BSA in the presence of the metal chelator iminodiacetic acid. This model quantitatively predicts the improved release characteristics of BSA as ligand concentration and ligand affinity increase. Introduction

Polymeric hydrogels, such as poly (ethylene glycol) (PEG), have been widely used as controlled delivery matrices[1]. The physicochemical properties of the hydrogel can be altered to control the release behaviors of protein drugs. Differing from drugs with small molecular weights, proteins have complex structures as well as multiple reactive polypeptides that commonly induce undesired protein-polymer interactions. These reactions can reduce protein stability or permanently immobilize protein within the artificial matrix, thereby lowering the fraction of active protein within the gel available for release.

In this abstract, a chelating agent, iminodiacetic acid (IDA), was used as a protecting agent to diminish protein-polymer interactions and thus enhance the cumulative release of bovine serum albumin (BSA) from PEG hydrogels. Divalent metal ions such as copper, zinc, and nickel were also used synergistically with the IDA to evaluate the effect of ligand affinity on the degree of protein protection.

A model based on the known dissociation constants was established to predict the total amount of protein released from the hydrogels.

Experimental Methods

Di-acrylated poly(ethylene glycol) (PEGDA) was used as a macromer to form the hydrogel network via photopolymerization. PEGDA (10 wt %), BSA (5 wt %) and the photoinitiator Irgacure 2959 (I-2959, 0.2 wt %) were mixed together in phosphate buffer solution (PBS, pH7.4). Required amounts of IDA and divalent metal ions were also added. The mixed solution was exposed to UV radiation at a wavelength of 365nm to rapidly form a protein-loaded hydrogel network of desired dimensions in a single step.

After photopolymerization, gels were placed in pH 7.4 PBS for in vitro protein release. Supernatant solution was sampled to quantify BSA cumulative release. Protein concentration was quantified by fluorescamine staining.

Results and Discussion

Photopolymerization is a simple yet effective method for preparing protein-loaded hydrogels. However, the protein is subjected to UV and free-radical exposure during hydrogel fabrication. Without proper protection, a substantial portion of the protein can be irreversibly immobilized within the hydrogel

networks. As shown in Figure 1, the amount of protein available for release depends on the initial protein loading concentration. The higher the absolute amount of loaded protein, the greater the fraction released. This is hypothesized to occur because the protein-reactive free radical is the limiting reactant.

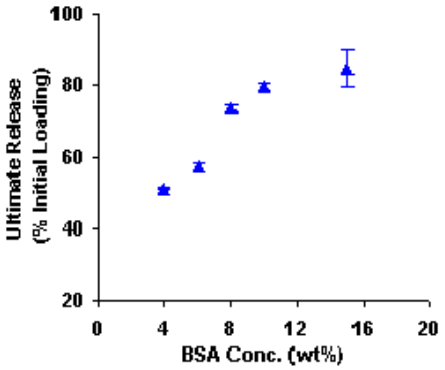

Figure 1. Percentage of BSA ultimately released from a PEG hydrogel as a function of initial loading concentration. (Ultimate release ≡ $M_{t=infinity}/M_{loading}$)

To increase the amount of protein available for delivery and therefore improve therapeutic efficacy, we incorporated IDA as a protecting agent to prevent BSA from becoming denatured and/or permanently immobilized within the network. The binding of proteins to ligands such as IDA provides a shielding effect and thus greatly decreases non-specific protein-polymer interactions. As shown in Fig. 2, when IDA and different concentrations of copper ions were added into the mixed solution prior to photopolymerization, BSA release from the crosslinked hydrogel can be enhanced to a final value of 100% at high copper concentrations. The increased amount of BSA released is mainly due to the affinity between BSA and IDA-Cu^{2+} complex. The addition of other divalent ions such as zinc and nickel also increase BSA cumulative release. However, due to the weaker affinities of Zn^{2+} and Ni^{2+} to IDA, the protective effect provided by these metals is lower than that provided by copper. The cumulative release shown in Figure 3 verify the well-known sequence of binding strengths to IDA[2], i.e. Cu^{2+}> Zn^{2+}> Ni^{2+}.

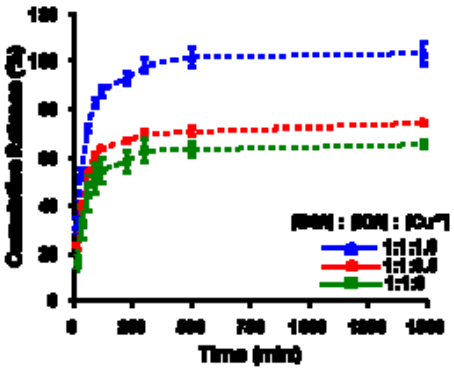

Figure 2. The effect of [IDA-Cu^{2+}] on enhancing BSA release from PEG hydrogels. (Cumulative release ≡ $M_t/M_{loading}$; BSA loading concentration: 5 wt %)

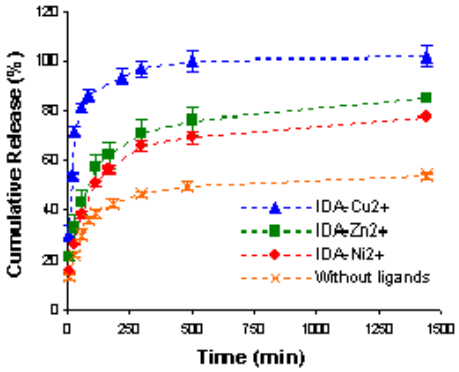

Figure 3. The effect of different divalent metal ions on enhancing BSA released from PEG hydrogels. All components were added in equal molar ratios. (BSA loading concentration: 5 wt %)

A model was developed to predict the protein ultimate release after the addition of protein-binding ligand.

$$K_d = \frac{[P]\times[L]}{[P\cdot L]} = \frac{([P]_0 - x)\cdot([L]_0 - x)}{x}$$

$$U_R = \frac{[P]}{[P]_0} = 1 - i\cdot\frac{([P]_0 - x)}{[P]_0} = 1 - \frac{i\cdot x\cdot K_d}{([L]_0 - x)[P]_0}$$

Where:

K_d = Dissociation constant.

U_R = Protein ultimate release.

i = Percentage of non-specific protein immobilization occurring without ligand protection([Ligand] = 0)

x = Conc. of ligand-bound protein

$[P]_0$ = Protein loading conc.

$[L]_0$ = Ligand loading conc.

BSA ultimate release can be plotted as shown in Figure 4 based on the known dissociation constant between BSA and IDA-Cu^{2+} (K_d = 0.0082mM)[3] and between BSA and IDA (K_d = 0.40mM). Comparison to experimental data confirms the model's accuracy and indicates that conditions leading to greater binding between ligand and protein result in greater protein release efficiencies.

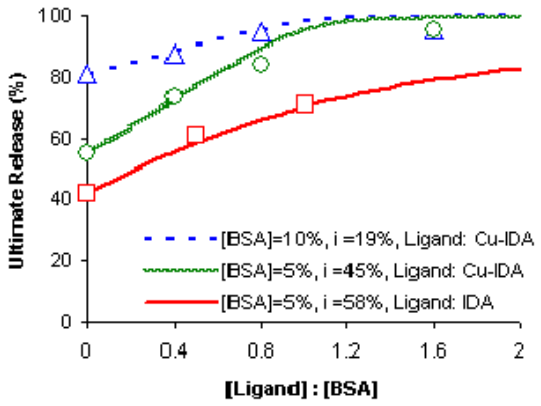

Figure 4. The prediction of experimental BSA release data by the theoretical model. i values were determined experimentally. (Symbols: experimental data; lines: theoretical predictions)

Conclusions

This study provides a plausible strategy for protecting proteins during in situ hydrogel formation via chain polymerization and crosslinking by reversibly binding them to soluble ligands. With the addition of IDA and divalent metal ions to the prepolymer solution, the fraction of BSA successfully released from crosslinked PEG hydrogels can be greatly increased. Specifically, protein (BSA) ultimate release can be increased by:

(1) Increased protein (BSA) loading concentration

(2) Increased ligand concentration (IDA)

(3) Proper selection of divalent metal ion (Cu^{2+}> Zn^{2+}> Ni^{2+})

References

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2. Ueda EKM. et. al. *J Chromatogr A*. 2003 (988):1-23.

3. Wu CY et al. *J Chromatogr A*. 2003 (996):53-70.

Acknowledgements

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