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Cover story

Maintaining protein activity during hydrogel cross-linking

Controlled release of therapeutic proteins remains a major challenge in formulation development. To safeguard bioactivity over extended periods of time, the protein molecules are often embedded in suitable carrier systems. Among these, *in situ* forming hydrogels are particularly attractive for protein delivery [1]. For protein loading, the hydrogel forming polymers are often cross-linked in concentrated protein solutions. Popular cross-linking reactions include radical polymerizations, Michael-type additions, and Diels–Alder reactions. A common feature of these reactions is the involvement of electrophiles such as acryloyl, vinyl sulfone or maleimide groups. For example, maleimide can act as a Michael acceptor or dienophile in Diels–Alder reactions. While these cross-linking reactions are quite effective, their lack of selectivity represents an important disadvantage. During covalent cross-linking, the involved electrophiles can react with nucleophilic groups of proteins such as cysteine, lysine, histidine, tryptophan or arginine residues [2]. These side reactions can impair the cross-linking process; on the other hand, protein molecules are covalently bound to the polymer network. This may lead to incomplete protein release, loss of bioactivity and increased immunogenicity.

The paper by Gregoritza, Goepferich and Brandl in this issue describes the use of polyanions to prevent side reactions during cross-linking [3]. In their study, a number of pharmaceutically relevant polyanions, namely alginate, dextran sulfate, heparin, hyaluronic acid and poly(acrylic acid), were screened for their capability to protect proteins during hydrogel formation. Lysozyme, which served as a model protein, was incubated with furyl- and maleimide-substituted methoxy poly(ethylene glycol). Without polyanions, side reactions between polymer and lysozyme were observed. For example, at pH 7.4, 61.1% of the total lysozyme amount was PEGylated; the residual activity decreased to 20.3%. Upon the addition of polyanions, protein conjugation and activity loss could be significantly reduced. For example, by adding dextran sulfate, PEGylation could be completely suppressed; the residual activity was 98.4%. In addition, hydrogels were loaded with lysozyme and bevacizumab. In the presence of polyanions, both proteins were mobile and their release was diffusion-controlled; without polyanions, a large fraction of the incorporated proteins was covalently bound to the polymer network and released during gel degradation.

The study by Gregoritza et al. presents a number of interesting observations. It could be demonstrated that the addition of polyanions is a simple, yet powerful, tool to reduce undesired protein conjugation and subsequent loss of bioactivity. Cross-linking reactions, if occurring between polymer and protein molecules, reduce protein stability and availability. However, such polymer/protein cross-linking reaction during *in situ* encapsulation can be avoided, if polyanions are added. Since the association of proteins with polyanions is through electrostatic interactions, it is reversible at high salt concentrations. Since the addition of polyanions is a relatively mild approach, it does not cause protein structural changes, protein aggregation, or fragmentation. The "shielding" effect of polyanions was shown to be effective at pH ranges from 5 to 9. Furthermore, typical cryoprotectants, such as sucrose or trehalose, did not interfere with the protective effect of the polyanions. This is particularly important when lyophilized proteins are incorporated into hydrogels. Another advantage of the approach is that only small amounts of polyanions (at the concentrations of 1~2 mg/ml) are required to achieve protective effects. Thus, the physicochemical properties of the delivery system, such as network mesh size, swelling behavior or degradability, are not affected. The paper is particularly important as it demonstrates that adding polyanions to protein drugs during polymer cross-linking can prevent unwanted polymer/protein cross-linking. The beauty of this approach is in its simplicity, and it opens up a new avenue of making sustained protein delivery systems through *in situ* cross-linking reactions, facilitating the development of hydrogel-based protein delivery systems.

References

[1] T. Vermonden, R. Censi, W.E. Hennink, Hydrogels for protein delivery, Chem. Rev. 112 (2012) 2853–2888.
[2] G.T. Hermanson, Bioconjugate Techniques, Academic Press, Inc., Amsterdam, Netherlands, 2013.
[3] M. Gregoritza, A.M. Goepferich, F.P. Brandl, Polyanions effectively prevent protein conjugation and activity loss during hydrogel cross-linking, J. Control. Release 238 (2016) 92–102.

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