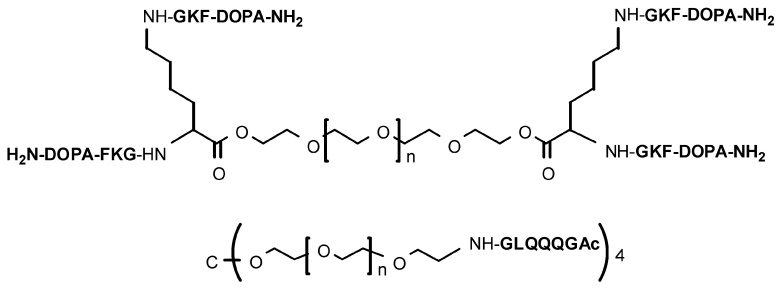
Communication

Rational Design of Transglutaminase Substrate Peptides for Rapid Enzymatic Formation of Hydrogels

Bi-Huang Hu, and Phillip B. Messersmith

J. Am. Chem. Soc., **2003**, 125 (47), 14298-14299• DOI: 10.1021/ja038593b • Publication Date (Web): 31 October 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 12 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/31/2003

Rational Design of Transglutaminase Substrate Peptides for Rapid Enzymatic Formation of Hydrogels

Bi-Huang Hu and Phillip B. Messersmith*

Biomedical Engineering Department, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208

Received September 18, 2003; E-mail: philm@northwestern.edu

Polymer hydrogels have been extensively investigated as materials useful for drug delivery, tissue repair, and tissue engineering.[1−3] With increasing frequency, polymer hydrogels are being designed for in situ gelation from a liquid precursor, allowing minimally invasive administration via syringe and needle.[4−9] Although solidification of such materials can be triggered by a variety of chemical and physical mechanisms,[10] from the biological perspective it is particularly attractive to utilize protein cross-linking enzymes, such as transglutaminase (TGase). TGase catalyzes an acyl-transfer reaction between the γ-carboxamide group of protein-bound glutaminyl residues and the ϵ-amino group of Lys residues, resulting in the formation of ϵ-(γ-glutamyl)lysine isopeptide side-chain bridges.[11] Although several biological fluids are known to undergo rapid TGase-catalyzed hydrogel formation,[12,13] previous attempts to use TGase to catalyze gelation of peptide modified synthetic polymers have resulted in slowly gelling systems.[14−16] In this paper, we describe the rational design of short peptide substrates of TGase, their conjugation with a biocompatible polymer, and gelation of the polymer-peptide conjugates within the few minutes desired for many medical applications. Furthermore, we report for the first time the incorporation of L-3,4-dihydroxyphenylalanine (DOPA), an adhesive amino acid found in marine mussel proteins,[17] into synthetic polymer hydrogels by TGase enzyme.

The Lys and Gln substrate peptides investigated in this study (Table 1) were designed to possess basic features of known biomacromolecular and synthetic peptide substrates of TGase. The Gln substrate peptides contained 2−5 contiguous Gln residues, based on evidence that peptides become better TGase substrates with increasing length of Gln repeats,[18,19] and that proteins containing two or more adjacent Gln residues are known to be good substrates.[20,21] A Leu residue was placed adjacent to the Gln near the C-terminus in several peptides, because this has been shown to result in a significant increase in Gln specificity.[22] Regarding the Lys substrate peptides, it has been shown that the composition and sequence of the amino acids adjacent to lysine residues in peptide and protein substrates can have an effect on the amine specificity.[23,24] For example, previous model peptide studies showed a significant increase in specificity when the residue adjacent to the Lys on the N-terminal side was changed from Gly to a more hydrophobic residue (Leu).[25] Hence, several of the peptides investigated in this study contained hydrophobic residues (Leu or Phe) in this position. The effect of DOPA incorporation, either immediately adjacent to or separated from Lys by a Leu/Phe, was also investigated. Finally, in all peptides a Gly residue was added on the C-terminal side to act as a spacer between the peptide and the polymer in the peptide-PEG conjugates, so that the peptide in the conjugate may be more accessible to enzyme.

The peptides were assayed as TGase substrates by monitoring the rate of the cross-linking reaction with a known dansyl labeled substrate. The use of fluorescently labeled compounds and RP-HPLC analysis with the aid of LC-ESI/MS allowed for identification of products and comparison of the kinetic constants of the candidate peptides (see Supporting Information). The specificity ($k_{cat}/K_{m,app}$) values determined under identical experimental conditions reflect the relative specificity of the enzyme toward the substrates; peptides with higher specificities are better substrates for TGase enzyme, and therefore the enzyme-catalyzed cross-linking reaction of that peptide will proceed more rapidly.

Table 1. Peptide Sequences and Their Substrate Specificity toward Tissue TGase

peptide sequence	$k_{cat}/K_{m,app}$[a]
Ac-KG-NH_2	10.6
FKG-NH_2	61.6
LKG-NH_2	48.4
DOPA-KG-NH_2	
Ac-FKG-NH_2	560
Ac-LKG-NH_2	482
DOPA-FKG-NH_2	1324
DOPA-LKG-NH_2	1179
Ac-GQQQLG-NH_2	34.1
DOPA-GQQQLG-NH_2	47.9
NH_2-GQLKHLEQQEG-NH_2	47.3

[a] min^{-1} mM^{-1}. For details, see Supporting Information.

Under the conditions of our experiments, the specificities of the Lys peptides varied by several orders of magnitude, whereas the specificities of the Gln peptides varied only modestly (Table 1). The acetylated dipeptide Ac-KG-NH_2 had a specificity of 10.6 (min^{-1} mM^{-1}) for TGase. Addition of a hydrophobic residue (F or L) to the N-terminus of the dipeptide resulted in an approximately 5-fold increase in specificity, and acetylation of the N-terminus of the F/LKG-NH_2 tripeptide further increased the specificity ca. 10-fold.

Incorporation of a DOPA residue directly preceding the Lys residue of the peptide KG-NH_2 caused the side-chain primary amine of the Lys to entirely lose its ability to serve as an acyl acceptor. However, addition of an N-terminal DOPA residue to the tripeptides Ac-F/LKG-NH_2 resulted in ca. 2.4-fold increases in specificity. Remarkably, the specificities of tetrapeptides DOPA-F/LKG-NH_2 were enhanced ca. 100-fold as compared to the dipeptide Ac-KG-NH_2. To our knowledge, this is the first successful demonstration of DOPA incorporation into TGase substrates.

More subtle differences were noted in the specificities of the Gln-containing peptides, with all three designed Gln peptides exhibiting good substrate properties. It is interesting to note that the specificities of short peptides Ac-GQQQLG-NH_2 and DOPA-GQQQLG-NH_2 compared favorably to the specificity of NH_2-GQLKHLEQQEG-NH_2, a peptide derived from the repeat motif found in the keratinocyte protein involucrin, which is known to be an excellent substrate for TGase.[20]

On the basis of their high specificities, DOPA-FKG and Ac-GQQQLG were selected and separately coupled to PEG to form PEG-peptide conjugates **1** and **2** shown in Scheme 1 (for details of

10.1021/ja038593b CCC: $25.00

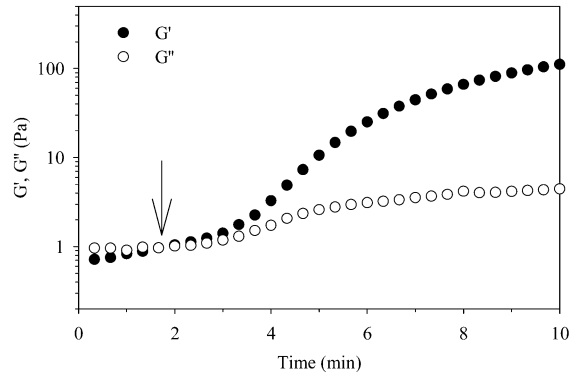
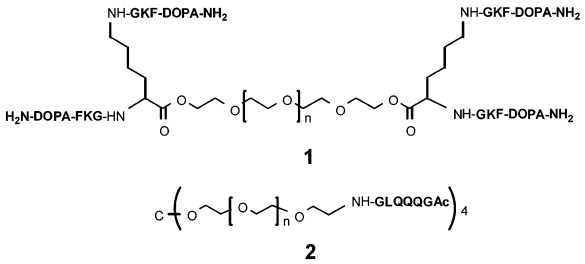


Figure 1. Oscillatory rheology of a solution containing **1**, **2**, and TGase. The arrow indicates the time at which the gel point was reached.

Scheme 1. Structures of PEG-Peptide Conjugates **1** and **2**



the synthesis, see Supporting Information). The PEG-peptide conjugates were analyzed and purified by RP-HPLC, and their structures were confirmed by MALDI TOF-MS analysis.

In the presence of TGase, an aqueous fluid containing equimolar amounts of PEG-peptide conjugates **1** and **2** formed a hydrogel within minutes under physiologic conditions. Rheological studies indicated that the hydrogel formed in less than 2 min, as indicated by the crossover of the storage (G') and loss (G'') moduli in the gelation experiment (Figure 1).[26] The elastic nature of the cross-linked gel was demonstrated by constant values of G' obtained over several decades of frequency in an oscillatory frequency sweep experiment, and the resulting hydrogel was found to be highly elastic, as indicated by constant values of G' at up to 100% strain during a strain sweep experiment (see Supporting Information). Formulations in which **1** was replaced with PEG end-functionalized with single Lys residues (Lys-PEG-Lys) did not form gels under identical conditions (data not shown), demonstrating the low reactivity of a single Lys residue, and emphasizing the importance of identifying Lys and Gln peptides with high TGase reactivity.

The conjugated peptides clearly retained their TGase substrate characteristics after coupling to PEG, as evidenced by the rapid gelation time observed. This finding is consistent with the results of several other studies, in which TGase peptides were shown to retain activity after conjugation to PEG.[14−16,27] In the present case, if we assume that only one Gln residue in each of the four Ac-GQQQLG peptides in **2** is reactive (LC-MS showed this to be the case), theoretical calculations show that a fractional peptide conversion of only 0.33 is necessary for network formation to occur.[15] Thus, the multifunctionality of the PEG-peptide conjugates facilitates rapid gel network formation.

The gelation time demonstrated here approaches that desired for tissue engineering and surgical adhesive applications, and the presence of DOPA suggests that these new hydrogel materials may prove useful in applications where adhesion to tissues is paramount. Although the enzyme concentration necessary for rapid hydrogel formation was considerably higher than that for TG-induced cross-linking of free peptide, this may be explained in part by the effect of gel network formation on the mobility of TGase enzyme. During incipient network formation resulting from partial cross-linking of the PEG-peptide conjugates, the solution viscosity rapidly increases while the mobility of the enzyme rapidly decreases. Higher enzyme concentration is therefore necessary to ensure sufficient enzyme is available to the cross-linking sites during the later stages of gelation, when enzyme mobility is increasingly restricted.

Acknowledgment. The authors gratefully acknowledge support from the NIH (DE13030 and DE14193), Dr. Laszlo Lorand for providing dns-ϵ-aca-QQIV, and Joel Collier for assistance with rheology experiments.

Supporting Information Available: Design, synthesis, and characterization of peptides and peptide-conjugates (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Andrade, J. D., Ed. *Hydrogels for medical and related applications*; American Chemical Society: Washington, DC, 1976; Vol. 31.
(2) Peppas, N. A., Ed. *Hydrogels in medicine and pharmacy*; CRC Press: Boca Raton, FL, 1987.
(3) Okano, T., Ed. *Biorelated polymers and gels*; Academic Press: Boston, 1998.
(4) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581−587.
(5) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature* **1997**, *388*, 860−862.
(6) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389−392.
(7) Collier, J. H.; Hu, B.-H.; Ruberti, J. W.; Zhang, J.; Shum, P.; Thompson, D. H.; Messersmith, P. B. *J. Am. Chem. Soc.* **2001**, *123*, 9463−9464.
(8) Schneider, J. P.; Pochan, D. J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. *J. Am. Chem. Soc.* **2002**, *124*, 15030−15037.
(9) Nowak, A.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D.; Pochan, D.; Deming, T. *Nature* **2002**, *417*, 424−428.
(10) Hennink, W. E.; van Nostrom, C. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 13−36.
(11) Lorand, L.; Conrad, S. M. *Mol. Cell. Biochem.* **1984**, *58*, 9−35.
(12) Fuller, G. M.; Doolittle, R. F. *Biochemistry* **1971**, *10*, 1311−1315.
(13) Williams-Ashman, H. G.; Pabalan, S. S.; Notides, A. C.; Lorand, L. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2322−2325.
(14) Sperinde, J. J.; Griffith, L. G. *Macromolecules* **1997**, *30*, 5255−5264.
(15) Sperinde, J. J.; Griffith, L. G. *Macromolecules* **2000**, *33*, 5476−5480.
(16) Sanborn, T. J.; Messersmith, P. B.; Barron, A. E. *Biomaterials* **2002**, *23*, 2703−2710.
(17) Waite, J. H. *Chemtech* **1987**, *17*, 692−697.
(18) Gorman, J. J.; Folk, J. E. *J. Biol. Chem.* **1980**, *255*, 419−427.
(19) Kahlem, P.; Terre, C.; Green, H.; Djian, P. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14580−14585.
(20) Etoh, Y.; Simon, M.; Green, H. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 51−56.
(21) Hohenadl, C.; Mann, K.; Mayer, U.; Timpl, R.; Paulsson, R.; Aeschlimann, D. *J. Biol. Chem.* **1995**, *270*, 23415−23420.
(22) Gross, M.; Whetzel, N. K.; Folk, J. E. *J. Biol. Chem.* **1975**, *250*, 4648−4655.
(23) Groenen, P.; Smulders, R.; Peters, R. F. R.; Grootjans, J. J.; Vandenijssel, P.; Bloemendal, H.; Dejong, W. W. *Eur. J. Biochem.* **1994**, *220*, 795−799.
(24) Grootjans, J. J.; Groenen, P.; Dejong, W. W. *J. Biol. Chem.* **1995**, *270*, 22855−22858.
(25) Gross, M.; Whetzel, N. K.; Folk, J. E. *J. Biol. Chem.* **1977**, *252*, 3752−3759.
(26) Ross-Murphy, S. B. *Polym. Gels Networks* **1994**, *2*, 229−237.
(27) Sato, H.; Ikeda, M.; Suzuki, K.; Hirayama, K. *Biochemistry* **1996**, *35*, 13072−13080.

JA038593B