Chemoselective Chemistry of Catechol for HA hydrogels and Conjugation of Biological Molecules

Mihyun Lee, Haeshin Lee
Department of Chemistry, KAIST, Daejeon, 305-701, Republic of Korea

Introduction

Development of chemoselective reaction is of great importance in many interdisciplinary fields such as pharmaceutics, bio/nanotechnology, tissue regeneration, and protein engineering. One representative of chemoselective reaction is click chemistry. The prime example of a click reaction is the copper-catalyzed Huisgen's 1,3-dipolar cycloaddition of azides and terminal alkynes (1). There is also a widely implemented chemoselective reaction so called native chemical ligation that has been used for total synthesis of proteins (2). One peptide fragment with N-terminal cysteine and the other peptide with thioester at C-terminus react to form ligated peptide creating a native peptide bond at the reaction site.

Herein, another useful chemoselective chemistry of catechol is presented. We found that catechol selectively reacts with amine groups at N-terminus of proteins and peptides (3). Utilizing this chemoselective property of catechol, we were able to conjugate N-terminal amine of a peptide GRNIAEIIKDI which is derived from laminin to hyaluronic acid-catechol (HA-Cat). Tryptic digestion experiment confirmed that the N-terminal amine of a peptide selectively reacted with HA-Cat conjugates. Our study indiates that the chemoselective property of catechol can be used for a wide variety of biomaterials.

Methods

Methacrylic anhydride (MA, Sigma-aldrich) was added to 1wt% HA(50 and 132kDa, Lifecore) solution in water at molar ratio of 1:5. The reaction pH was maintained between 8 and 11 and the reaction was kept in the dark at 4°C for 8 hours. The product was dialyzed (MWCO 8000) against water for 48 hours and freeze-dried.

For the preparation of MA-HA-Cat, HA was dissolved in phosphate buffered saline buffer (PBS, pH6). 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (Sigma-aldrich) and N-hydroxysuccinimide (Sigma-aldrich) were added to HA solution at molar ratio 1:1 and mixed for 9 hours. The product was dialyzed against PBS.

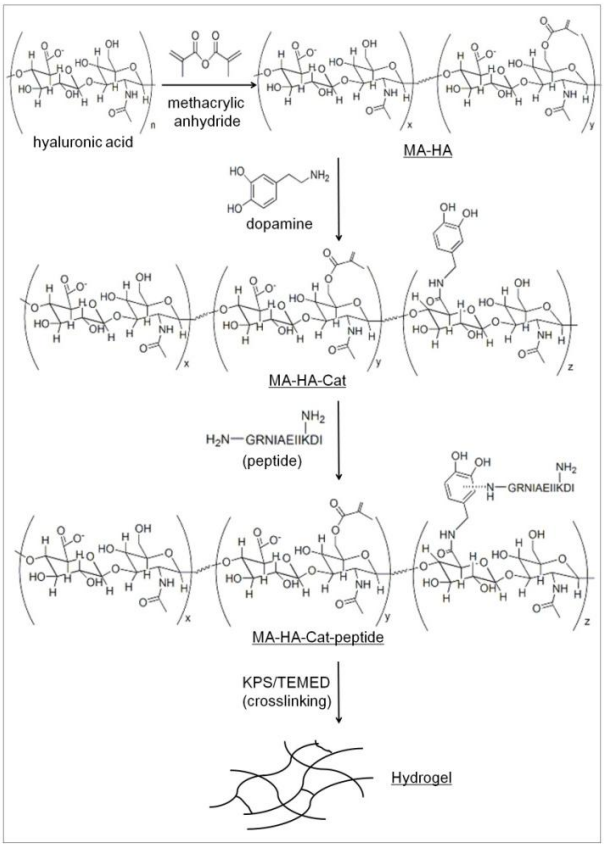
A peptide (GRNIAEIIKDI) was added to MAHA solution in PBS (pH6) at molar ratio of 1:1(peptide:Cat) for peptide binding. The reaction was kept for overnight followed by dialysis against PBS and water. The product was digested by trypsin and analyzed by LC/MS.

The sample was crosslinked to form hydrogel via radical polymerization as previously described (4).

Results

The amount of modification per HA disaccharide unit was determined by integrating the methacrylate (acrylate peaks at 5.6 and 6.0 ppm) and HA peaks (methyl resonance peak at 1.8 ppm). The synthesized HA-MA was found to be 53% modified.

Catechol content in the MA-HA-Cat was calculated from UV absorbance. The intensity measured was 0.456,



Scheme1. Preparation of peptide inserted HA hydrogel

from which the Catechol content was calculated at 1.55%.

Several peptide fragments were eluted in LC/mass spectroscopy (table1). We found that no peptides including GR were eluted.

retention time (min)	mass	sequence
18.375	800	NIAEIIK
22.3	1028	NIAEIIKDI
17.9	246	DI

Table1. LC/MS analysis of digested sample

From table1, we suggest that catechol group selectively reacts with amine group at N-terminus of peptides, not with lysine (K).

References

1) Huisgen.R., et al., Chem.Beri.Recueil, 1967; 100; 2494:2507
2) Philip.E.D., et al., Science, 1994; 266; 776:778
3) Haeshin L., et al., Science, 2007; 318; 426:430
4) Marion H.M., et al., Polymer, 2007; 48; 1915:1920