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Development of Peptide-Photooxygenation Catalyst Conjugates for Myostatin Inactivation

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Introduction

Myostatin is a protein belonging to transforming growth factor β (TGF-β) superfamily. Since myostatin negatively regulates the growth of skeletal muscle, the inactivation of myostatin activity causes an increase in muscle mass. Hence, the inactivation is expected as a promising therapeutic strategy for muscular atrophic diseases such as muscular dystrophy, cancer cachexia and disused muscular atrophy. Previously, we discovered a 23-residues myostatin-binding peptide **1** (Figure 2) from *N*-terminal sequence of a prodomain protein which forms interactions with myostatin in a latent myostatin complex [1]. This peptide reversibly binds with myostatin and inhibited its activity. To drastically improve the inhibitory effect, we developed peptide-photocatalyst conjugates which inactivate myostatin *via* photooxygenation irreversibly and catalytically (Figure 1).

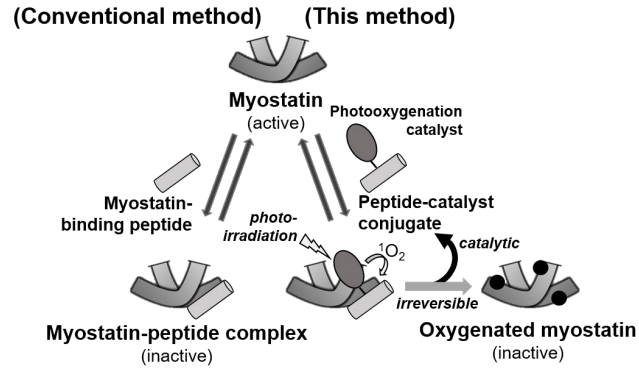

Fig. 1. Inhibition of myostatin using a conjugate of myostatin-binding peptide and photooxygenation catalyst.

Results and Discussion

We synthesized conjugate **2** with an on/off switchable photooxygenation catalyst [2] at the position 12 (Figure 2) [3]. The amino acid residue at the position 12 has been reported to show a high tolerance against structural modifications in our previous structure-activity relationship studies [4]. Since Trp is sensitive to oxidation, Trp1 of **1** was also replaced with 3,3-diphenyl propionic acid which was used as a surrogate for Trp1 in our previous study [5]. Myostatin was oxygenated with conjugate **2** under near-infrared light irradiation conditions (wavelength: 730 nm). This

	1	8	12	16	23
peptide 1	H-WRQNTRYSRIEAIKIQILSKLRL-NH_2				
conjugate 2	**U**RQNTRYSRIE**X**IKIQILSKLRL-NH_2				
conjugate 3	**Z**RQNTRYSRIEAIKIQILSKLRL-NH_2				
conjugate 4	**U**RQNTRY**X**RIEAIKIQILSKLRL-NH_2				
conjugate 5	**U**RQNTRYSRIEAIKI**X**ILSKLRL-NH_2				
conjugate 6	**U**RQNTRYSRIEAIKIQILSKLRL**X**-NH_2				

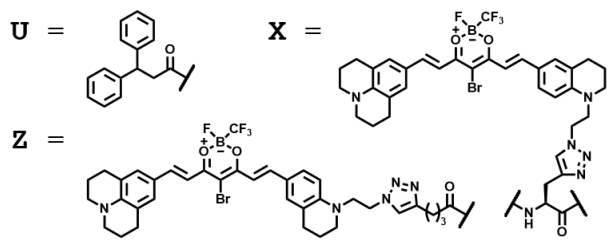

*Fig. 2. Structures of myostatin-binding peptide **1** and peptide-photocatalyst conjugates **2-6**.*

wavelength enables a high light transmission in a living tissue. No oxygenation occurred in the absence of **2** or light irradiation. Moreover, the oxygenation was significantly suppressed under degassing conditions. These results indicated that conjugate **2** induced the photooxygenation of myostatin. Then, to evaluate the inactivation of myostatin by **2**, the myostatin activity was measured by a luciferase reporter assay. As a result, the oxygenated myostatin was significantly less active than native myostatin. No inactivation of myostatin was observed in the absence of **2** or light irradiation. Therefore, myostatin was effectively inactivated by photooxygenation with **2**.

Next, we optimized the position of photooxygenation catalyst on the myostatin-binding peptide. Based on our previous Ala scan study of peptide **1** [6], Ser8 and Gln16 were selected as modification sites. Trp1 and the C-terminus were also selected since they are termini of the peptide chain. Conjugates **3**-**6** with the photooxygenation catalyst at each position were synthesized (Figure 1) [7]. Comparing the myostatin photooxygenation by conjugates **2**-**6**, all conjugates exhibited the similar photooxygenation activities. Then, to compare the myostatin selectivity, we examined the photooxygenation of off-target models such as amyloid-β and substance P. As a result, **2**-**6** induced much less photooxygenation of off-targets than methylene blue which is a nonspecific photosensitizer, while especially **2**, **4** and **5** exhibited a better selectivity than **3** and **6**. Probably because **2**, **4** and **5** have the photooxygenation catalyst in the middle part of the peptide chain, the peptide chain may prevent the catalyst part from approaching off-target molecules. Comparing the myostatin-inhibitory activities of **2**-**6**, particularly **5** with the photooxygenation catalyst at the position 16 showed the strongest myostatin inhibitory activity among all conjugates. The inhibitory activity of **5** (IC_{50} 2.1 nM) was twice higher than that of **2** (IC_{50} 4.0 nM). In addition, **5** inhibited myostatin more than 1500-fold efficiently compared to original peptide **1** (IC_{50} 3500 nM), suggesting that irreversible and catalytic inactivation of myostatin by photooxygenation is highly effective. Finally, we evaluated the cytotoxicity of the conjugate by a WST-1 assay using HEK293 cells. Conjugate **5** showed no significant cytotoxicity under both non-irradiated and irradiated conditions despite its high concentration of 3 μM, suggesting that **5** has no cytotoxicity and phototoxicity. This could be attributed to the high target selectivity of **5**.

In conclusion, we developed the new conjugates of myostatin-binding peptide and photooxygenation catalyst and optimized the catalyst-attachment position. The conjugates selectively oxygenated myostatin with near-infrared light irradiation, resulting in its efficient inactivation. These findings would contribute to a new photooxygenation-based myostatin-targeting therapy. The protein inactivation based on target-selective photooxygenation would open new therapeutic modalities for diseases.

Acknowledgments

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