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Efficient synthesis of three-helix bundle proteins using ligase-mediated trimerization of HIV-1 fusion inhibitor T20 on a dendrimer scaffold

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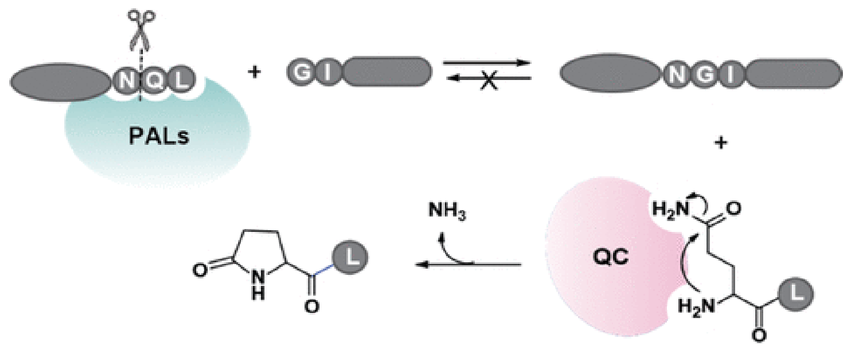
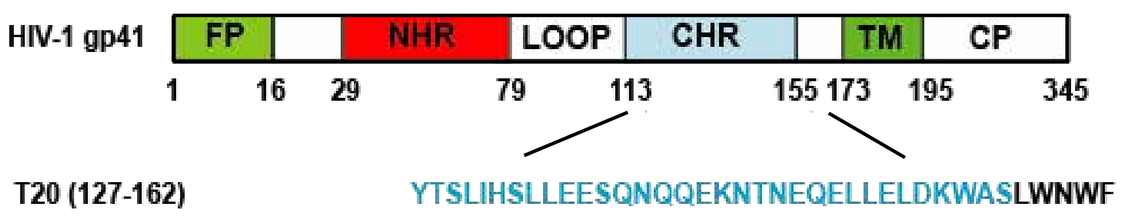
INTRODUCTION

Helix bundles are stable quaternary proteins and hold promise for developing therapeutics and synthetic vaccines. However, their synthesis, particularly by enzymatic methods, continues to be challenging. Peptide asparaginyl ligases (PAL) have been shown to be useful for precision protein conjugations, modifications and semi-synthesis. Butelase-1, which is the prototype PAL discovered in 2014 [1], is a transpeptidase because the reverse reaction could decrease the ligation yield in certain applications. To overcome this drawback, we have recently developed PAL-QC enzymatic cascade method in which a second enzyme, glutaminyl cyclase (QC) is added in the reaction mixture to prevent the reverse reaction by a glutaminyl dipeptide liberated from the PAL-mediated reactions as an inactive pyroglutamyl dipeptide [2]. Here, we report a successful synthesis of a highly stable three-helix bundle (3HB) of an HIV-1 fusion inhibitor using a PAL-QC cascade ligation approach. We used a modified 41-residue T20 (enfuvirtide) on a dendrimeric lysine-based scaffold to form a 3HB-T20 quaternary mimics by the PAL-QC ligation. Addition of QC resulted in a 78% ligation yield in 3 h, and which is much higher than a reaction without QC. A combination of CD analysis and proteolytic stability showed that the T20-trimer exhibits a 3HB structure that is highly stable to proteolytic degradation, and which was not observed in the monomer.

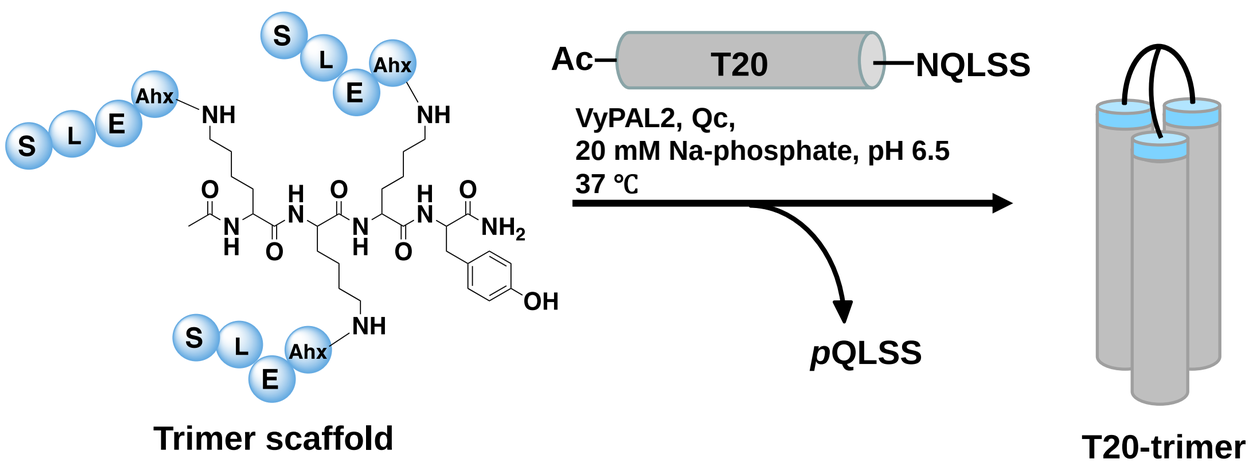


General scheme of PAL-QC enzymatic cascade method

SCHEMES



Scheme 1. Schematic illustration of HIV-1 gp41 and amino acid sequence of T20



Scheme 2. The synthesis of T20-trimer using the PAL-QC cascade ligation method

RESULTS

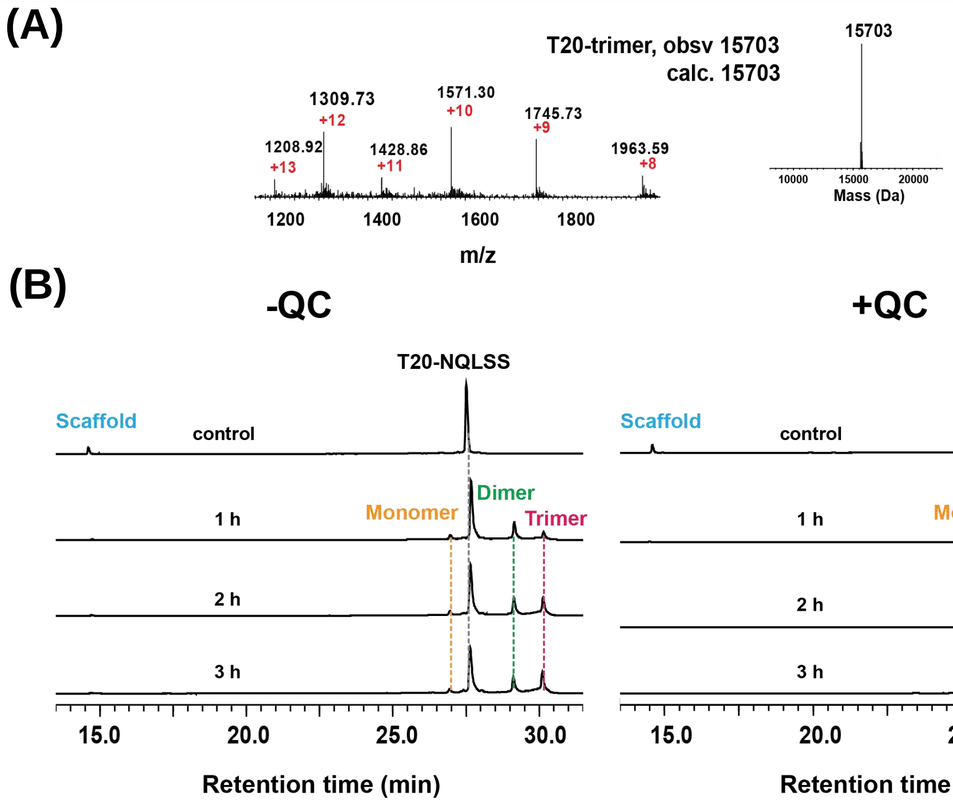
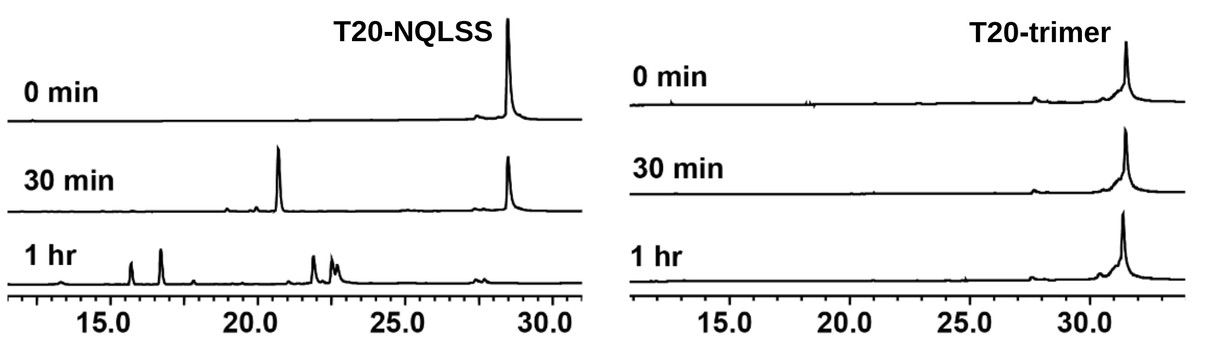


Figure 1. VyPAL2-mediated T20 trimerization with and without QC. Reaction conditions: T20-NQLSS 0.7 mM, scaffold 0.15 mM, 1.4 uM VyPAL, 0.7 uM QC in 20 mM Na-phosphate buffer, pH 6.5, 37 °C. (A). Mass of T20-trimer. (B). HPLC profile of conjugation reaction.

RESULTS



(A) Monomer T20-NQLSS digestion **(B) T20-trimer digestion**

Figure 2. Stability test of T20-NQLSS and T20-trimer against proteinase K digestion. 0.1 eq. of Proteinase K was added to the testing solution for 0, 30 and 60 min digestion.

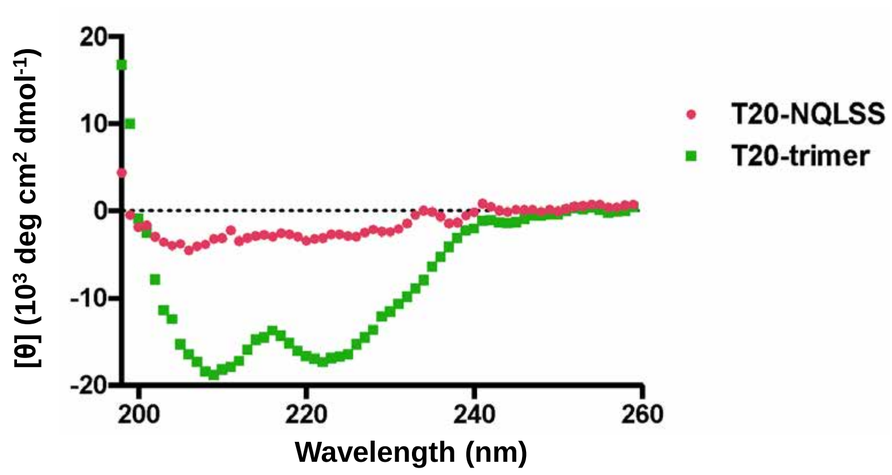


Figure 3. CD spectra of monomer- and trimer-T20 indicate that the trimer adopts an α-helix structure while the monomer does not. Solutions of 10 μM of peptides were prepared in phosphate buffer pH 7.4.

SUMMARY

In sum, our results demonstrated that PAL-QC cascade enzymatic ligation is an effective method to prepare protein multimers for therapeutic, diagnostic and biotechnological applications.

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