Design and synthesis of a new substrate of transglutaminase

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BACKGROUND

Transglutaminases (TGases) are a class of Ca^{2+}-dependent enzymes that catalyze a posttranslational acyl transfer reaction from the ɣ-carboxamide group of a peptide-bound glutamine residue to the Ɛ-amino group of a peptide-bound lysine residue[1]. The resulting product contains an isopeptidic amide bond that forms a crosslink between a variety of proteins. Transglutaminase II (TGase II) and factor XIII (FXIIIa) are specific biological transglutaminases that are essential for an organism to create barriers and stable structures in the body. TGase II is widely expressed and plays a major role in events such as cell growth, differentiation, wound healing and apoptosis *via* enzymatic modification of extracellular matrix proteins, transcription factors and signaling molecules[1]. Its crosslinking activity has been connected to arterial stiffening, which can lead to development of isolated systolic hypertension. In contrast, FXIIIa – a related mammalian TGase – stabilizes fibrin clots, is imperative for blood coagulation and is linked to osteoblast differentiation[1].

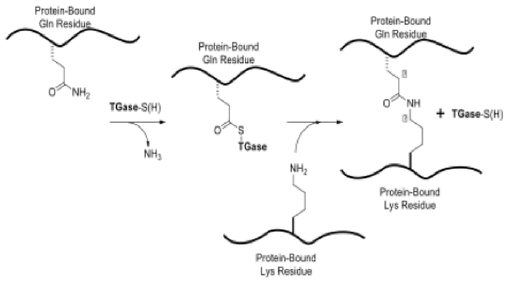


Figure 1. TGase-mediated protein crosslinking.

PURPOSE

The primary goal of this research project is to expand a current inhibitor probe library by synthesizing a new substrate peptide sequence ($7F19H_3F_7$) that will have a high affinity and substantial reactivity for FXIIIa and no selectivity for TGase II. This is conducted in order to differentiate between different biological processes that are linked between these two very closely related transglutaminases. The design of this substrate is based on a phage-displayed peptide library previously constructed by Hitomi *et al*[1]. The objective was to replace a methionine residue with a histidine in the peptide sequence $7F19M_3F_7$ that has already been characterized to have high specificity for FXIIIa. This newly incorporated amino acid residue will aid in the solubility of the peptide in the polar buffers used to test kinetic reactivity of the probes *in vitro*.

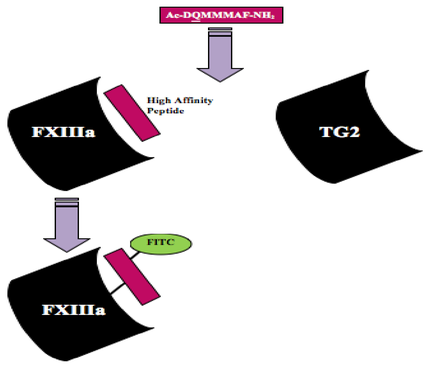


Figure 2. Selective affinity of fluorescent irreversible inhibitor probe $7F19M_3F_7$ for FXIIIa.

MATERIALS AND METHODS

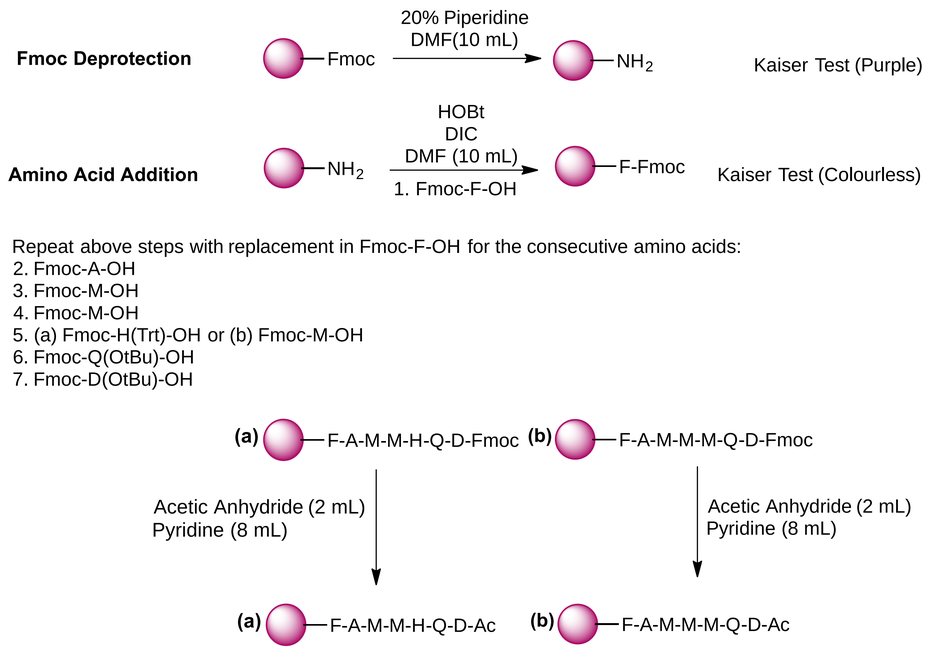
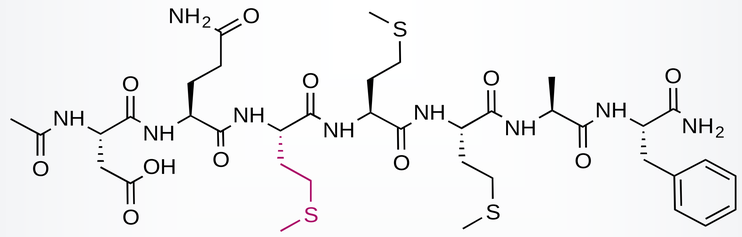


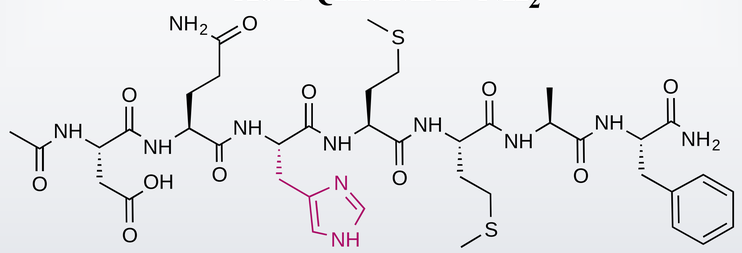
Figure 3. Synthesis of Ac-DQHMMAF-NH_2 and Ac-DQMMMAF-NH_2

Ac-DQMMMAF-NH_2



Chemical Formula: $C_{38}H_{59}N_9O_{11}S_3$
Exact Mass: 913.3496

Ac-DQHMMAF-NH_2



Chemical Formula: $C_{39}H_{57}N_{11}O_{11}S_2$
Exact Mass: 919.3680

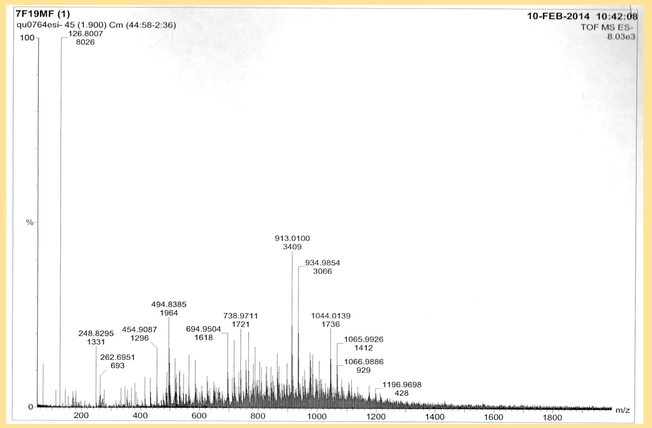
Figure 4. Chemical structures of synthesized transglutaminase inhibitors

Cleavage and purification of the peptide

Trifluoroacetic acid (TFA) is a strong acid used to detach the peptide from the resin support in order to yield the desired peptide[2]. The peptide is precipitated with ether, collected by centrifugation and lyophilized. Lyophilization is a process of drying in which water is sublimed from the product after it is frozen and is useful in preserving heat-sensitive material[3].

Reversed phase high-performance liquid chromatography (HPLC) is a technique used to separate the components in the crude peptide mixture in order to identify as well as quantify each component[4]. Silica containing non-polar hydrocarbon chains coat the column and a polar solvent (80% QH_2O and 20% ACN) is passed through. Non-polar compounds in the mixture will form attractions via van der Waals dispersion forces with the hydrocarbon groups. Polar molecules will travel through the column the fastest. The retention time is the time taken for a specific compound to travel through the column to the detector[4]. Ultra-violet absorption is used to detect when a substance has passed through the column. The peptide sequence can be isolated from the impurities by associating the desired peptide with the largest peak produced by the detector, as the area under a peak is proportional to the amount of the particular compound in the initial mixture.

RESULTS



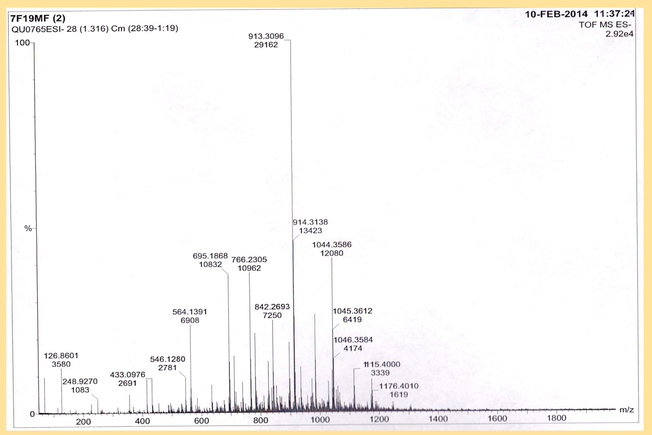


Figure 5. Mass of peptide (Ac-DQMMMAH-NH_2) confirmed using mass spectral analysis with electrospray ionization (-). m/z = 913.31. Overall yield is 24%.

CONCLUSIONS

The peptide sequence Ac-DQMMMAF-NH_2 was purified and identified via the mass spectral analysis. Once the peptide Ac-DQHMMAF-NH_2 is additionally purified, the kinetic reactivity of each synthesized substrate can be tested. If the additional histidine modification in the peptide $F19H_3F_7$ results in a higher solubilized protein, then additional kinetic parameters, such as the Michaelis constant (K_M) and the catalytic constant (k_{cat}) may be determined. This newly synthesized substrate may allow researchers to better differentiate biological processes occurring between TGase II and FXIII, as well as provide a sequence for the design of valuable selective probes for chemical knockout experiments intended to identify a specific enzyme and initiate steps toward drug design.

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