Enhancing the Gastrointestinal Stability of Salmon Calcitonin thought Peptide Stapling

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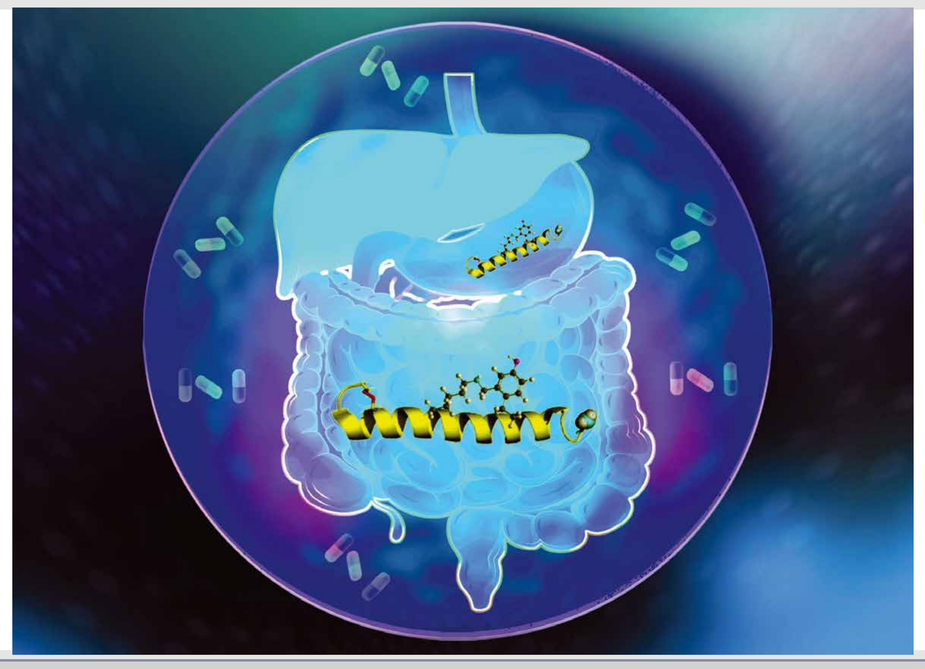
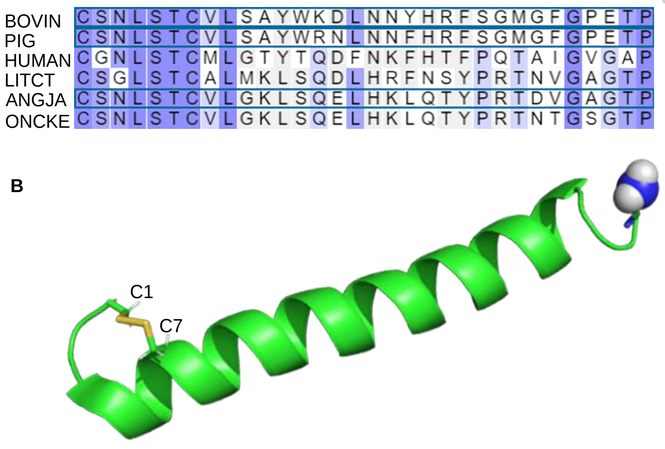
Abstract

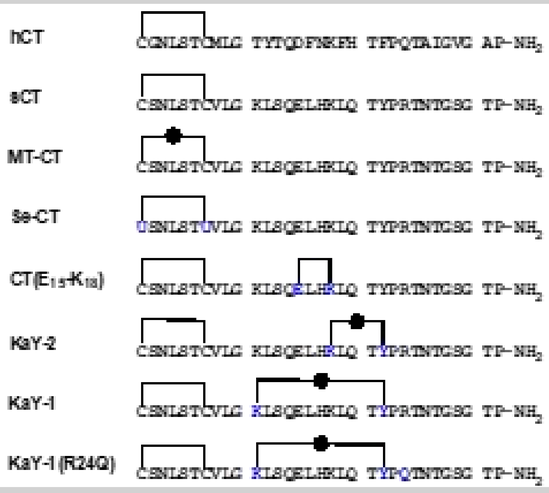
Salmon calcitonin (sCT) is a polypeptide hormone available in the clinic. sCT is degraded in the gastrointestinal tract in minutes. In this work, a stapled analogue of salmon calcitonin, KaY-1(R24Q), was developed using the cooperative stapling between Lys and Tyr, with R24Q substitution. The analogue exhibited an improved stability in simulated gastric and intestinal fluid and retain the ability to activate calcitonin receptor. This work will serve as a starting point for the development of an oral sCT drug.



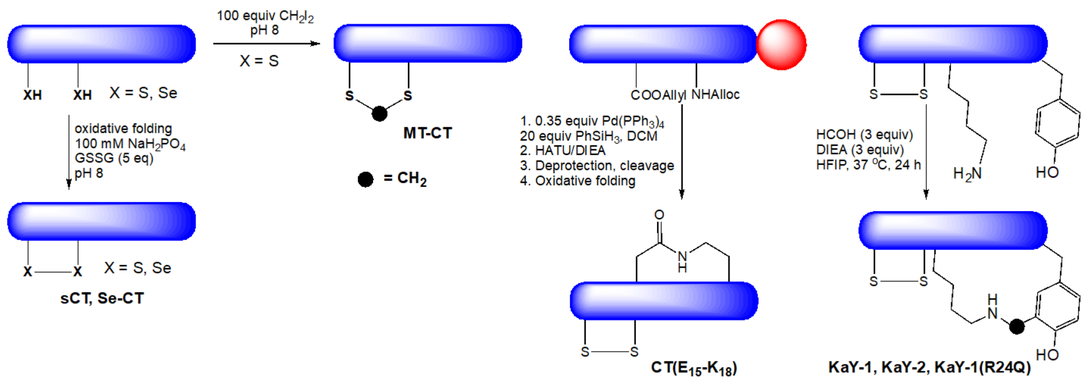
Method

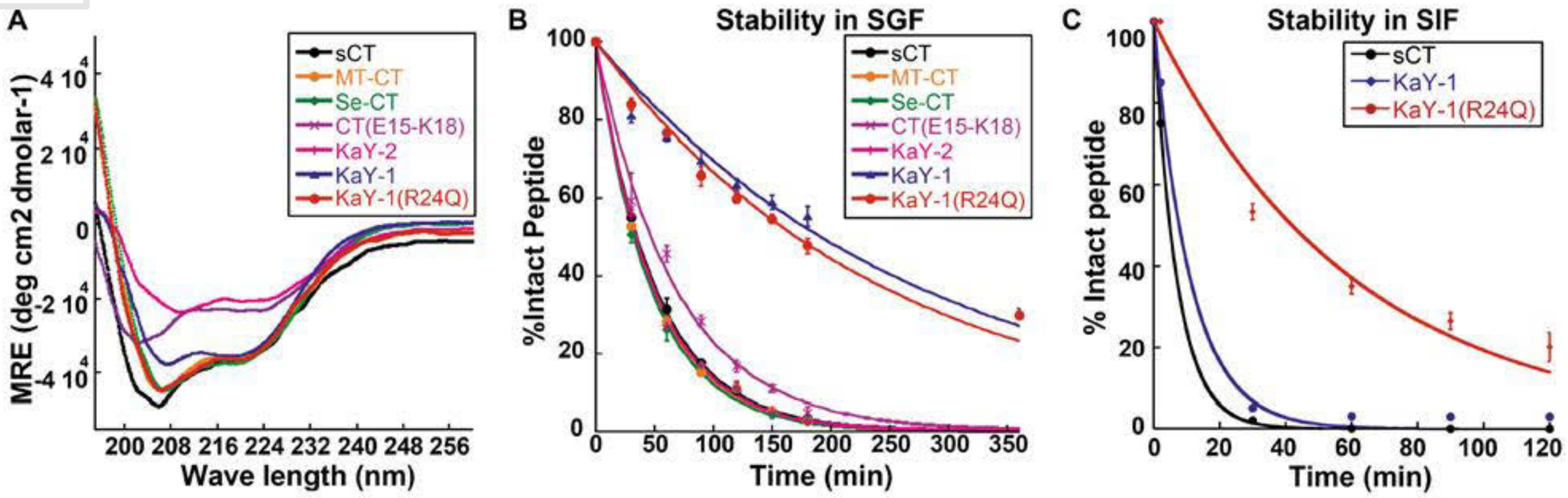


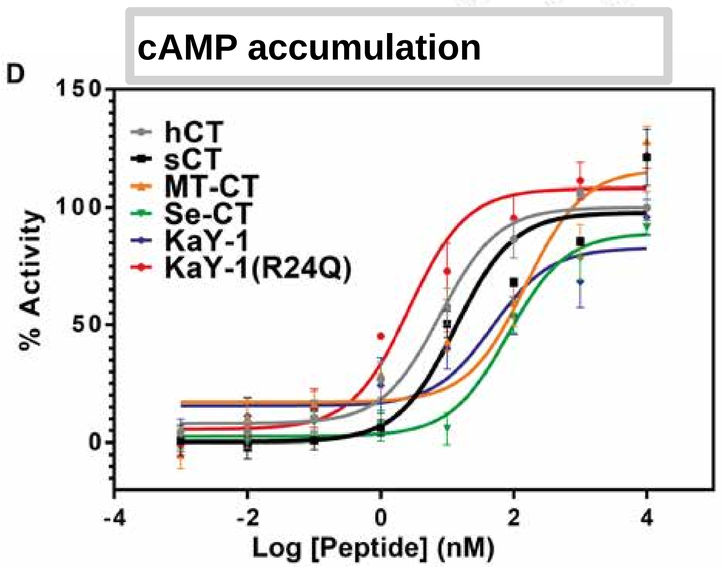
Designing of analogues using modification and helix stapling



Chemical protein synthesis for macrocyclic peptide







CT analogue	SGF $t_{1/2}$ (min)	SIF $t_{1/2}$ (min)	cAMP EC_{50} (nM)	Ca^{2+} EC_{50} (nM)
hCT	n.d.	n.d.	7.8±1.6	2.9±0.6
sCT	35.6±0.1	4.9±0.7	13.5±4.5	87.0±17.5
MT-CT	33.5 ±0.2	n.d.	1666±60	7304±700
Se-CT	32.6±0.5	n.d.	82.7±16.5	112.5±18.3
CT(E_{15}-K_{18})	47.3±1.9	n.d.	n.d.	n.d.
KaY-2	34.5±0.3	n.d.	n.d.	n.d.
KaY-1	191.2±66.1	7.1±0.7	47.6±24.1	165.5±34.8
KaY-1(R24Q)	170.6±32.2	41.9±6.1	2.7±0.8	215.8±43.8

(**A**) All analogues obtained α- helicity. (**B**) KaY-1 and KaY-1(R24Q) have 5 fold longer $t_{1/2}$ in SGF. (**C**) KaY-1(R24Q) has 6 fold longer $t_{1/2}$ in SIF (**D**) KaY-1(R24Q) enable cAMP accumulation with EC_{50} 2.7 nM. (**E**) KaY-1(R24Q) enable Ca2+ accumulation in cytosol with EC_{50} 216 nM

Conclusions

The present study demonstrated that KaY-1(R24Q) obtained by stapling reaction between Lys11 and Tyr22 retained helical structure, exhibited increased stability in both SGF and SIF, and retained activity. Our results provided a starting point for the development of oral sCT analogue for the treatment of postmenopausal osteoporosis, Paget`s disease and hypercalcemia.

Ghareeb, H., & Metanis, N. (2023). Enhancing the gastrointestinal stability of salmon calcitonin through peptide stapling. Chemical Communications, 59(44), 6682-6685.