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Modulation of the antagonistic properties of an insulin mimetic peptide by disulfide bridge modifications

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INTRODUCTION

Insulin is a peptide crucial for maintaining metabolic balance within the organism, it elicits its effects through binding to the transmembrane insulin receptor (IR). Investigating insulin mimetics, which can either stimulate or inhibit the receptor, presents a promising field of research for diabetes and cancer treatments. We synthesized five variants of a previously reported 20-amino acid insulin-mimicking peptide S592 (Table 1) [1]. These new peptides **1-5** (Table 2) differ from each other by the structure of the covalent bridge connecting positions 11 and 18. In addition to the peptide with a disulfide bridge, we prepared a derivative with a dicarba bridge and three derivatives with a 1,2,3-triazole, differing from each other by the presence of sulfur or oxygen in their staples.

Table 1. Sequences of referred peptides.

Code	Sequence
S592	SLEEEWAQIECEVWGRGCPSY
S371	GSLDESFYDWFERQLGKK
S661	GSLDESFYDWFERQLGGGSGGSSLEEEWAQIQCEVWRGCPSY-amide
S961	GSLDESFYDWFERQLGGGSGGSSLEEEWAQIQCEVWGRGCPSY
S519	SLEEEWAQVECEVYGRGCPSGSLDESFYDWFERQLG
Cys→Ser in S661	GSLDESFYDWFERQLGGGSGGSSLEEEWAQIQSEVWGRGSPSY-amide
Cys→Ser in S592	SLEEEWAQIQSEVWGRGSPSYC
peptide 1	SLEEEWAQIECEVWGRGCPS-amide
IM459	#SLEQEWaKIECEVYGKCPPKKAyKWFERQLK-amide
IM172N22	#SLEEEWAQIECEVYGRCPPSES-amide
S597-N20	SLEEEWAQIECEVYGRGCPS-amide

The Cys residues forming the disulfide bridge are highlighted in red. # denotes *N*-terminal phenylacetylation, a means 2-aminoisobutyric acid, y is *O*-methyltyrosine

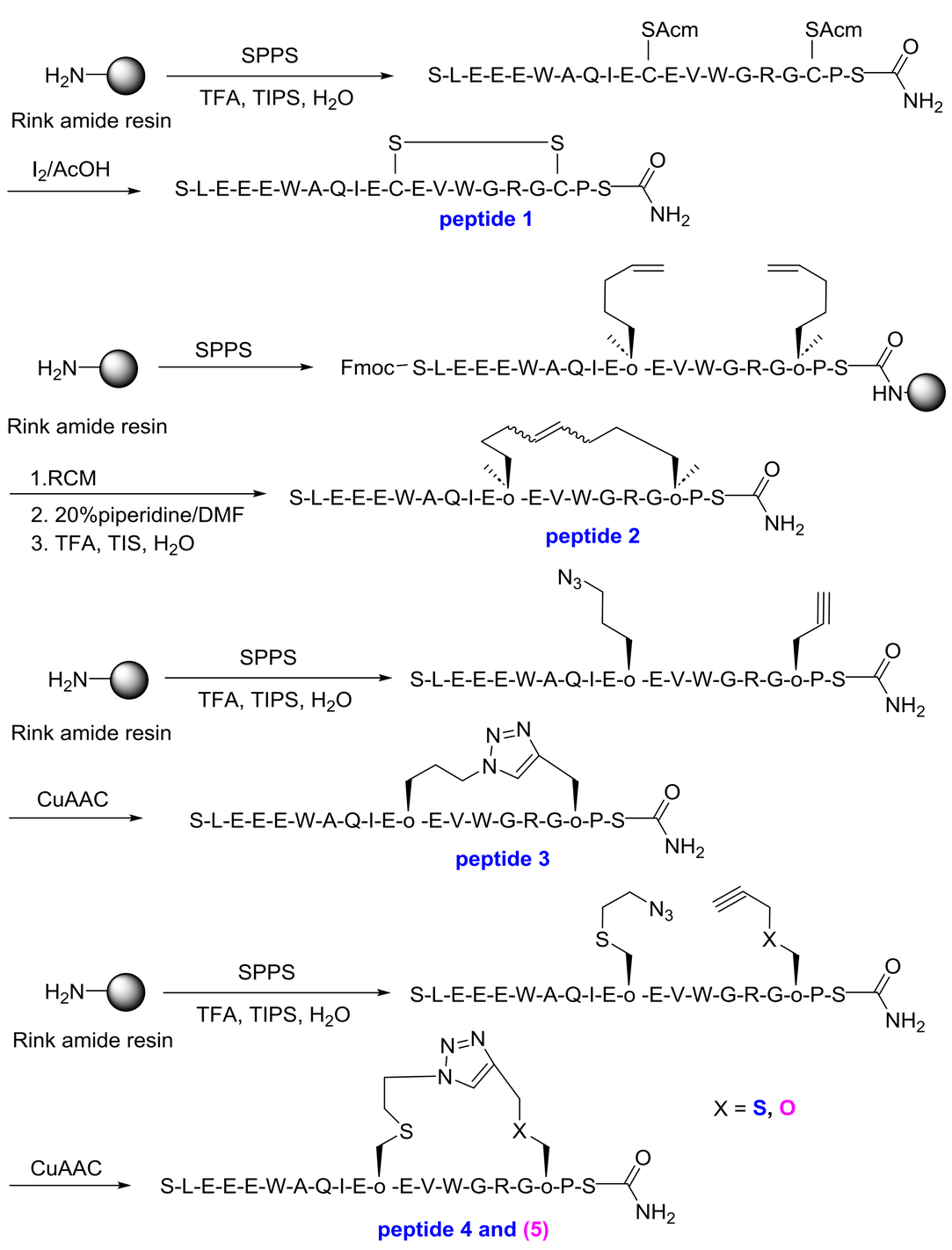

Figure 1. Synthetic route for cyclic peptides preparation. o denotes C^{α} atom of non-standard amino acids.

SYNTHESIS

Peptides were synthesized by the solid-phase peptide synthesis (SPPS) on Rink Amide AM resin using standard Fmoc protocol on the multiple Peptide Synthesizer Spyder Mark IV (EP 17206537.7) developed in the Development Center of the IOCB (Figure 1). Cleavage of peptides was achieved by treatment with TFA:TIS:H_2O. Peptide with Acm-protected cysteines was dissolved in 40% AcOH at 10^{-3} M. Iodine (25 eq.) in AcOH was added, and the solution was stirred at RT. Then, 1 M ascorbic acid was added until the iodine colour disappeared. Ring-closing olefin metathesis (RCM) was performed on resin-bound peptide with the N-terminal amino acid protected with Fmoc. RCM was carried out using 6 mM Grubbs catalyst 1st generation (20 mol% regarding the resin substitution) in DCE. The resin was agitated under nitrogen at rt for 2 h, protected from light. A Cu(I)-catalyzed azide-alkyne cycloaddition (click reaction) was conducted on the linear peptide, which was dissolved in H_2O/tBuOH (2 : 1, v/v), and then $CuSO_4 \cdot 5H_2O$ (14 eq.) and ascorbic acid (13 eq.) were added. The reaction was stirred overnight, without light at RT.

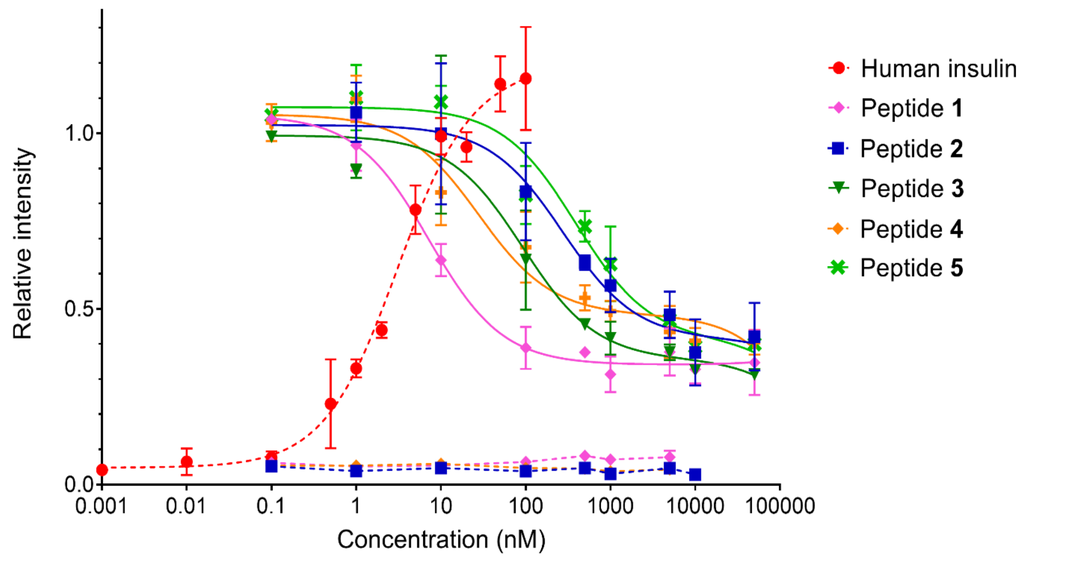

Figure 2. Relative abilities of peptides **1**–**5** to antagonize insulin-stimulated receptor phosphorylation (full lines). Dashed lines show stimulation of IR-A with insulin and selected peptides **1**, **2** and **4** alone.

Table 2. Binding affinity for IR-A and ability to stimulate IR-A autophosphorylation by human insulin and peptides **1-5**.

Peptide	Type of the intramolecular staple (number of atoms in the staple)	K_d ± S.D. [nM] for binding to IR-A (n≥3)	EC_{50} [nM] (best fit values) for stimulation (S) or antagonism (A)	EC_{50}/K_d
Insulin		0.31 ± 0.02	3.0 (S)	10
1	disulfide (4)	3.9 ± 1.0	7.2 (A)	1.8
2	dicarba (8)	49 ± 6	274 (A)	5.6
3	triazole (7)	15 ± 3	92 (A)	6.1
4	triazole-sulfur-sulfur (10)	25 ± 7	28 (A)	1.1
5	triazole-sulfur-oxygen (10)	75 ± 29	389 (A)	5.5

CONCLUSIONS

We prepared five variants of a 20-amino acid insulin-mimicking peptide targeting Site 2 of the insulin receptor (IR). Peptide **1**, with a natural disulfide bridge, exhibited the strongest binding, with affinity only 13 times lower than human insulin (Table 2). Other derivatives had 4–19 times weaker binding than peptide **1**, indicating a correlation between increased bridge length and reduced affinity. Although none of the peptides activated IR, all inhibited insulin-induced activation, behaving as partial antagonists with about 30% residual receptor activity at high concentrations (Figure 2). Peptides with sulfur in their bridges showed better binding and antagonistic effects, while those without sulfur had lower affinity and reduced antagonism. This study demonstrates that functional properties of IR peptide ligands can be modulated using unnatural disulfide bridge mimetics, potentially leading to effective nonpeptide insulin mimetics in the future.

REFERENCES

1. M. Lubos, J. Pícha, I. Selicharová, J. Žák, M. Buděšínský, K. Mitrová, L. Žáková, J. Jiráček, *J. Pept. Sci.*, 2023, 29:e3478.

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