Hyperdisulfide and Cell-Penetrating Cytoprotective Peptides from Medicinal Plants

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INTRODUCTION

A longstanding interest of our laboratory is to study disulfide-rich peptides from medicinal plants as drug leads and as an inspiration for designing orally-active compounds. Plants produce disulfide-rich peptides, also known as cysteine-rich peptides (CRPs), as part of their host-defense mechanism against microbes and insects. Most CRPs contain 15-25% of cysteine per molecule, or about one cysteine per 4 to 7 amino acid residues. Recently, we discovered hyperdisulfide peptides containing >30% cysteine per molecule, or a cysteine in every three amino acids. Here, we report the discovery of β-ginkgotides from *Ginkgo biloba*, as a "first-in-class" hyperdisulfide-constrained peptide family from plants. They contain a conserved six-cysteine core with a highly clustered cysteine spacing and a motif of C-CC-C-CC, an arrangement that has not been reported in CRPs. β-ginkgotides are highly resistant against heat, acid and protease-mediated degradation. Bioinformatics data-mining revealed that β-gB1 contains the canonical LC3-interacting region (LIR) motif crucial for selective autophagy. Our results showed that β-gB1 is a cell-penetrating adaptogen which can maintain cellular homeostasis through selective autophagy by promoting autophagosome formation. We also showed that β-gB1 is cytoprotective by protecting intracellular proteins against stress-mediated damage from hypoxia and hypoxia-reoxygenation-induced cell death. Furthermore, the hyperdisulfide scaffold of β-gB1 holds promise for the engineering of peptidyl therapeutics with enhanced structural and metabolic stability.

Figure 1: Ginkgo biloba nut

RESULTS

β-Ginkgotide from *Ginkgo biloba*

- **30% cysteine in the sequence**
- **New cysteine motif and connectivity**
- **Bipolar and highly compact structure**
- **Stable to thermal, acidic and enzymatic degradation**

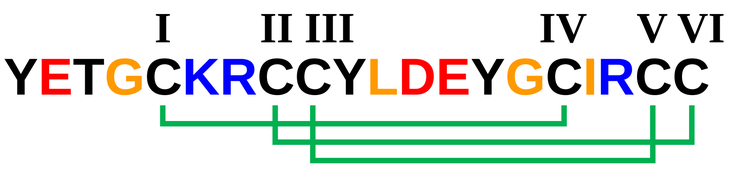


Figure 2: Primary sequence and disulfide connectivity of β-gB1.

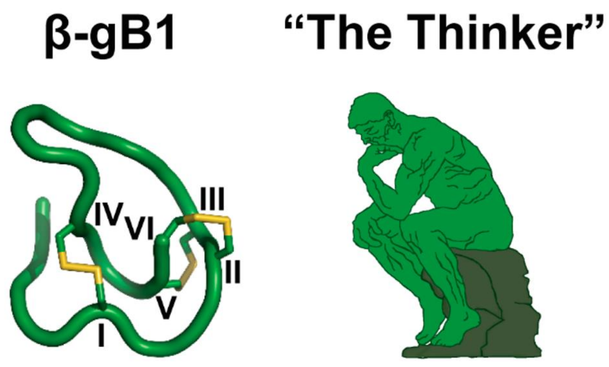


Figure 3: NMR structure of β-gB1 resembles the Rodin sculpture "The Thinker" (Le Penseur).

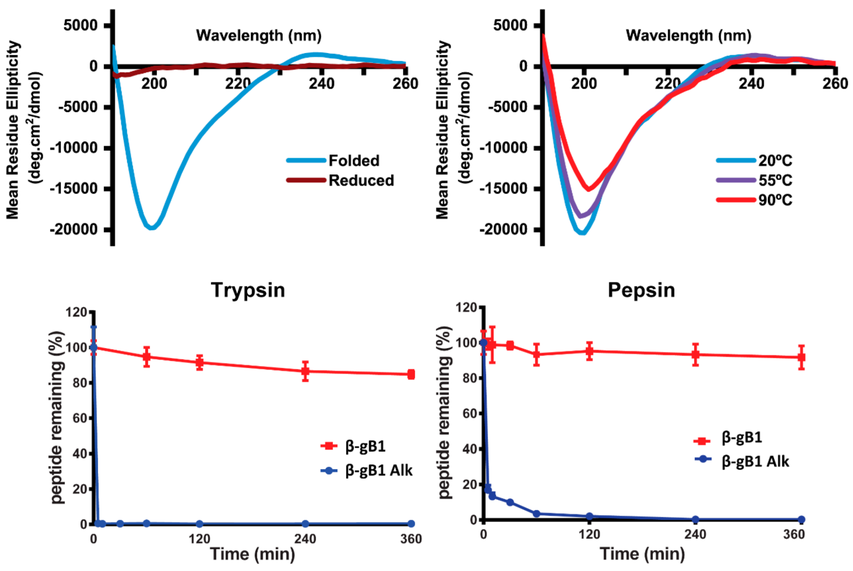
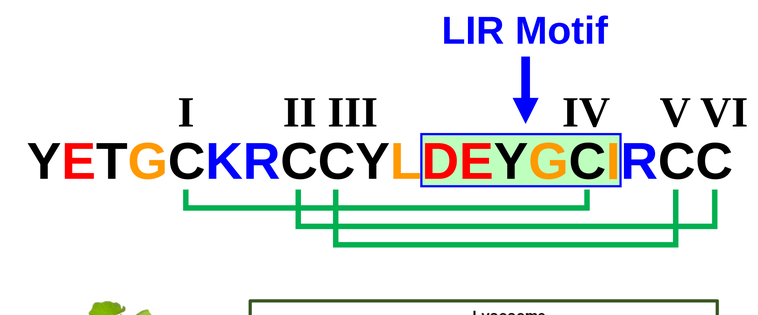
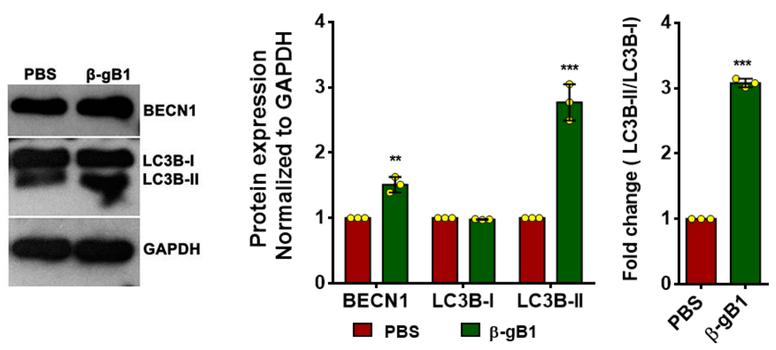


Figure 4: β-gB1 is stable to thermal and enzymatic degradation.

β-Ginkgotide is adaptogenic

- **β-gB1 loop 2 contains the canonical LC3-interacting region (LIR) motif**
- **Interacts with Atg8 family proteins and induces autophagosome formation**
- **Maintains cellular homeostasis through selective autophagy**





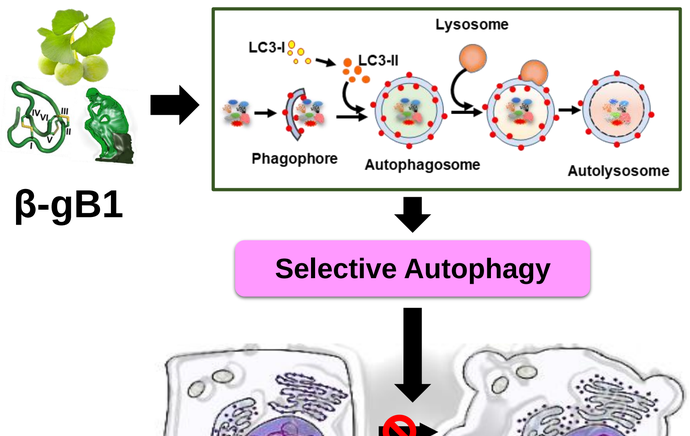


Figure 5: β-gB1 induces autophagosome formation in neuronal-like SH-SY5Y cells.

β-Ginkgotide is cytoprotective

- **Protects cells from hypoxia and hypoxia-reoxygenation-induced cell death**

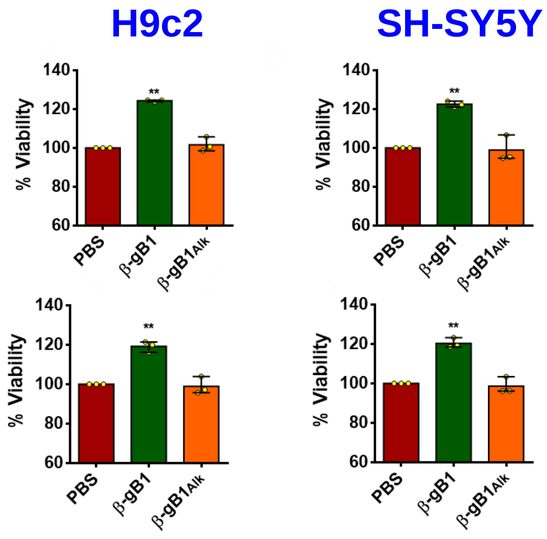


Figure 6: Effects of 1 μM β-gB1 on cell survivability under hypoxia (top) and hypoxia-reoxygenation (bottom) conditions.

CONCLUSION

- **Hyperdisulfide β-gB1 is cytoprotective against hypoxia stress**
- **β-gB1 is adaptogenic to induce selective autophagy for maintaining cellular homeostasis and promoting cell survival**
- **β-gB1 is non-toxic to cells**

Acknowledgement

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References

1. Dutta, B.; Huang, J.; To, J.; Tam, J. P., LIR Motif-Containing Hyperdisulfide β-Ginkgotide is Cytoprotective, Adaptogenic, and Scaffold-Ready. *Molecules* 2019, 24, (13), 2417.
2. Wong, K.H.; Tan, W.L.; Serra, A.; Xiao, T.; Sze, S.K.; Yang, D.; Tam, J.P. Ginkgotides: Proline-Rich Hevein- Like Peptides from Gymnosperm *Ginkgo biloba*. *Front. Plant Sci.* 2016, 7, 1639.
3. Wong, K.H.; Tan, W.L.; Xiao, T.; Tam, J.P. beta-Ginkgotides: Hyperdisulfide-constrained peptides from Ginkgo biloba. Sci. Rep. 2017, 7, 6140.