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.

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.

β-gB1 is cytoprotective
• Protects cells from hypoxia and hypoxia-reoxygenation-induced cell death

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

Figure 6: Effects of 1 μM β-gB1 on cell survival 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

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References

www.sbs.ntu.edu.sg