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606/28. PEXOPHAGY IN SUGAR-STARVED EMBRYONIC AXES OF GERMINATING LUPIN (LUPINUS SPP.) SEEDS

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Summary:

Objectives, Description, Main Results & Conclusions

Plant autophagy is a process by which cells degrade and recycle their own components such as organelles and protein complexes. Under adverse conditions, including nutrient deficiency, autophagy is enhanced to increase the pool of respiratory substrates and hence increase the potential of cell survival. We found that advanced autophagy occurs under sugar starvation conditions in isolated embryonic axes of white lupin (Lupinus albus L.; up to 14% total lipid content in seed dry matter) and Andean lupin (Lupinus mutabilis Sweet; about 20% total lipid content in seed dry matter) cultured in vitro for 96 h. Simultaneously, we observed disruption of storage lipid breakdown, which was reflected in higher lipid content in the sugar-starved (-S) than in sucrose-fed (+S) axes. Our results indicate that the disturbance in storage lipid breakdown is caused by the pexophagy, i.e. a selective autophagic degradation of peroxisomes - the key organelles in storage lipid degradation. Evidence for such conclusion are: i) peroxisome localization in the autophagic bodies, ii) higher content of total lipid, iii) higher transcript levels of genes coding for proteins of the pexophagy machinery, and iv) lower content of the peroxisome marker Pex14p and its increase caused by an autophagy inhibitor (concanamycin A) in -S axes than +S axes. We did not find significant differences in pexophagy and storage lipid breakdown between axes of two investigated lupin species despite the different lipid content in seeds. Importantly, our results show that autophagy is an adaptive response to adverse conditions also in very young plant organs like embryonic axes. The research was financed by the National Science Centre, Poland, grant no. 2016/23/B/NZ3/00735.

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606/40. b-CONGLUTINS' MOBILE ARM IS CRUCIAL IN THE NUTRACEUTICAL PROPERTIES OFBLUE LUPIN

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Summary:

Objectives

Sweet lupin species, particularly narrow-leafed lupin (NLL; Lupinus angustifolius L.), have gained significant attention in recent years for their nutraceutical properties, specifically their anti-inflammatory and anti-diabetic effects. β-conglutin proteins, particularly the NLL β1, β3, and β6, have been extensively studied for their health benefits [1], with some studies reporting their cytotoxic effect on breast cancer cells and prevention of malignant transformation of healthy cells, reducing metastasis and recurrence in breast cancer cell lines [2]. These nutraceutical properties may be attributed to the unique structural domain of β-conglutin proteins, characterized by an N-terminal mobile arm [3] absent in other vicilin proteins of legume species. This study focused on the anti-inflammatory activity and oxidative regulatory capacity of the NLL β5 and β7 conglutins.

Concise description of the work (materials & methods)

Biochemical and molecular biology techniques were employed using in vitro and ex vivo systems: LPS-stimulated in vitro (HepG2) model culture cells and ex vivo (isolate PBMC from blood samples of T2D-diagnosed patients and healthy control subjects). We purified recombinant forms of complete (β5 and β7) and truncated (tβ5 and tβ7, missing the N-terminal mobile-arm domain) conglutin proteins using affinity chromatography.

Main Results

The results revealed that β5 and β7 proteins reduced the levels of pro-inflammatory mediators and mRNA expression levels of iNOS, TNFα, and IL-1β (Figure 1). In addition, the complete forms of β5 and β7 decreased the protein levels of pro-inflammatory cytokines such as TNF-α, interleukins (IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, IL-27), and other pro-inflammatory mediators (INFγ, MOP, S-TNF-R1/-R2, and TWEAK), while exerting a regulatory effect on cellular oxidative balance through glutathione levels, catalase, and superoxide dismutase enzymatic activities. Interestingly, the truncated forms (tβ5 and tβ7) did not exhibit these molecular effects [3].

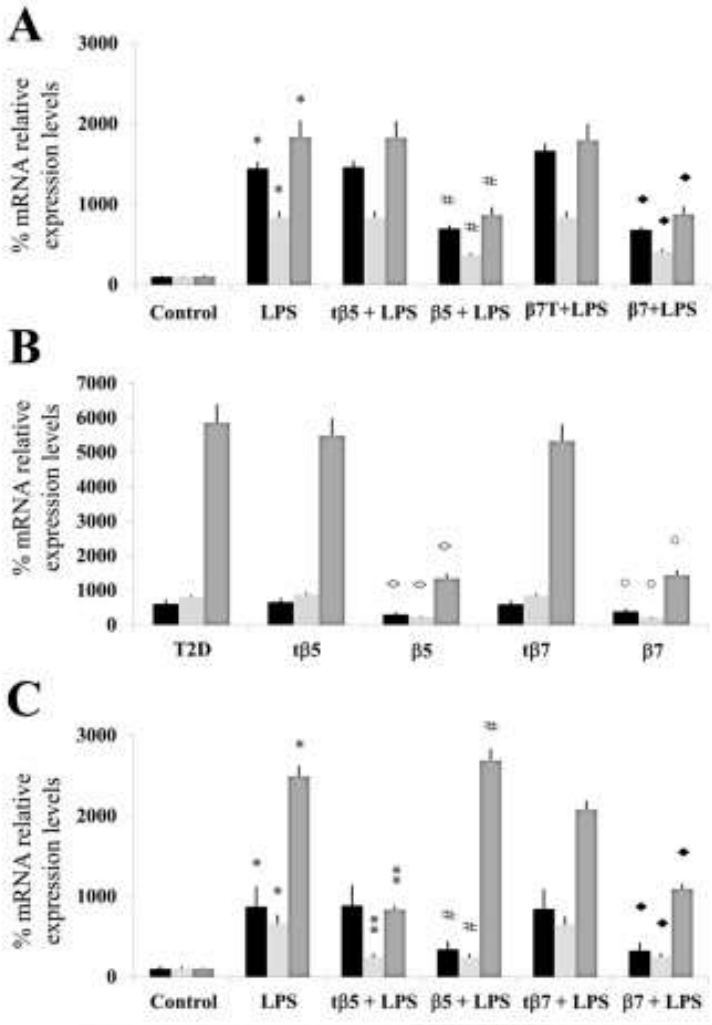

Figure 1. Example of the effect of β5 and β7 compared to truncated forms in all experimental groups: Assessment of mRNA expression levels of TNF-α, IL-1β, and iNOS genes. Culture cells of HepG2 (A), T2D (B), and healthy control subjects (C). Each group was incubated for 24 h in LPS, LPS+tβ5, LPS+β5, LPS+tβ7, or LPS+β7. Bars show TNF-α (black), IL-1β (light gray), and iNOS (gray) color. *p< 0.05 LPS vs. control; **p< 0.05 LPS+tβ5 or tβ7 vs. LPS; #p< 0.05 LPS+β5 vs. LPS; ▯p< 0.05 LPS+β7 vs. LPS. ◊p< 0.05 LPS+β5 vs. T2D, and op< 0.05 LPS+β7 vs. T2D in T2D cell cultures. Data represent the mean ± SD of three independent experiments. [3]

Conclusions

Our findings highlight the anti-inflammatory and oxidative cell state regulatory properties of β5 and β7 conglutins. Furthermore, we identified the N-terminal mobile-arm domain as a critical element of β-conglutin proteins contributing to these nutraceutical properties.

Consequently, these newly discovered anti-inflammatory proteins (NLL β5 and β7) might play a key role as potential functional food components for preventing and treating inflammation-related diseases and cancer. Furthermore, our study highlighted the importance of the unique β-conglutin' mobile arm as a key structure in mediating these beneficial effects.

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606/128. IDENTIFICATION OF MENDEL'S POD COLOUR CHARACTER IN PEA: CHARACTERISATION OF THE ALLELE CONDITIONING YELLOW POD COLOUR

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Summary:

Objectives, Description, Main Results & Conclusions

The inheritance of yellow-podded versus green-podded peas was studied by Mendel more than 150 years ago, but, until now, the allelic variation underlying this difference remained unknown. A report of a novel, spontaneous gp mutant (Pellew and Sverdrup, 1923) suggested that at least two independent gp alleles existed. We undertook genetic complementation crosses with 19 yellow-podded lines present in the John Innes Pisum germplasm collection, one of which is described as "Pellew's gp", and we found that all were allelic to gp carried by the type-line, JI0128. This showed that Mendel's yellow-podded character is conditioned by variation at a single locus. We used genetic mapping of Axiom markers in a large F2 population to define the position of gp within a 4.4 cM interval, encompassing approximately 8 Mb. We used exome capture to compare genome structures within this interval, in 227 Gp accessions and the 19 gp accessions. We found that all 19 gp lines carry the same large 100 kb deletion, while all 227 Gp accessions do not, indicating that only one gp haplotype exists in the germplasm we studied, and that gp must have been crossed into different backgrounds by breeders and researchers in the past. Three candidate genes associated with the deletion were assessed. After crossing a null TiLLING mutant for one of these candidates with a gp line, yellow-podded F1 progeny were obtained. This genetic complementation test showed that we have identified a new allele of the gp gene. We used backcross lines to gain a better understanding of the mutant phenotype. It was already known that thylakoid membranes are underdeveloped in gp lines (Price et al., 1988). Additional effects will be described.

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