Summary

The glycogen synthase kinases 3 family remains conserved throughout the eukaryotic evolution, from yeasts to plants and mammals. We studied the LjSK1 kinase of the Lotus japonicus plant, which is similar to the mammalian GSK3 and had previously been shown to be involved in nodule development. Purification of the enzyme was based on standard liquid protein chromatography methods and enzyme kinetics and mutagenesis studies were performed to compare LjSK1 with homologous mammalian kinases. Using a prime-phosphorylated peptide as substrate, LjSK1 showed optimal kinase activity at pH 8.0 and 20 ˚C, following Michaelis-Menten kinetics with kinetic parameters Km and Vmax, 8.2 μM and 111.6 *nmol/min/mg* respectively, for ATP.

Site directed mutations were carried out and Lys167was proven to be a key amino acid of the enzyme catalytic triad, while at the same time it was clarified that the residues related to the specificity of the substrate in the human enzyme, do not have the same key role in the regulation of the specificity of LjSK1.Additional studies performed on the truncated LjSK1$_{90\text{-}467,}$ indicated a different regulatory mechanism of LjSK1, by proteolysis of a domain located in the N-terminus, as the removal of 89 residues almost doubled its catalytic efficiency. At the same time, the effect of Lupeol, Betulinic acid and Hederacoside C triterpenes on the activity of LjSK1 was studied, revealing a strong inhibition.

The 3D structure of LjSK1$_{90\text{-}467}$ was determined at 2.90 Å resolution, providing, for the first time, structural data for a plant GKS3 like kinase. Comparative structural analysis to the human GSK3β homologue, revealed significant differences at the N- and C-termini of LjSK1$_{90\text{-}467}$, supporting the notion for a different regulatory mechanism in plant GSK3-like kinases. Similarly, structural similarities of important functional domains, such as the catalytic center and the ATP binding site, explain the similarity in the function of these two proteins.

We also evaluated the alteration of LjSK1 kinase activity in planta, by overexpressing mutant variants K167A and Y298A, as well as truncated LjSK1$_{90-467}$ in hairy roots of modified plants and a phenotype in nodulation and lateral root development was verified. Finally, by employing synthetic biology techniques, a new synthetic calcium-dependent protein kinase, CALM-LjSK1$_{90-467}$, which carries functional domains of both LjSK1, and human calmodulin, was produced. The aim of this new protein was to have a protective effect against plant infestation. Kinetic studies with CALM-LjSK1$_{90-467}$showed a significant increase of enzymatic activity in the presence of Ca^{2+}.