Cloning and expression of frutalin, an α-D-galactose binding lectin, in *Pichia pastoris*, *Escherichia coli* and baculovirus/insect cells system

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Frutalin is an α-D-galactose-binding jacalin-related lectin expressed in breadfruit seeds (*Artocarpus incisa* seeds). One of the most interesting biological properties of frutalin is its potential use as a cancer diagnostic marker due to its ability to bind galactose complexes expressed in the cancer cells membranes. This application will require large amounts of frutalin, with the highest level of purity, however the extraction of frutalin from plants is time-consuming and results in a heterogeneous mixture of different lectin isoforms. The heterologous expression and production of frutalin in model organisms may not only improve its availability but will also help to elucidate its role as a cancer diagnostic marker.

The aim of this work is to express and produce frutalin in three different expression systems and to compare the molecular and biological properties of each recombinant frutalin obtained with the native lectin properties. Frutalin codifying gene was cloned and expressed with its native C-terminal in the following hosts: *Pichia pastoris* (yeast), *Escherichia coli* (bacteria) and baculovirus/insect cells system (Sf9 cells line). Recombinant frutalin was detected in all the cultures of the different organisms by Western blot analysis. Optimization of frutalin expression and purification was conducted for the *P. pastoris*[1] and *E. coli* systems. Frutalin was expressed in the three expression systems as a single chain protein, since the four amino-acid linker peptide „T-S-S-N", which connects α and β chains, was not cleaved. In native frutalin the linker is excised to separate α and β chains forming a tetrameric protein in which each monomer is made of one α chain and one β chain bound by non-covalent linkages. Further characterisation of the yeast frutalin revealed that it is probably also a tetrameric protein, is partly *N*-glycosylated and has a carbohydrate-binding specificity similar to the reported for the plant lectin. Despite of specifically binding to Me-α-galactose, recombinant yeast frutalin showed much less affinity to bind this monosaccharide than native frutalin and did not agglutinate rabbit erythrocytes as native does. This result shows that linker cleavage might not be essential for lectin sugar specificity. On the other hand, recombinant *E. coli* cell-free extracts showed to have hemagglutinating activity but, as expected, the recombinant frutalin is not glycosylated. This indicates that the *P. pastoris* glycosylation pattern may inhibit the hemagglutinating activity, since the presence of the linker in *E. coli* recombinant frutalin did not inhibit its hemagglutinating activity. Also, no hemagglutinating activity was detected on the Sf9 infected cells extracts or their culture media. Thus, recombinant frutalin presents different properties depending on the expression system used. Data on global comparative expression and functional analysis will be presented.

[1] C. Oliveira, W. Felix, R.A. Moreira, J.A. Teixeira, L. Domingues, Expression of frutalin, an α-D-galactose-binding jacalin-related lectin, in the yeast *Pichia pastoris*, Protein Expression and Purification 60 (2008) 188-193.