Expression of a functional pro-lectin from seeds of *Dioclea grandiflora* and insights into Diocleinae post-translational modification

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An active, recombinant pro-lectin (r-pro-DGL) was obtained from the seeds of *D. grandiflora* using the model prokaryote *Escherichia coli*. Produced in soluble form, the recombinant protein presented the same apparent molecular mass (25kDa) and sequence on mass spectrometry as the natural precursor. With a specific activity of 262,144 (HU/mg), pro-DGL is atypically functional compared to other legume lectins expressed in heterologous systems. Unlike the mature protein, r-pro-DGL did not recognize glucose and was only slightly inhibited by mannose. Due to the high degree of homology between sequences and structures of Diocleinae pro-lectins and certain lectins of ancient legume subtribes, some authors have proposed the typical post-translational process of this subtribe determines the specificity of these proteins. However, we demonstrate that binding site topology and the conformation of the surrounding loops are the main factors determining monosaccharide specificity. Based on these findings, it may be concluded that lectin precursors of the Diocleinae subtribe are active after deglycosilation and capable of forming oligomers and cross-linking cell membranes and glycoconjugates.

**Keywords:** Dioclea grandiflora, cloning, recombinant expression, post-translational processing.

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