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Equivalence of Native and Recombinant ArtinM-Carbohydrates Recognition Evaluated by Eletrogravimetric Technique

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Abstract – Lectins are proteins largely used in biological events studies, due to its capacity to bind carbohydrates reversibly and specifically. The ArtinM, a lectin isolated from jack fruit seeds, possess a strong immune action. A methodology to obtain ArtinM recombinant was developed due to restrictions caused by the fruit harvesting and the difficult task of purifying large quantities of native ArtinM. In this work was determined the association equilibrium constants of the interactions between both ArtinM forms and the trimannoside present in the horseradish peroxidase structure by quartz crystal microbalance technique, in order to evaluate the equivalence between both forms of ArtinM.

Lectins are non-imune proteins that bind carbohydrates reversibly and specifically. The seeds of jack fruit from *Artocarpus integrifolia* specie are known to contain a lectin, called ArtinM [1] , capable of activate immune system by inducting neutrophil migration and IL-12 p40 production by macrophages, moreover it inducts mitosis in B and T cells and accelerates healing in skin and corneal injury [2]. Due to restrictions in studies, caused by the fruit harvesting and the difficult task of purifying large quantities of native ArtinM (jArtinM), Roque-Barreira and co-workers [1] developed the recombinant protein (rArtinM). However, an evaluation of the equivalence between both forms is needed previously to substitution of the native protein. Therefore, the aim of this work was to evaluate the equivalence between two forms of ArtinM by studying the lectin-carbohydrate recognition using the Quartz Crystal Microbalance (QCM) technique.

QCM is a promising technique in biosensors and biomedical area because it allows rapid and cost-effective analysis, highly sensitivity and reliability, as well as measuring tiny mass changes in real time by label-free detection of molecules. Moreover, native biomolecules can be used in QCM experiments [3]. The coupling of a biological sensing element at transducer surface is the basis of a biosensor. In this work the lectin ArtinM was immobilized on gold surface, which was deposited on both sides of the transducer, the piezoelectric quartz crystal, by the self assembled monolayer of alkanethiols methodology. Here were used 11-mecaptoundecanoic acid to bind with the lectin and 2-mercaptoethanol as spacer, in the proportion 1:100, respectively, in order to avoid steric hindrance problems. Then all residual carboxyl groups were blocked by reaction with gelatin. Then, this functionalized transducer was used to determine the association equilibrium constants of the interactions between both ArtinM forms and the trimannoside present in the horseradish peroxidase (HRP) structure. The obtained constants, $(3.4 \pm 0.2)x10^3$ M^{-1} and $(6.7 \pm 0.5)x10^3$ M^{-1} to jArtinM-HRP and rArtinM-HRP interactions respectively. The results demonstrate that the recombinant protein is interacting with the trimannoside similarly to native protein does, proving the binding equivalence of the jArtinM and rArtinM carbohydrate-binding active sites.

References

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