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4 P4: Structural and biophysical studies on the lectin domain of GalNAc-T6 for therapeutic applications

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The expression of glycoproteins containing immature truncated O-glycans such as the Thomsen-Friedenreich antigen (Ser/Thr-O-Galβ1–3GalNAc; T-antigen) and the Lewis antigen (sialyl-T-antigen) is a characteristic feature observed on almost all malignant epithelial cells. Therefore, there is a particular interest in their application not only as prognostic markers but also as therapeutic targets [1]. These antigens can be recognized by lectins, a group of highly specific carbohydrate-binding proteins that have been proposed as useful tools for antitumor drug-targeting [2].The three-dimensional structure of several lectins with antitumor properties has been determined in our laboratory by X-ray crystallography.
N-α-acetylgalactosaminyltransferase-6 (GalNAc-T6) is an enzyme present also in humans which contains a catalytic domain and a lectin domain with a binding site for N-acetylgalactosamine (GalNAc), one of the saccharides exposed by cancer cells (Tn-antigen). Unlike other lectins with these properties, the lectin domain of GalNAc-T6 presents a structural fold found also in other human proteins, unlocking the opportunity to use protein engineering tools to design new anticancer therapeutics [3]. The three-dimensional structure of GalNAc-T6 has not been determined so far, neither has been its substrate specificity. Therefore, the production of a recombinant form containing only the lectin domain can contribute to these two critical points that need to be considered to evaluate its possible use in cancer therapies.
The lectin domain of this enzyme was expressed by cloning the C-terminal portion of the DNA coding sequence and introducing it into Pichia pastoris for its recombinant production. Biophysical methods such as spectrofluorimetry and isothermal titration calorimetry were used to analyze the ability of the engineered protein to bind the T-antigen monosaccharides. The binding dissociation constant (Kd) of the protein-carbohydrate interaction was determined. The stability of the protein was also studied through its thermodynamic parameters of unfolding using differential scanning calorimetry. Crystallization screenings were set up using a broad variety of precipitants in order to produce crystals to be used to study the three-dimensional structure of the engineered protein using X-ray diffraction. The crystals that were grown were taken to the European Synchrotron Radiation Facility (ESRF) in Grenoble (France) to carry out the diffraction experiments. Although we were able to collect data up to a resolution of 2.8 Å (854,648 reflections) all the crystals we have examined so far were found to be twinned making the assignment of a definitive space group uncertain. We are currently working on correcting this problem using both the appropriate software and attempting to grow better crystals.
Our goal is to produce an engineered human protein that specifically recognizes cancer specific carbohydrates and is thus suitable for protein therapeutics applied in drug-delivery methods for cancer treatment. The present structural and biophysical data are the prerequisite for future studies regarding the biological and clinical properties of the lectin.

[1] Stowell, S. R. Tongzhong J. and Cummings R. D. Protein Glycosylation in Cancer. Annu Rev Pathol **2015**. 10: 473–510.

[2] Sharon, N., and Lis, H. Lectins: from hemagglutinins to biological recognition molecules. A historical overview. Glycobiology. **2004**. 14: 53–62.

[3] Berois, N., Mazal, D. et al. UDP-N-Acetyl-D-Galactosamine: N-acetylgalactosaminyltransferase-6 as a New Immunohistochemical Breast Cancer Marker. Journal of Histochemistry & Cytochemistry. **2006**. 54(3): 317–328.