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4 P3: Purification and structural studies of a Tremella fuciformis mushroom lectin

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Lectins are carbohydrate-binding proteins of non-immune origine widely distributed in living organisms. They play a role in different biological processes, serve as storage proteins, are fundamental during fungi and plant morphogenesis and development and take part in their defense processes [1]. Due to their carbohydrate specific binding, some lectins are able to recognize, in a reversible way, the sugar moieties present on the surface of erythrocytes (N-acetylgalactosamine, D-galactosamine), causing a phenomena called hemagglutination. Furthermore some lectins have been found to possess antitumoral properties [2]. Specifically they recognize the Tn-antigenic determinant (Galβ1-3GalNAcα) on the malignant cells surface causing apoptosis, cytotoxicity, inhibition of tumor growth and preventing the proliferation of tumor cells. Considering the fact that this kind of residues are masked on healthy cells, the highly specific carbohydrate-lectin interaction can be exploited to target malignant cells. The Tn-antigen is the most specific human cancer-associated structure, expressed in about 90% of human carcinomas. Although the function and biological properties of several lectins have been determined, there are still many lectins that remain to be structurally and functionally characterized.
As reported in the literature, some Tremella fuciformis proteins have been investigated for their potential therapeutical properties [3] and in the light of this, we have examined the crude extract proteins of this fungus to assess the presence of lectins.

A lectin of 22 KDa was isolated and purified from the dried fruiting bodies and used for testing several crystal screening conditions. Crystals were grown in 0,1 M TRIS pH 8.5, 1,5 potassium phosphate dibasic and preliminary data sets were collected at the ESRF of Grenoble. The space group is $P2_1$ and the cell parameters are a= 61,6 Å, b= 61,8 Å, c= 67,8 Å with β= 106,87 °. The highest resolution of these crystals is 1,5 Å and the total number of reflections collected were 740651. Dynamic Light Scattering (DLS) analysis reveals that TFL is a monomer under normal conditions. The distribution plot shows a size distribution of 2,9 nm ± 0,2 nm, with a polydispersity index (PDI) of 0,4 ± 0,1. Thermal protein stability was examined by means of differential scanning calorimetry, while chemical and pH-induced unfolding was investigated using fluorescence spectroscopy. Isothermal titration calorimetry yielded preliminary data on sugar binding, justifying a more detailed study to be undertaken in the future. It has also been observed that Tremella fuciformis lectin shows no cytotoxicity on malignant and healthy cells and its antitumoral properties are currently being investigated.

[1] A. Varrot, S.M. Basheer, A. Imberty, Current opinion in structural biology , 2013, 23, 678-685.
[2] Ju T., Otto VI, Cummings R.D., Angew. Chem. Int. Ed Engl., 2011, 50(8), 1770-91.
[3] Hung C.L., Chang A-J.,Kuo, X-K and Sheu F., J.Agric.Food Chem., 2014, 62(7), 1526-35.