

## PURIFICATION AND STRUCTURAL STUDIES OF A *TREMELLA FUCIFORMIS* MUSHROOM LECTIN

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Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origine widely distributed in living organisms including animals, plants and fungi. They play a role in different biological processes mediating cellular signaling, differentiation, tissue metastasis and host-pathogen interactions. Moreover they serve as storage proteins, are fundamental during fungi and plant morphogenesis and development and take part into their defense processes [1].

Thanks to their carbohydrate specific binding, some lectins are able to recognize, in a reversible way, the sugar moieties on the erythrocytes cell surface (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 (Gal $\beta$ 1-3GalNAc $\alpha$ ) 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 only malignant cells, also because the Tn-antigen is the most specific human cancer-associated structure, expressed in about 90% of the human carcinomas.

For the reasons described above, during the last decades lectins have been extensively investigated for their potential therapeutical effects and biotechnological applications, especially fungal lectins which have unique carbohydrate specificities. However, although the function and the biological properties of many lectins have been determined, their structural characterization lags behind.

As reported in the literature, some *Tremella fuciformis* proteins have been investigated for their potential therapeutical properties and have shown to possess anticancer, anti-inflammatory, antioxidant and neuroprotective activities. In the light of above the crude extract proteins have been checked to assess the presence of lectins [3].

To this purpose, the mushrooms dried fruiting bodies of *Tremella fuciformis* were homogenized and extracted in a phosphate buffer at 4°C and neutral pH. The crude extract was then precipitated using a high concentration of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and dialysed against TRIS buffer in order to remove the precipitant. A lectin was eluted from a hog gastric mucin affinity column and purified first with a DEAE-cellulose column and then with a size exclusion SEPHACRYL G-100 column.

An electrophoresis gel was required to precisely define the lectin molecular weight, which is 22 kDa. The purified lectin has been used for testing several crystal screening conditions.

[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.