Structural studies of POL (*Pleurotus ostreatus* Lectin), a fungal lectin of medical interest

Laura Destefanis[a], Michele Bovi[a], Massimiliano Perduca[a], Hugo L. Monaco[a].

[a] *Biocrystallography laboratory, Department of Biotechnology, University of Verona, Verona, Italy. laura.destefanis@univr.it*

Lectins are proteins widely diffuse in nature that interact non-covalently with carbohydrates [1]. Of all the mushroom proteins, lectins are probably the most extensively investigated because it has been observed that they can exhibit antitumour activity on human cancer cells [2]. Among them, a lectin from the fruiting bodies of the edible oyster mushroom *Pleurotus ostreatus* was isolated since it appears to be able to inhibit the growth of human neoplastic cells [3]. It was named POL, *Pleurotus ostreatus* lectin and in our laboratory it is purified using two chromatographic steps: a hog gastric mucin column followed by a Sephacryl S-100 gel filtration column. Two alternative ways of elution from the affinity column (with lactose 0.2 M and with EDTA 5 mM) give the same yield (1-1.5 mg) of protein starting with 500 g of mushrooms. Crystals of 0,1-0,3 mm can be grown in two crystallization conditions: 1) 0.1 M Na Hepes pH 7.5 in the presence of 0.8 M potassium/sodium tartrate tetrahydrate and 2) 1.6 M Ammonium sulphate, 0.1 MES pH 6.5 and 10% v/v Dioxane. We have collected X-ray diffraction data at various beamlines of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. The structure was solved by Single Isomorphous Replacement (SIR) with anomalous dispersion. The model was built with the program Coot and refinement was carried out with data collected from apo crystals at 2.05 Å using RefMac 5. The unknown preliminary amino acid sequence of the polypeptide chain was obtained from the electron density maps. The asymmetric unit contains one monomer with two domains of 22 β-strand only: 10 forming the domain near the N-terminus and 12 the C-terminus nearer, with a conformation that resembles the β-barrel fold. β-sheets are radially arranged around a central tunnel packing face-to-face. Since there seems to be an enzymatic activity associated to POL, the purified lectin was routinely checked with DLS experiments to ensure that the eventual enzymatic activity was only due to the lectin and not to other contaminants [4]. The presence of a single peak confirmed the purity of the sample and so it was decided to perform enzymatic assays with four nitrophenol derivatives. The most reactive substrate for POL was 4-nitrophenyl-β-D-glucopyranoside with a Vmax=87.21 nmol sec-1 mg-1, kcat=43s-1 and K_m=240 µM. Since in the POL electron density maps there was a region too big for water fitting the presence of a metal cofactor was suspected. Experiments with the spectrofluorimeter, analyzing fluorescence protein quenching upon the addition of a metal, were carried out and confirmed the presence of Calcium bound to the lectin. As POL density maps did not reveal any density regions that could be ascribed to a carbohydrate, it will be necessary to crystallize the lectin with specific inhibitors bound at the active site (for example nojirimycin). In addition, POL was also tested on human pancreatic cancer cells (MiaPaCa-2) and its therapeutic effect was evident. The antitumoral activity of POL might be exploited to direct PLGA, poly(lactic-*co*-glycolic acid) nanoparticles, to different melanoma cell lines, and also to prepare POL-filled nanoparticles emulsions or patches applicable on melanomas. For this purpose, since the total yield of purified POL is very low, attempts of heterologous expression in *Pichia pastoris* and *E. coli* ,with the protein sequence optimized for the expression in this bacterial system, are still in progress.

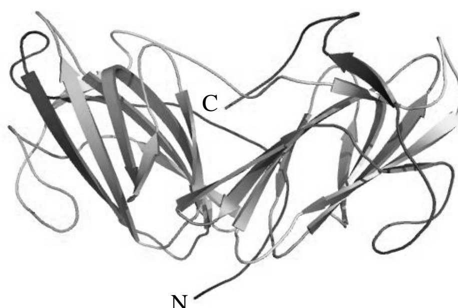

Figure 1. Ribbon model of one monomer of POL.

[1] H. Lis, N. Sharon. Lectins: Carbohydrate-Specific Proteins That Mediate Cellular Recognition. *Chem Rev.* **1998.** 98(2):637-674.
[2] J. Erjavec, J. Kos, M. Ravnikar. Proteins of higher fungi--from forest to application. *Trends Biotechnol*. **2012.** 30(5):259-73.
[3] H.Wang, J. Gao,TB. Ng. A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. *Biochem Biophys Res Comm*. 275: 810-816.
[4] R. Brechtel, H. Watzig, H. Rudiger. The lectin from the mushroom *Pleurotus ostreatus*: a phosphatise-activating protein that is closely associated with an α-galactosidase activity. *Plant Sci*. **2000**. 160:1025-1033.