Protease Inhibitors from Mushrooms as Potential Bioactive Metabolites of Medicinal Value

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During the last several years proteolytic enzymes have been firmly established as major regulatory components in a number of cellular, tissue, and physiological processes. The most important factors influencing proteolytic enzymes themselves and pathways of proteolysis are natural protease inhibitors which form complexes with target proteases to inactivate and/or to regulate their activity. They have been extensively investigated from the aspects of physiological functions, tools for analysing protease enzymology, models for protein–protein and protease–protein interactions, and of medical applications. There is growing interest in new inhibitors of proteases, not only synthetic but also naturally occurring (mushroom-generated ones among them), owing to their role in treatment of various human diseases including cancer (chemopreventive), hemorrhagic disorders and thrombophilia (anti- and procoagulant), or infections (antibacterial or antivirus). The most potent of the different types of protease inhibitors are the serine proteases, cysteine proteases, and metalloproteases. Among known protease inhibitors from mushrooms are yeasts, inhibitors of proteinases A and B, and low molecular weight inhibitors of serine proteinases from fruiting bodies of mushrooms –*Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *Lentinus edodes* (Berk.) Sing.—as well as water extracts of some species of *Basidiomycetes* that show evidence of some proteinase inhibitory activity. As fungi constitute a very populous and very distinct kingdom of living organisms, it may be expected that a number of new natural protease inhibitors, especially those with medicinal applications, will be (or better—should be) isolated from them.

Searching for new bioactive metabolites of basidiomycetous mushrooms, we recently isolated and characterized some low and high molecular weight proteinaceous natural protease inhibitors of serine proteases and metalloproteases. They were isolated from fruiting bodies and mycelia of a few edible, cultivable mushrooms (*Pleurotus ostreatus, Lentinus edodes*) and a few nonedible, but potentially medicinal mushrooms (*Schizophyllum commune* (Fr.:) Fr., *Trametes versicolor* (L.) Pilát, *Abortiporus biennis* (Bull.) Sing.). Isolation of inhibitors was achieved by typical ion-exchange and size-exclusion chromatography. Preliminary characterization of their inhibitory activity (against trypsin, chymotrypsin, and proteinase K for serine protease, and thermolysin and collagenase for metalloprotease inhibitors respectively), pH and temperature optima for activity, and molecular mass was done using classical analysis. It appeared that low molecular weight inhibitors of serine proteases were typical for both mycelium and fruiting bodies, and high molecular weight inhibitors were characteristic for fruiting bodies only. Inhibitors of metalloprotease were found mainly in fruiting bodies. We found no inhibitors of cysteine protease in the investigated mushrooms, despite one case in the scientific literature. Preparations for experiments with laboratory animals and with a range of human cells are in progress.

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