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CHAPTER 6

SUMMARY

Lectins are proteins or glycoproteins of non-immune origin that specifically and reversibly bind to carbohydrates or glycoconjugates. Because of their diverse roles and applications, there is an expanding emphasis on the isolation, purification and characterization of lectins from different sources. Literature reports suggest that mushrooms are rich source of lectins. In the present study, a new lectin whose hemagglutinating activity cannot be inhibited by various simple sugars and sugar derivatives, polysaccharides and

glycoproteins tested was purified and characterized from the fruiting body of an edible mushroom *Lentinus cladopus* Lév.

During the investigation, the lectin was assayed by the hemagglutination assay method of Devi *et al*. (2009) involving a standard serial dilution procedure using 2% suspension (v/v) of rabbit RBC. The lectin activity was taken as the hemagglutination titre, the reciprocal of the highest dilution of the lectin sample at which complete hemagglutination occurred. It was expressed in terms of hemagglutination unit (HAU) that is the minimum amount of lectin required for complete agglutination under the standard assay condition. Protein was estimated following the method of Lowry *et al*. (1951) using crystalline bovine serum albumin as the standard. The carbohydrate specificity of the lectin activity was determined by hemagglutination inhibition assay taking various carbohydrates and glycoproteins according to a slight modification of the method of Sawhney *et al*. (1996). The crude lectin preparation was found to have a typical specific activity of 1,136.0 HAU/mg protein. In an attempt to facilitate high resolution purification steps that follow for purification of the desired lectin and also to rule out pseudohemagglutination as the possible cause of the apparent hemagglutination activity, the crude lectin preparation was subjected to ammonium sulphate fractionation. The ammonium sulphate (20-60%) fraction was found to possess a typical specific hemagglutination activity of 3,233.7 HAU/mg protein with a 2.8-fold purification and an activity recovery of 160% as compared to the starting crude preparation thereby indicating that the hemagglutination activity observed in the crude preparation is not due to pseudohemagglutination but due to a lectin activity. Further the ammonium sulphate fraction was subjected to a set of high resolution chromatographic steps. The Q-Sepharose ion exchange chromatography with specific protein elution by a discontinuous gradient of salt ranging from 0.1 to 0.5M NaCl

yielded three pooled activity peak fractions. Only the pooled activity peak fraction eluted at 0.2M NaCl having specific activity of 4,512.5 HAU/mg protein with 4-fold purification and a recovery% of 69 (with reference to the starting crude lectin extract) was taken for further purification. In the next step, the lectin sample was subjected to DEAE-Cellulose chromatography with specific protein elution at continuous gradient of 0.02 to 0.25M NaCl and yielded a lectin fraction having a specific activity of 70,400.0 HAU/mg protein that was 62-fold purified with recovery% of 36.7. The lectin fraction was finally purified to electrophoretic homogeneity by Q-Sepharose column chromatography with specific protein elution at continuous gradient of NaCl and yielded a lectin fraction having a specific activity of 2,04,800.0 HAU/mg protein that was 180-fold purified with recovery% of 16.0.

Following the successful purification, the lectin from the fruiting body of *Lentinus cladopus* Lév. was characterized with respect to certain physiochemical properties. The subunit molecular weight of the lectin, determined by SDS-PAGE with reduction coupled with silver staining, was found to be 20kDa. The molecular weight under native condition, determined by gel filtration through a Sephadex G-75 column, was found to be 40kDa. This native molecular weight compares well with many of the mushroom lectins reported in the literature. Combining the result of native molecular weight determination with that of the molecular weight determination by SDS-PAGE, the purified lectin was found to be a dimeric protein made up of apparently chemically identical subunits. This subunit structure is also in agreement with many of earlier reports on the subunit structure of mushroom lectins. *Lentinus cladopus* lectin, purified in the present investigation, is distinctive in that its hemagglutinating activity cannot be inhibited by various carbohydrates and glycoproteins tested. The activity of the lectin, with or without prior treatment with EDTA, was not influenced by absence or

presence of metal ion ($Ca^{2+}/Mg^{2+}/Mn^{2+}$). This is in conformity with the non-metal-ion requirement for expression of activity by many mushroom lectins reported in the literature. The pH optimum of the lectin activity was found to be relatively broad ranging from 7.5 to 9.0 which compares well with the pH optima of other mushroom lectins. Full activity of the lectin was observed at the assay temperature from 10 to 50°C. At higher assay temperature activity declined and complete loss of activity occurred at assay temperature of 90°C. These observed effects of assay temperature are comparable to or in conformity with literature reports on other mushroom lectins. With respect to thermal stability, the purified lectin subjected to 10 min incubation at 3.1 μg protein/mL in PBS pH 7.4 exhibited a moderate stability with $t_{1/2}$ of 55°C. The lectin could agglutinate all RBC belonging to human blood group A, B and O, with O group being 2 times more specific than either A or B group. However, the observed hemagglutination activity of the lectin towards the human blood groups was 2-4 times lesser than that observed towards rabbit RBC. These results are comparable with those of many other lectins reported in literature. Based on the results of peptide mass fingerprinting of the lectin and subsequent analysis by Ludwig NR and Mascot Sequence matching software (Matrix Science), no match was found with any of the proteins in the database and it was concluded that the lectin, purified from the fruiting body of *Lentinus cladopus* Lév. in the present investigation is a new protein not characterized earlier. Based on the *de novo* sequencing of a major internal peptide, derived from the purified lectin, that yielded the *de novo* sequence NEQFLSGTK, and subsequent analysis by NCBI nr95 (MS-BLAST) (http://dove.embl-heidelberg.de/Blast2/msblast.html), no match was found with any of the proteins in the database and it was again concluded that the lectin, purified in the present investigation, is a new protein not characterized earlier