Thesis Title Modification of Purification Process and Application of Lectin from Sunn Hemp (Crotalaria juncea) Seeds

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Abstract

Lectins are carbohydrate-binding proteins which precipitate glycoconjugates and/or agglutinate cells. Sunn hemp is a famous plant for biofertilizer and the seeds contain lectin, hence it is a suitable source for lectin production. However, the conventional processes for seed extraction and lectin purification are too complicated to scale up the production. This thesis aims at modification of the production process for the lectin from sunn hemp seeds and survey for the lectin application.

The seeds were extracted by 50 mM acetate buffer, pH 4.3, containing 1.0 M sodium chloride with the ratio of seed dry weight to buffer volume of 1 : 10 g/ml and the extraction time was 2 hours at 4 °C. The extraction conferred with maximal quantity and purity and especially required only one step. The lectin was further purified by

affinity binding to agarose beads, which has been incubated with hydrochloric acid for 2 hours, with the ratio of bead volume to extraction volume of 1 : 1.25 - 1 : 1.88 and the pH range between 5.0-7.4. The lectin was then eluted from the beads by 50 mM lactose. The modified processes conferred the lectin with high purity considerred from a single protein band in SDS-PAGE.

Some properties of the purified lectin from sunn hemp seeds were studied. Molecular weight of the lectin was 144,000 daltons and the lectin specifically bound D-galactose. Haemagglutination by the lectin required calcium, magnesium and manganese ions. The stability of lectin decreased with increasing temperature and no haemagglutination was observed at the temperature higher than 70 °C. The lectin agglutinated both human erythrocytes and lymphocytes with no blood group specificity. The haemagglutination study of group O blood from eleven panel subjects and one parabombay patient indicated individual variation of the lectin binding capacity. Besides, the lectin showed no agglutination with five species of bacteria and two of yeasts. However, from eleven types of glycoprotein tested, the lectin bound casein and some glycoproteins in human plasma. These findings might convey essential information for the lectin production in the future.