OPTIMISATION OF PEROXIDASE ADSORPTION ON CONCANAVALIN A-AGAROSE

M.V. MIRANDA, M.L. MAGRI, R.B. CABRERA, H.M. FERNÁNDEZ LAHORE and O. CASCONE

Cátedra de Microbiología Industrial y Biotecnología. Facultad de Farmacia y Bioquímica. Univ. de Buenos Aires. Junín 956 (1113) Buenos Aires, Argentina.
ocasco@ffyb.uba.ar

Abstract— **Many publications describe methods for peroxidase purification from plant material. When the goal was to obtain a high purity enzyme every purification method included an affinity chromatography step using Concanavalin A as the ligand. However, the adsorption step was carried out under quite different conditions with regard to pH, ionic strength and metal cation content in the binding buffer, thus leading to a rather confuse situation. To establish the best conditions for purification of peroxidases from horseradish root (HRP) and soybean hull (SBP), equilibrium adsorption isotherms were fitted to the Langmuir-type model, where ionic strength, pH and cation concentration were chosen as the variables. For SBP, our results showed that Kd rounded 10^{-6} M in all cases (pH 5.0 - 7.0, 1 and 5 mM Mn^{2+} / Ca^{2+}, 0 - 0.75 M NaCl). For HRP, Kd ranged between 10^{-5} M and 10^{-6} M depending on the parameters. Under optimised binding conditions, 84.3% SBP was recovered after elution carried out with 0.74 M α-methyl-D-mannopyranoside and 0.37 M NaCl. For HRP, the recovery was lower (75%) and 0.36M α-methyl-D-mannopyranoside was necessary for the elution step.**

Keywords-- **Peroxidase, Adsorption, Concanavalin A-Agarose, Chromatography.**

I. INTRODUCTION

Peroxidases (EC 1.11.1.7) are ubiquitous oxidoreductases that use hydrogen peroxide or organic hydroperoxides as oxidants. Most peroxidases are glycoproteins containing N-linked oligosaccharide chains (Dunford and Stillman, 1976; Gray and Montgomery, 1997). Horseradish peroxidase, extracted from *Armoracia rusticana* roots, is a commercially important enzyme that occurs as a large family of isoenzymes (Kay *et al*., 1967). Later, a peroxidase extracted from soybean hulls, an inexpensive food industry by-product usually used as poultry feed, was described. This peroxidase was extracted from soybean seeds as a single isoenzyme (Gillikin and Graham, 1991). The unique thermal properties and activities of soybean peroxidase make it particularly suited for different industrial applications (McEldoon and Dordick, 1996).

Several methods for peroxidase purification have been reported. Both plant peroxidases are obtained by homogenising crude material with buffer or water; the homogenate is filtered and further purified by precipitation, centrifugation and different chromatographic steps depending on their intended application. When the goal was to obtain a high purity enzyme every purification method included an affinity chromatographic step. In this way, the revised publications use a lectin chromatography to achieve a pure enzyme preparation taking advantage of the fact that HRP and SBP are glycoproteins (Paradkar and Dordick, 1993; Gillikin and Graham, 1991; Casl and Kostrencic, 1987; Tijssen, 1985). In order to reduce the global process cost - especially for SBP - we reported other possibilities based on partitioning in aqueous two-phase systems, but in every proposed scheme an affinity chromatography step was necessary to obtain high-purity products (Miranda *et al*., 1998).

Plant lectins bind monosaccharides with a relatively low affinity (Ka ~ 1 x 10^3 M), and oligosaccharides much more tightly (Ka ~ 1 x 10^6 M) [Sanders, *et al.*, 2001). Concanavalin A (Con A), from the jack bean *Cannavalia ensiformis*, is the most extensively studied member of the lectin family. It consists of 26.5 kDa subunits that readily form tetramers. The association of subunits is pH-dependent. Each subunit has binding sites for one Mn^{2+} or Mg^{2+}, one Ca^{2+} and one saccharide. Mn^{2+} or Mg^{2+} must be bound before Ca^{2+} binding, and both metal ions must be present for saccharide binding (Reeke *et al.*, 1974). Con A binds molecules containing α-D-mannopyranosyl and α-D-glucopyranosyl residues, and has been extensively utilised for isolation, fractionation, structural characterisation and immobilisation of glycoproteins carrying these kind of residues. Con A-agarose is commercially supplied. The complex Con A-peroxidase is desorbed with α-methyl-D-mannopyranoside, a sugar that competes with the enzyme at the binding sites.

The aim of this work was to establish the best conditions for purification of peroxidases from horseradish root and soybean seed coat on Con A-agarose affinity chromatography. Ionic strength, pH and cation concentration were chosen as the variables for this study.