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Differential Chromatographic Behavior of Some Lignolytic Enzymes from White-Rot Basidiomycetes on Immobilized Metal Chelates

Amin Karmali

Centro de Investigação de Engenharia Química e Biotecnologia do Instituto Superior de Engenharia de Lisboa, R. Conselheiro Emídio Navarro, 1949-014 Lisboa, Portugal

The lignocellulosic materials are the most abundant on earth, which has attracted great attention for production of useful substrates from this waste (Volc and Kubatova, 1994; Takakura and Kuwata, 2003). The white-rot fungi (Basidiomycetes) have the ability to degrade lignocellulosic substrates by synthesizing several lignolytic enzymes of industrial and medical interest such as xylanases (EC 3.2.1.8), celulases (EC 3.2.1.4), glucose 2-oxidase (EC 1.1.3.10), peroxidase (EC 1.11.1.7), pyranose 2-dehydrogenase, superoxide dismutase (EC 1.15.1.1), and laccase (EC 1.10.3.2). White rot fungi are believed to be the most effective lignin-degrading microorganisms in nature.

The overproduction of lignolytic enzymes (pyranose 2-dehydrogenase, glucose 2-oxidase, laccase, xylanases, and superoxide dismutase) from several fungal strains—(*Agaricus bisporus* (J.Lge) Imbach, *Trametes versicolor* (L.:Fr.)Pilát, *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd, *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm., and *Fusarium* sp.)—was carried out by optimizing the composition of the culture media. As far as the composition of culture media is concerned, several agricultural wastes were used, such as rice husks, corn cobs, and rice bran. The effect of specific inducers for overproduction of enzymes was also investigated using these fungal strains.

In order to devise a simple and rapid one-step purification procedure for lignolytic enzymes, the chromatographic behavior of these enzymes (laccases, glucose 2-oxidase, and superoxide dismutase) on immobilized metal chelates was investigated as a function of pH, nature of metal ion, length of spacer arm, ligand concentration, and nature of matrix. The adsorption of enzymes was investigated by using Metal (II)–iminodiacetic acid metal chelates containing Cu (II), Ni (II), Zn (II), Co (II), and Ca (II) as a function of pH. The adsorption to immobilized metal chelates was pH dependent, as evidenced by the fact that high adsorption was observed at pH 8.0. The adsorption of enzymes on metal (II)-IDA chelates was due to the available histidine residues on enzyme molecules, shown by the fact that the addition of imidazole in the buffer system abolished the binding of enzymes to these columns.

Once the experimental conditions of immobilized metal affinity chromatography (IMAC) for enzyme purification were optimized, they were purified in one step by IMAC on Cu(II)-IDA agarose column at pH 6.0 and 8.0 with a high recovery of enzyme activity as well as a high degree of purity. Purified preparations of enzymes were apparently homogeneous on native PAGE and SDS-PAGE. The differential chromatographic behavior of enzymes on metal(II)-IDA chelates is apparently due to the number and spatial distribution of available histidine residues on these enzyme molecules.

REFERENCES

Volc D. G. J. and Kubatova E. 1994. *Appl Environ Microbiol,* **60**, 2524-2532.

Takakura Y. and Kuwata S. 2003. *Bios Biotechnol Biochem,* **67**, 2598-2607.