ABSTRACT

PURIFICATION OF YEAST ALCOHOL DEHYDROGENASE BY IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY

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In this thesis, poly(2-hydroxyethyl methacrylate-glycidyl methacrylate) [poly(HEMA-GMA)] cryogels were prepared by radical cryocopolymerization of HEMA with GMA as a functional comonomer and N,N'-methylene-bisacrylamide (MBAAm) as a crosslinker. Iminodiacetic acid (IDA) functional groups were attached via ring opening of the epoxy group on the poly(HEMA-GMA) cryogels and then Zn^{2+} ions were chealeted with this structures. Characterization of cryogels was performed by FTIR, SEM, EDX and swelling studies. These cryogels have interconnected pores of 30-50 μm size. The equilibrium swelling degree of Zn^{2+} chealeted poly(HEMA-GMA)-IDA cryogels was approximately 600 %. Zn^{2+} chealeted poly(HEMA-GMA)-IDA cryogels were used in the adsorption of alcohol dehydrogenase from aqueous solutions and adsorption was performed in continuous system. The effects of pH, alcohol dehydrogenase concentration, temperature, ionic strength and flow rate on adsorption were investigated. The maximum amount of alcohol dehydrogenase adsorption was determined to be 9,94 mg/g cryogel at 1,0 mg/mL alcohol dehyrogenase concentration and acetate buffer at pH 5,0 with flow rate of 0,5 mL/min. Desorption of adsorbed alcohol dehyrogenase was carried out by 1,0 M NaCI at pH 8,0 phosphate buffer and desorption rate was found to be 93,5 %. Additionally, these cryogels was used for purification of alcohol dehydrogenase from yeast with a single-step. The purity of desorbed alcohol dehydrogenase was shown by silver-stained SDS-PAGE. This purification process can successfully be used for the purification of alcohol dehydrogenase from unclarified yeast homogenates.

Key words: Alcohol dehydrogenase, yeast, cryogel, IMAC, iminodiacetic acid