Title: Chromatographic behaviour of monoclonal antibodies against wild-type amidase from Pseudomonasaeruginosa on immobilized metal chelates

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Abstract: The aim of this work was to devise a one-step purification procedure for monoclonal antibodies (MAbs) of IgG class by immobilized metal affinity chromatography (IMAC). Therefore, several stationary phases were prepared containing immobilized metal chelates in order to study the chromatographic behaviour of MAbs against wild-type amidase from Pseudomonas aeruginosa. Such MAbs adsorbed to Cu(II), Ni(II), Zn(II) and Co(II)-IDA agarose columns. The increase in ligand concentration and the use of longer spacer arms and higher pH values resulted in higher adsorption of MAbs into immobilized metal chelates. The dynamic binding capacity and the maximum binding capacity were 1.33 +/- 0.015 and 3.214 +/- 0.021 mg IgG/mL of sedimented commercial matrix, respectively. A K(D) of 4.53 x 10(-7) M was obtained from batch isotherm measurements. The combination of tailor-made stationary phases of IMAC and the correct selection of adsorption conditions permitted a one-step purification procedure to be devised for MAbs of IgG class. Culture supernatants containing MAbs were purified by IMAC on commercial-Zn(II) and EPI-30-IDA-Zn(II) Sepharose 6B columns and by affinity chromatography on Protein A-Sepharose CL-4B. This MAb preparation revealed on SDS-PAGE two protein bands with M(r) of 50 and 22 kDa corresponding to the heavy and light chains, respectively.

Author Keywords: Monoclonal Antibodies of IgG Class; Wild-Type Amidase from Pseudomonas Aeruginosa; Immobilized Metal Affinity Chromatography; Zn(2+) Ions; Epichlorohydrin

KeyWords Plus: Ion Affinity-Chromatography; Tomato Pomace; Purification; Proteins; Binding; Mutant

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