A New Matrix for Affinity Chromatography and its Application in the Separation of a Human Monoclonal Antibody

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A method for the preparation of a p-aminobenzene sulphonyl ethyl containing crosslinked Sepharose 4B (ABSE-Sepharose 4B-CL) is described. Trypsin, bovine serum albumin (BSA) and concanavalin A (Con A) were immobilized onto this matrix by diazotation. Conditions for the coupling reaction were investigated. The activity of immobilized trypsin reached 1.4×10^4 U/g, and 25 mg BSA can be coupled onto 1 g ABSE-Sepharose 4B-CL under the optimal conditions. An affinity medium with immobilized Con A as ligand was prepared by this method, and was used in the separation of a human monoclonal antibody.

INTRODUCTION

It is nearly 90 years since Emil Starkenstein first introduced affinity chromatography by the use of immobilized starch to separate amylase. Although many complicated mathematic models were established (Chatkcn, 1987), the procedure and hardware of affinity chromatography remain very similar to that used decades ago. One of the most important factors in the development of affinity chromatography is the development of solid supports. It includes two aspects, one is the selection of material and the other is the method of attachment.

Agarose is a linear polysaccharide composed of alternating β-galactose residues and 3,6-anhydro-β-galactose units. It is one of the few materials fulfilling almost all of the requirements of an ideal carrier, and has become the most commonly used support in affinity chromatography, especially after the introduction of spherical agarose by Pharmacia (Uppsala, Sweden) under the trade-name Sepharose. One of the main disadvantages of the agarose matrix is that it does not contain an active functional group for ligand attachment. To solve this problem, several derivatives and related methods for activation were introduced into practice, including epoxy-activated agarose, activated thiol-agarose, cyanogen bromide activated agarose and CH or AH activated agarose. However, as used in ligand attachment, all of these matrices need either a long reaction time or a high temperature, which may seriously damage the structure of the ligand or reduce recovery. For example, the most commonly used cyanogen bromide activated agarose, used for the immobilization of proteins, requires 2 h at room temperature or 16 h at 4°C, while pH between 8 and 10 (Turkova, 1978).

In this work, a new matrix containing a p-aminobenzene sulphonil ethyl (ABSE) group was synthesized from commercial Sepharose 4B. This matrix can be easily activated by diazotation, and biological macromolecules can be immobilized onto the matrix in 1 h at 0°C. Two kinds of ligand probe were used to evaluate the new matrix and the result obtained was satisfactory. Finally, a medium with concanavalin A (Con A) as affinity ligand was prepared and the separation of a human monoclonal antibody was performed.

EXPERIMENTAL

Materials. Commercially available spherical agarose, Sepharose 4B was purchased from Pharmacia (Uppsala, Sweden). Concanavalin A, Type 3 was from Sigma (St. Louis, MO, USA). p-D(sulphato ethyl sulphonyl) aniline (SESA) was an industrial product from Shanghai Eighth Chemical Dyestuff Factory (Shanghai, China). Bovine Serum Albumin (BSA) and N-Benzoyl-D,L-arginine-p-nitroanilide hydrochloride (BAPNA) were purchased from DonFeng Biochemical Corporation (Shanghai, China). Epoxy propane chloride was analytical grade reagent from Tianjin Chemical Plant (Tianjin, China). Crystallized bovine pancreas trypsin was donated by the Shanghai Institute of Biochemistry (Shanghai, China): the specific activity measured by Erlanger's method (Erlanger et al., 1961) with BAPNA as substrate was 3.0×10^4 U/g. Monoclonal antibody recognizing human colonic cancer associated antigens was from the General Hospital of Shenyang (Shenyang, China).

Apparatus. The experiments were performed on a Bio-Rad automated Econo System (New York, NY, USA) which consists of a Model EP-1 Econo Pump, a Model EM-1 Econo UV Monitor with a portable optics module equipped with two interchangeable filters (254 and 280 nm), a Model 2110 Fraction Collector and a Model ES-1 Econo System Controller. Chromatograms were recorded and processed on a Hewlett Packard Model 3394A integrator. The loading of samples onto the affinity column was carried out by a Gibson Minipin 3 Peristaltic pump (Middleton, USA) in a refrigerator (3-7°C).

Methods. The crosslinking (CL) of Sepharose 4B with epoxy propane chloride was carried out according to Porath (Porath et al., 1971). The protein content was assayed using the