THE PURIFICATION OF CARCINOEMBRYONIC ANTIGEN BY GLUTARALDEHYDE CROSS-LINKED CONCANAVALIN A[1]

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Summary: The partial purification of carcinoembryonic antigen from liver metastases by affinity chromatography with Concanavalin A is reported. Lyophilized perchloric acid extract of tumor tissue was resuspended and fractionated on Concanavalin A insolubilized by cross-linking with glutaraldehyde. Use of the insolubilized Con A in the batchwise method allowed for 65% recovery of the CEA with a 25-fold purification with regard to protein. The perchloric acid extracts contained 0.097 mg CEA per mg protein while the purest fraction from the insolubilized Con A contained 2.6 mg CEA per mg protein.

Introduction

Carcinoembryonic antigen (CEA) refers to a group of glycoprotein antigenic determinants which are found in high concentration in human fetal gastrointestinal tissue and various malignancies but are usually not detectable or present only in small quantities in normal adult tissue (1-5). The possibility for diagnostic value has led to extensive biochemical and clinical investigations of CEA thus necessitating large amounts of purified material. Others have reported methods for the purification of CEA which require several steps following the extraction of the material from primary tumors or metastases (6-8). Affinity chromatography utilizing anti-CEA antibodies covalently bound to Sepharose

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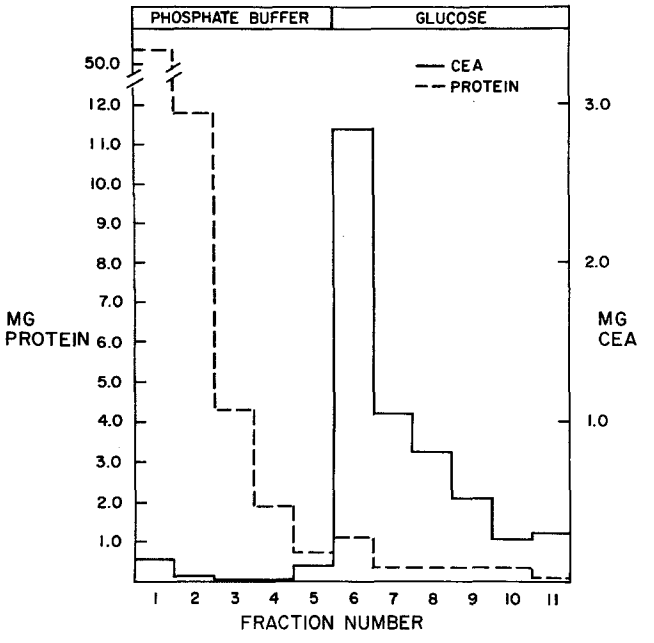
4B allows for the purification of CEA extracts in one step (9). Recent reports have indicated that the phytohemagglutinin Concanavalin A (Con A) binds specifically to CEA (4,10). Others have reported the binding of purified CEA to insolubilized Con A (11,12). Rogers et al. found that CEA which had been purified by Sepharose 4B and Sephadex G-200 chromatography could be resolved into 3 separate fractions, and that these 3 fractions contained 2 distinct antigenic determinants (11). We now report the partial purification of CEA in perchloric acid extracts of liver metastases by affinity chromatography with Con A insolubilized by modification with glutaraldehyde.

Materials and Methods

Con A (Sigma, Grade IV) was insolubilized by modification of the procedure described by Donnelly and Goldstein (13). Glutaraldehyde (0.1 ml of a 12.5% solution) was added to a solution of Con A (800 mg in 55 ml 0.1 M phosphate buffer, pH 7.2) at 4°C. The reaction mixture was stirred for 1 hr at 4°C. The resulting precipitate was collected by centrifugation for 5 min at 7800 g. The supernatant was allowed to react with an additional 0.1 ml glutaraldehyde for 1 hr. This process was repeated until essentially all of the Con A had been insolubilized (a total of 0.6 ml of 12.5% glutaraldehyde was required). The precipitates were washed 5X with 0.02 M phosphate buffer in 1.0 M NaCl (pH 7.2), combined, and stored at 4°C under buffer. All subsequent steps involved the use of 0.02 M phosphate buffer in 1.0 M NaCl unless otherwise stated.

Crude CEA was obtained from hepatic metastases of a primary adenocarcinoma of the colon. Homogenization, perchloric acid extraction, and lyophilization of the tumor tissue were carried out as described by Krupey et al. (8). Lyophilized material was resuspended in buffer at a concentration of 12.1 mg/ml protein as determined by a modification of the Lowry method (14). Further purification of the CEA was obtained by the use of glutaraldehyde insolubilized Con A in the batchwise mode. A total of 75 mg protein from the crude CEA preparation was added to the insolubilized Con A in a volume of 12 ml and allowed to stir for 1 hr at room temperature. Insolubilized Con A and the material bound to it were recovered by centrifugation for 5 min at 7800 g. The supernatant was decanted and stored at 4°C. An additional 12 ml of buffer was added to the pellet and allowed to stir for 15 min at room temperature followed by centrifugation as described above. This process was repeated until the insolubilized Con A had been washed 4X with buffer. The first supernatant and the phosphate buffer washes comprised fractions 1 through 5. CEA bound to the insolubilized Con A was released by washing with 0.1 M glucose in 1.0 M NaCl (13) 6X for 15 min at room temperature. Supernatants from the glucose washes were designated fractions 6 through 11 and were dialyzed against 0.01 M phosphate in 0.9% NaCl (pH 7.2) to remove glucose. The protein content of each fraction was determined by a modification of the Lowry method (14), while CEA concentrations were determined by the Hansen-Z-gel radioimmunoassay marketed by Hoffman-La Roche (Nutley, N. J.).



Results and Discussion

In fraction 6, the ratio of CEA:protein was increased 25-fold over that in the crude extract of CEA prior to adsorption with insolubilized Con A (Fig. 1). Fraction 6 contained 1.4% of the protein and 39% of the CEA present in the original extract of CEA. Cumulatively, 84% of the CEA and 96% of the protein in the unfractionated CEA extract were recovered in the combined fractions. Double diffusion in agar gel of Con A and samples from these fractions gave single bands of precipitation. Double diffusion of samples from fractions 6 through 11 and anti-CEA goat antiserum (the kind gift of Dr. Charles W. Todd, City of Hope, Ca.) also resulted in single precipitin bands.

The quantitative relationship between carbohydrate and protein portions of CEA is not clear at this time. Terry et al. have summarized the findings in this area suggesting that CEA from hepatic metastases contains about 50% carbohydrate with total yields of carbohydrate plus protein generally not

exceeding 90% (4). Banjo et al. have reported that the molecule does not contain fatty acids (15). The intermolecular heterogeneity of CEA is also well documented (4,9,15). We are currently investigating the possibility that different molecular species may be separated between fractions 6 and 11.

The perchloric acid extracts used in this study contained about 3% CEA by weight which is consistent with the findings of Krupey et al. for liver metastases (8). The recovery of 65% of the applied CEA in a ratio of 2.6 mg CEA:1 mg protein indicates that the binding of crude CEA extracts to glutaraldehyde insolubilized Con A results in the removal of most protein contaminants.

The results of this study demonstrate that affinity adsorption by Con A offers an alternative means to molecular sieving for the isolation and initial purification of CEA from crude preparations. Among the advantages offered by this system are that large amounts of CEA can be processed in relatively small volumes, and, as shown by David and Reisfeld (12), antigenically inactivated CEA is not bound to Con A. Furthermore, there is in this system the potential for resolving antigenically distinct forms of CEA such as those described by Rogers et al. (11).

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