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Note

Chromatography of non-human albumins on Cibacron Blue–agarose

Application to the separation of albumin from rat alpha-fetoprotein

PHILIP C. KELLEHER and CAROL J. SMITH
Department of Medicine, University of Vermont College of Medicine, Burlington, Vt. 05405 (U.S.A.)
and
RALPH PANNELL*
Department of Biochemistry, University of Vermont College of Medicine, Burlington, Vt. 05405 (U.S.A.)
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A major difficulty in the purification of many serum proteins is their separation from albumin. Human, rat and mouse alpha-fetoproteins (AFP) are physicochemically similar to the albumins of the respective species so that separation by conventional physical and chemical techniques is difficult[1]. Travis *et al.*[2] described the removal of albumin from human serum by affinity chromatography on a column of agarose–Cibacron Blue conjugate. We attempted to apply their method as a step in the purification of non-human AFP and found a far lower affinity of rat, mouse and bovine albumin for Cibacron Blue than of human albumin under the same conditions. This paper describes conditions for chromatography of murine and bovine albumin on Cibacron Blue–agarose.

EXPERIMENTAL

Cibacron Blue–agarose[2] was packed in a 240 × 9 mm column (bed volume, 15 ml) and equilibrated with one of the following buffers: buffer 1, 10 m*M* Tris–HCl, pH 7.5; buffer 2, 50 m*M* Tris–HCl, pH 7.5, 10 m*M* NaCl; buffer 3, 50 m*M* Tris–HCl, pH 7.5, 50 m*M* NaCl; buffer 4, 50 m*M* Tris–HCl, pH 7.5, 500 m*M* NaCl. All buffers contained 10 m*M* NaN_3. Buffers 3 and 4 have been used to prepare albumin-free human serum proteins[2,3].

Pooled one-day-old newborn rat or mouse sera, pooled hepatoma-bearing rat sera containing AFP, pooled normal adult mouse sera, partially purified rat AFP preparations, or culture media containing rat AFP and fetal bovine serum were dialyzed against one of the above buffers prior to chromatography. Single donor normal adult human sera were dialyzed against buffer 1 prior to chromatography. The equilibrating buffer was used to elute by descending flow the proteins which did

* Present address: Department of Microbiology, University of St. Louis Medical School, St. Louis, Mo. 63104, U.S.A.

not bind to Cibacron Blue. The albumin bound to Cibacron Blue was eluted with 50 m*M* Tris–HCl, pH 7.5, containing 200 m*M* NaSCN. The column was regenerated with two column volumes of the equilibrating buffer.

Some of the rat serum samples were treated with activated charcoal[4] prior to dialysis against the equilibrating buffer for chromatography.

Murine AFP and murine and human albumins in the sera and chromatographic fractions were quantified by radial immunodiffusion[5,6]. Bovine albumin was quantified by the method of Lowry *et al.*[7] after precipitation with 5% trichloroacetic acid and solubilization with 95% ethanol[8]. Proteins eluted from the Cibacron Blue–agarose column were monitored by the procedure of Lowry *et al.*[7].

RESULTS

All of the rat and mouse AFP applied to the Cibacron Blue–agarose column was recovered in the unbound fraction at all buffer concentrations. The binding capacities of rat, mouse and bovine albumins for Cibacron Blue, however, were affected by the composition of the equilibrating buffer. The amount of albumin bound to the Cibacron Blue was inversely proportional to the ionic strength of the buffer (Table I). The relative proportions of AFP and albumin in the samples did not affect the binding of albumin to Cibacron Blue. Recoveries of rat, mouse, bovine and human albumins (unbound and bound fractions) from the column were 95–100%. Repeated use of the Cibacron Blue–agarose column did not result in a decreased binding of albumin. Seventy five percent of the bound rat albumin was eluted when 50 m*M* Tris–HCl buffer, pH 7.5, containing 2 *M* NaCl rather than 200 m*M* NaSCN was used.

TABLE I

EFFECT OF BUFFER COMPOSITION ON BINDING OF RAT, MOUSE AND BOVINE ALBUMINS TO CIBACRON BLUE–AGAROSE

Aliquots (0.25 ml) of rat, mouse and human sera and culture media were chromatographed as described in the text.

Buffer	*Composition*	*Albumin bound (%)*			
		Rat	*Mouse*	*Bovine*	*Human*
1	10 m*M* Tris–HCl	98–100*	99**	100**	100**
2	50 m*M* Tris–HCl, 10 m*M* NaCl	69– 82	93	—	—
3	50 m*M* Tris–HCl, 50 m*M* NaCl	54– 61	—	60	—
4	50 m*M* Tris–HCl, 500 m*M* NaCl	39– 46	51	18	—

* Range of values from six determinations.
** Average of values from three determinations.

The binding capacities (mg albumin/ml column bed volume) of human, rat, mouse and bovine albumins for Cibacron Blue were 2.0, 4.8, 0.83 and 0.79, respectively, when chromatographed using buffer 1.

Treatment of rat serum samples with activated charcoal prior to Cibacron Blue affinity chromatography did not improve the binding of rat albumin to Cibacron Blue with buffer 3. In addition, charcoal treatment resulted in a loss of 10 to 24% of the AFP after Cibacron Blue affinity chromatography.

DISCUSSION

Albumins from various species are physicochemically similar. Species variability in the affinity of various albumins for Cibacron Blue is not surprising, however, in view of differences found between human and bovine albumins in the binding of sulfonphthalein dyes[9] and in other conformational parameters which affect ligand binding[10].

Travis and Pannell[3] found the use of the high-ionic-strength buffer 4 necessary to prevent the blue dextran–agarose from behaving as a cation exchanger. Gold *et al.*[11] utilized the high-ionic-strength buffer in the purification of human AFP; they removed 79% of the albumin present in the sample and recovered 75% of the AFP. Young and Webb[12], using a buffer ionic strength of 160 m*M* in the chromatography of fetal human serum, removed 97% of the albumin and recovered 91% of the AFP.

With buffer conditions of low ionic strength we removed over 98% of rat and mouse albumins from all AFP-containing preparations by Cibacron Blue affinity chromatography. In most instances no albumin could be detected in the AFP preparations. Rat albumin present in newborn serum and tumor-bearing adult serum had the same chromatographic behavior on Cibacron Blue–agarose columns. Purification of rat or mouse AFP by negative immunoadsorbent affinity chromatography is facilitated by removal of albumin since albumin is the major contaminating normal serum protein against which a high-titer antiserum must be prepared[13].

Cultures of the AFP-secreting rat hepatoma cell line UVM-RH777 are grown in nutrient mixture F12 supplemented with 10% fetal bovine serum in this laboratory. We also demonstrated that the quantitative removal of bovine serum albumin from rat AFP-containing culture media necessitated the use of low-ionic-strength buffer in Cibacron Blue affinity chromatography. Passage of these culture media over Cibacron Blue–agarose allowed us to visualize the rat AFP in various electrophoretic procedures without additional purification.

Albumins exist in serum unbound and bound to bilirubin, fatty acids and drugs. Activated charcoal treatment, which has been used to defat albumin[3], was attempted to increase the unbound fraction for binding to Cibacron Blue. The fact that Cibacron Blue binding of rat albumin was not improved by charcoal treatment but by lowering the buffer ionic strength suggests that endogenous ligands were not interfering with Cibacron Blue binding but that the affinity of rat albumin for Cibacron Blue is sufficiently low for buffers of moderate ionic strength to decrease the protein–dye interaction.

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