CHARACTERIZATION OF THE INTERACTION OF ALBUMIN WITH ISOLATED RAT LIVER CELLS TO REVEAL THE MECHANISM OF ALBUMIN-MEDIATED HEPATIC TRANSPORT

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The binding of bovine serum albumin (BSA), containing $^{125}$I-BSA, to isolated rat hepatocytes was studied over a 300-fold concentration range of BSA to characterize the interaction between albumin and the liver cells in albumin-mediated hepatic transport. The binding of BSA with a high affinity to the cell surface of hepatocytes was not found in the binding behavior. The bound fraction of BSA with hepatocytes was about 1% over those concentration range of BSA.

Keywords — albumin; hepatocyte; hepatic transport; binding; albumin-mediated transport; liver cell; uptake

INTRODUCTION

Since the kinetic studies in isolated perfused rat liver by Weisiger et al. and Forker and Luxon showed that fatty acid and bile acid which bound to the albumin could be taken up into the liver, several studies, which support the albumin mediated hepatic transport, have been reported concerning isolated perfused rat liver using rose bengal and bromosulfophthalein (BSP). The albumin mediated transport of iopanoic acid was also reported in isolated rat hepatocytes in primary culture by Barnhart et al. Mizuma et al. also showed the effect of albumin on BSP uptake by isolated rat hepatocytes. This albumin-mediated hepatic transport is very interesting because it has been widely accepted that only the unbound substances are available for uptake. However, the mechanism of the albumin mediated hepatic transport has not been revealed. To elucidate the mechanism, the study of the interaction between albumin and hepatocytes in vitro may be useful. Weisiger et al. and Ockner et al. reported specific binding of albumin to hepatocyte, while Stremmel et al. found that the liver cell plasma membranes did not contain a membrane protein with a high affinity for albumin and that the albumin was only loosely associated with the membrane.

In the present study, we investigated the binding of albumin to isolated rat hepatocytes in order to characterize the interaction between albumin and hepatocytes.

MATERIALS AND METHODS

**Materials** — $^{125}$I-Bovine serum albumin (1.3 mCi/mg) and [carboxyl-$^{14}$C] dextran (avg. $M_t$ 70000) (0.51 mCi/g) were purchased from New England Nuclear (Boston, Mass, U.S.A.). BSP sodium salt, bovine serum albumin (BSA) (fraction V, Lot 101F-003345), fluorescein isothiocyanate dextran (FITC-dextran) (avg. $M_t$ 64200), dextran (avg. $M_t$ 65600) and collagenase (type I) were obtained from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). Silicone oil (KF961, 100cs) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Spectrapor 2 membrane tubing was obtained from Spectrum Medical Industries, Inc. (Los Angeles, CA, U.S.A.). All other chemicals were of analytical grade.

**Isolation of Hepatocytes** — Enzymatically isolated rat hepatocytes were prepared from male Wistar rats of 195—240 g body weight according to the method of Lin et al. which was a slightly modified method of Baur et al. All the experiments were performed with cell suspensions containing more than 90% viability estimated by trypan blue exclusion.

**Uptake of BSP by Hepatocytes** — The uptake of BSP by isolated rat hepatocytes suspended in the incubation medium containing 137 mM NaCl, 5.2 mM KCl, 0.9 mM MgSO$_4$, 0.12 mM CaCl$_2$, 5 mM glucose, 3 mM Na$_2$HPO$_4$ and 15 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethansulfonic acid), pH 7.4, was studied by

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the centrifugal filtration method. After preincubation of 1.5 ml of the cell suspension containing $8 \times 10^6$ cells/ml at 37 °C for 5 min, the reaction was started by the addition of 1.5 ml of BSP solution preincubated at 37 °C. Following the reaction, the experimental procedures were carried out according to those described previously. The amount of BSP taken up was determined by absorption of the supernatant fluid obtained by centrifugation at 579 nm using a Hitachi 557 spectrophotometer. The volume of fluid adhered to the cells was determined as described previously. All the data for uptake were corrected by the amount of BSP in the volume of adherent fluid.

**Binding of BSA to Hepatocytes** — Binding was studied in two ways as follows.

Method 1: Three fifths ml of BSA solution incubated at 37 °C containing $^{125}$I-BSA (0.2 μCi/ml) was added to 0.6 ml of isolated rat hepatocyte suspension containing $8 \times 10^6$ cells/ml incubated in a glass tube for 5 min at 37 °C. One fifth ml of the mixture was used to determine the total concentration of BSA in the mixture. Immediately after the addition of BSA solution to the hepatocyte suspension and after the mixture was incubated for 30 min at 37 °C, the mixture was centrifuged for 1 min at 1600 rpm in a Kubota centrifuge KN-70. Then, 0.2 ml of the supernatant fluid was taken to determine the unbound concentration of BSA in the medium.

Method 2: Binding was studied by the centrifugal filtration method. BSA solution (0.675 ml) incubated at 37 °C containing $^{125}$I-BSA (3.73 μCi/ml) was added to 0.675 ml of hepatocyte suspension containing $8 \times 10^6$ cells/ml incubated in a glass tube for 5 min at 37 °C. One fifth ml of the mixture was used to determine the total concentration of BSA in the reaction mixture. Immediately after the addition, the mixture was centrifuged for 5 s in a Beckman microfuge B according to the centrifugal filtration method and 0.2 ml of the supernatant fluid was taken to determine the unbound concentration of BSA in the medium. After the supernatant fluid was removed, the centrifuge tube was washed twice with distilled water and the hepatocytes, under a silicone layer, were resuspended with 1 ml distilled water to determine the bound concentration of BSA in the mixture.

The influence of BSA concentration on the cellular adherent fluid volume was studied using 5 μM dextran containing $^{14}$C-dextran.

**Binding of Dextran to Hepatocytes** — Binding was studied using the same procedure as described in method 1. Three fifths ml of dextran solution containing $^{14}$C-dextran (0.1 μCi/ml) was used instead of BSA solution containing $^{125}$I-BSA (0.2 μCi/ml).

The adsorption of BSA to the experimental plastic ware, such as tips of pipettes, was significant. Therefore, in these binding experiments of BSA or dextran to hepatocytes, glass ware, except the centrifuge tube for a Beckman microfuge B, was used to minimize the adsorption of BSA. All the experiments were performed at 37 °C in the same buffer used in the uptake of BSP by hepatocytes.

**RESULTS**

**Effect of Albumin on Uptake of BSP by Hepatocytes**

In a previous paper, we reported the albumin-mediated hepatocellular uptake of BSP using isolated rat hepatocytes. In this study, the effect of albumin concentration on the uptake of BSP by hepatocytes was investigated. The initial uptake rate of BSP by hepatocytes was plotted against unbound BSP concentration in the medium (Fig. 1). The initial uptake rate was obtained by a method described in the previous paper, which was a modification of the method of Schwenk et al. The unbound BSP concentration was estimated by the correction for BSP.

![FIG. 1. Effect of Albumin on the Uptake of BSP by Isolated Rat Hepatocytes](image-url)

Initial uptake rate of BSP by hepatocytes was plotted as a function of unbound BSP concentration. (●), no BSA; (△), 0.2% BSA; (□), 0.6% BSA; (○), 1.5% BSA. Temperature, 37°C; hepatocytes, $4 \times 10^6$ cells/ml. Each point is the mean ± S.E. from 5—6 experiments.
binding to cell membranes and to BSA in the case of the presence of BSA as already described. The presence of BSA obviously increased the uptake rate of BSP by hepatocytes.

**Binding of Albumin to Hepatocyte**

The binding of BSA containing $^{125}\text{I}}$-BSA to hepatocytes was studied by the centrifugation method (method 1) at two different reaction times, 30 min and less than 30 s. In either case, the bound fraction of BSA to hepatocytes was very small and showed almost a constant value in the concentration range of BSA (0.01 to 3%). The binding of BSA to hepatocytes was not dependent on the reaction time (Fig. 2). Thus, the ratio ($R$) of the radioactivity of $^{125}\text{I}}$-BSA between the supernatant fluid and the whole medium was compared with the ratio of the radioactivity of $^{14}\text{C}}$-dextran between the supernatant fluid and the medium (Fig. 3). Dextran is known not to be taken up by the cells and has been used as a cellular adherent fluid volume indicator. $R$ for BSA was slightly but obviously less than 1.0, while $R$ for dextran was larger than 1.0. The correction for the cell volume in the mixture was not done, which gave $R$ over 1.0 in the case of dextran. This indicates, simultaneously, that there is little interaction between dextran and hepatocytes. On the other hand, $R$ for BSA in hepatocyte suspension showed the value to be slightly below 1.0. This suggests some interaction between BSA and hepatocytes which is not found between dextran and hepatocytes. The interaction of BSA to hepatocyte should be characterized. However, it is difficult to determine the amount of BSA bound to hepatocytes by the centrifugation method because of its low binding capacity. Therefore, the binding of $^{125}\text{I}}$-BSA to hepatocytes was studied according

![FIG. 2. Binding of Albumin to Isolated Rat Hepatocytes](image)

**Binding was measured at 37°C by the centrifugation method. Hepatocyte concentration: 4 × $10^6$ cells/ml. The reaction time: (O) less than 15 s; (●) 30 min. The unbound BSA fraction (%) was expressed as the ratio of the concentration of BSA in the supernatant fluid after centrifugation to the total BSA concentration in the mixture of hepatocytes and BSA containing $^{125}\text{I}}$-BSA, which was corrected for the cell volume, 7.87 μl/10^6 cells quoted from Bock et al. Each point is the mean ± S.E. from three experiments.**

![FIG. 3. Comparison of the Binding Behavior of Albumin and Dextran to Isolated Rat Hepatocytes](image)

**Binding was measured at 37°C by the centrifugation method. Ratio of radioactivity ($R$) in the supernatant fluid after centrifugation to that in the mixture of hepatocytes and albumin or dextran containing $^{125}\text{I}}$-BSA or $^{14}\text{C}}$-dextran, respectively, was plotted against total BSA concentration. Hepatocyte concentration: 4 × $10^6$ cells/ml. (O), BSA; (●), dextran. Each point is the mean ± S.E. from three experiments for BSA and four experiments for dextran.**

![FIG. 4. Binding of BSA to Hepatocytes](image)

**Binding was measured at 37°C by the centrifugal filtration method. The concentration of bound BSA was plotted against total BSA concentration. The values were corrected for the amount of BSA in the adherent fluid, assuming that dextran does not have any interaction with hepatocytes. Each point is the mean ± S.E. from five experiments.**
Table I. Effect of Albumin on the Cellular Adherent Fluid Volume Required on the Study of Ligand Uptake by Hepatocytes by Silicone Filtration Method

<table>
<thead>
<tr>
<th>Albumin concentration (%)</th>
<th>Cellular adherent fluid volume (μL/4×10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.9±0.8</td>
</tr>
<tr>
<td>0.001</td>
<td>11.2±0.7</td>
</tr>
<tr>
<td>0.2</td>
<td>10.0±0.2</td>
</tr>
<tr>
<td>0.6</td>
<td>12.3±1.6</td>
</tr>
<tr>
<td>1.5</td>
<td>11.1±0.7</td>
</tr>
</tbody>
</table>

The experimental conditions are described under Materials and Methods. ¹⁴C-dextran was used to estimate the cellular adherent fluid volume. The values of cellular adherent fluid volume were given mean ± S.E. from four experiments.

to the centrifugal filtration method (method 2) described under Materials and Methods, which showed the amount of BSA bound to hepatocytes. The amount of BSA bound to hepatocytes corrected for the cellular adherent fluid volume, was plotted against total BSA concentration in Fig. 4. The amount of BSA bound to hepatocytes increased linearly with total BSA concentration over a wide range of concentration of BSA (10⁻⁴ to 2%). The cellular adherent fluid volume was not influenced by the albumin concentration (Table I). The cellular adherent fluid volume, 11.1±0.4 μL/4×10⁶ cells (mean ±S.E.) was comparable to the value, 10.4±1.3 μL/4×10⁶ cells (mean ±S.E.) obtained using FITC-dextran as a cellular adherent fluid volume indicator. The value reported by Eaton and Klaassen et al.¹⁶ was about 0.6—1.0 μL/mg protein, which can be converted to 5.8—9.6 μL/4×10⁶ cells using the value of 2.41 mg protein/10⁶ cells obtained by Bezooijen et al.¹⁷ This value is comparable to our results. These results show clearly that BSA binds to hepatocytes but the binding was not of high affinity.

DISCUSSION

Further support for the effect of albumin on uptake of BSP by isolated rat hepatocytes is presented in Fig. 1. To elucidate the mechanism of the albumin-mediated hepatocellular uptake, it is important to characterize the interaction of albumin with hepatocytes. Weisiger et al. reported that the binding of albumin to isolated rat hepatocytes consists of a single class of relatively high-affinity binding sites and a second binding component which is not saturable.¹¹ We investigated the binding of albumin to isolated rat hepatocytes, using ¹²⁵I-BSA. Weisiger et al. measured the binding of ¹²⁵I-albumin to isolate hepatocytes after 30 min incubation.¹¹ We employed two different reaction times, 30 min and less than 30 s, using the centrifugation method for the binding experiments. The binding of BSA to hepatocytes was non-saturable at both incubation times. No high-affinity binding was observed in the binding profile (Fig. 2). R obtained for ¹²⁵I-BSA was slightly less than 1.0 and R for ¹⁴C-dextran was more than 1.0, as shown in Fig. 3. This indicates that there is some interaction between BSA and hepatocytes. The evidence for this interaction of BSA with hepatocytes was further substantiated by the data presented in Fig. 4 which measured the bound amount of BSA to hepatocytes directly by the centrifugal filtration method. The amount of albumin bound to hepatocytes increased linearly with total albumin concentration over a 300-fold concentration range of albumin. The binding ratio of albumin to hepatocytes was about 1%. A single class of relatively high-affinity binding sites as was reported by Weisiger et al.¹¹ was not observed, even at a very low concentration of albumin in our binding experiments. The present result is consistent with the result reported by Stremmel et al. that albumin was only loosely associated with the hepatocyte plasma membrane.²³ Consequently, no specific albumin receptor on the liver cell plasma membrane was found in this study. Two possibilities were proposed by Forker and Luxon²⁴ concerning the albumin mediated uptake mechanism. One possibility is that the diffusion of ligand across the unstirred fluid layer in the Disse space is accelerated by the binding of ligand to albumin. The second is that the interaction of the ligand-albumin complex with the cell surface

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enhances the dissociation of ligand from the ligand–albumin complex and consequently provides a higher local concentration of unbound ligand than that predicted by an equilibrium state in free solution. Forker et al. reported in a following paper that an effect of albumin on the rate of limiting diffusion of ligand across an unstirred layer in the Disse space appeared unlikely in view of saturation phenomenon in transport. However, the binding characteristic of albumin to hepatocytes shown in the present study would not exclude the second possibility.

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