CONCANAVALIN A BINDING PROTEINS OF LYMPHOID CELL SURFACE*

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One of the central problems in cellular immunology has been to understand how lymphoid cells are triggered to differentiate. Concanavalin A (Con A) is known to stimulate DNA synthesis of lymphoid cells in vitro (1). Although this mitogen appears to bind to the surface of both T and B cells in equal amounts (2), only T cells are selectively activated to synthesize DNA by soluble Con A (3). Insoluble Con A-agarose beads which bind both B and T lymphoid cell surfaces also induce DNA synthesis as soluble Con A, suggesting that the specific receptor for Con A stimulation resides in the plasma membrane (4).

Recently, specific adherence of intact cells to large plastic beads or nylon fibers coated with hormones or antigens has been used to study specific receptors (5, 6). Moreover, isolation of the insulin receptors (7) was more readily achieved with agarose beads in which insulin is separated from the matrix backbone by relatively long spacers ("arms"). Using a similar technique of affinity chromatography of intact lymphoid cells, isolation of a specific cell surface receptor protein for Con A is described in this communication.

Materials and Methods

A suspension of chicken lymphoid cells was prepared from the spleen, as previously described (8, 9). Con A was freshly prepared from the jack bean by the methods of Agrawal and Goldstein (9) and covalently attached to agarose. Agarose derivatives of Con A ("Con-A-arm-Sepharose") were prepared by reaction with activated *N*-hydroxysuccinimide esters of diaminodipropylaminosuccinyl-agarose, as described by Cuatrecasas and Parikh (10). Con A-arm-Sepharose contained 25 mg of Con A/ml gel. The capacity of Con A-arm-Sepharose to bind glycoproteins was measured by ^{125}I-labeled ovomucoid (1 μCi/μg) in the presence of 1% bovine serum albumin (BSA). At the saturation point, 10–15 μg ovomucoid were bound to 0.01 ml Con A-arm-Sepharose. In the presence of 0.2 M D-mannose, less than 20% as much ovomucoid was bound.

The cell surface proteins were labeled with ^{125}I as described by Marchalonis et al. (11) and subjected to the binding experiments to Con A-arm-Sepharose as shown in Fig. 1. ^{125}I-labeled cells (4×10^7 cells) were incubated at 37°C with Con A-arm-Sepharose (5 mg Con A/0.2 ml) for 30 min as described above (Fig. 1). The beads with bound cells were gently collected on stainless steel mesh (no. 200) which does not retain unbound cells (8). The bound lymphoid cells were quickly lysed with cold 1% Nonidet P-40 (NP-40) in TKM (0.05 M Tris-HCl, pH 7.6, 0.025 KCl, and 0.005 M $MgCl_2$) containing 0.005 M $CaCl_2$ and 0.004 M phenyl-methyl-sulfonyl-fluoride (PMSF) to inhibit proteases. Solubilized membranes and free nuclei were quickly washed through the stainless steel mesh with 0.5% NP-40, leaving behind ^{125}I-labeled surface proteins bound to Con A-arm-Sepharose. The beads were scraped off the mesh and transferred into a cone-shaped plastic tube, exhaustively washed with 0.5% NP-40 TKM Ca^{++}, and treated with 1 ml 0.2 M mannose in 0.1% NP-40 TKM Ca^{++} and PMSF overnight in the cold room in order to elute Con A binding proteins. The eluted fraction was concentrated

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by lyophilization and analyzed in sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis as described elsewhere (8). The radioactivity of the fractions was determined in a Nuclear Chicago autogamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). For the internal standard of the molecular weight in the gel, ^{131}I-labeled proteins with known molecular weights were run simultaneously.

RESULTS AND DISCUSSION

Specific adherence of chicken lymphoid cells to Con A-arm-Sepharose is shown in Fig. 1. Within 30 min of incubation at 37°C (Fig. 1 *a*), many lymphoid cells adhered to Con A-arm-Sepharose, and they later detached as incubation continued for more than 6 h. In the dish which was incubated at 4°C (Fig. 1 *b*), far less lymphoid cells adhered to the beads, indicating that attachment of lymphoid cells to Con A-arm-Sepharose is dependent upon energy metabolism of the cells, which may be required for redistribution of surface receptors like "capping" (12). Addition of 0.2 M mannose, which competes with the cells for Con A binding sites, prevented, although not completely, the binding of the adherent cells (Fig. 1 *c*). In contrast, cells incubated in the presence of BSA-arm-Sepharose remained free (Fig. 1 *d*), suggesting the specific reaction of Con A-arm-Sepharose with binding sites on the cell surface.

In order to facilitate the isolation of proteins that bind to Con A, cell surface proteins were labeled with ^{125}I by lactoperoxidase (11). ^{125}I-surface-labeled cells were bound to Con A-arm-Sepharose by incubating at 37°C for 30 min and ^{125}I-labeled surface proteins bound to Con A were isolated and analyzed as described in the Materials and Methods. As shown in Table I, the amount of radioactivity bound to Con A-arm-Sepharose was far greater than that retained by BSA-arm-Sepharose, which was predictable from the visual observations as shown in Fig. 1. ^{125}I-labeled proteins remaining bound to Con A-arm-Sepharose were eluted with 0.5–1.0 ml 0.2 M mannose in 0.1% NP-40 TKM Ca^{++} containing 0.1% BSA. SDS-acrylamide gel electrophoresis (SDS-AGE) of the eluted proteins (Fig. 2 *a*) revealed two principal proteins (peptide I and II) whose mobilities corresponded to mol wt of approximately 30,000 and 20,000 daltons. They were remarkably different from the gel profiles of the whole membranes. Chloroform-methanol (2:1 = vol/vol) treatment of the eluted fraction indicated that it consisted primarily of proteins; less than 10% radioactivity was recovered in the chloroform layer. The eluted proteins were not serologically precipitable with antichicken Ig (8), thus excluding the possibility of Ig nature of Con A binding proteins. If cell surface Ig was labeled, carbohydrate residues on the Fc portions apparently were not exposed for Con A binding.

Although the initial adherence of cells to Con A-arm-Sepharose beads was specific, quantitative elution of bound proteins by a competitive inhibitor, D-mannose, was difficult to achieve. Two consecutive elutions of 0.2 ml Con A-arm-Sepharose with 1.0 ml 0.2 M mannose eluted at maximum 20% of the bound radioactivity. One half of this radioactivity was not precipitated by 10% TCA. However, treatment of the beads with 10 M urea-1% SDS-0.5 M Tris (pH 8.5) released all the remaining radioactivity of which 90% was TCA pre-

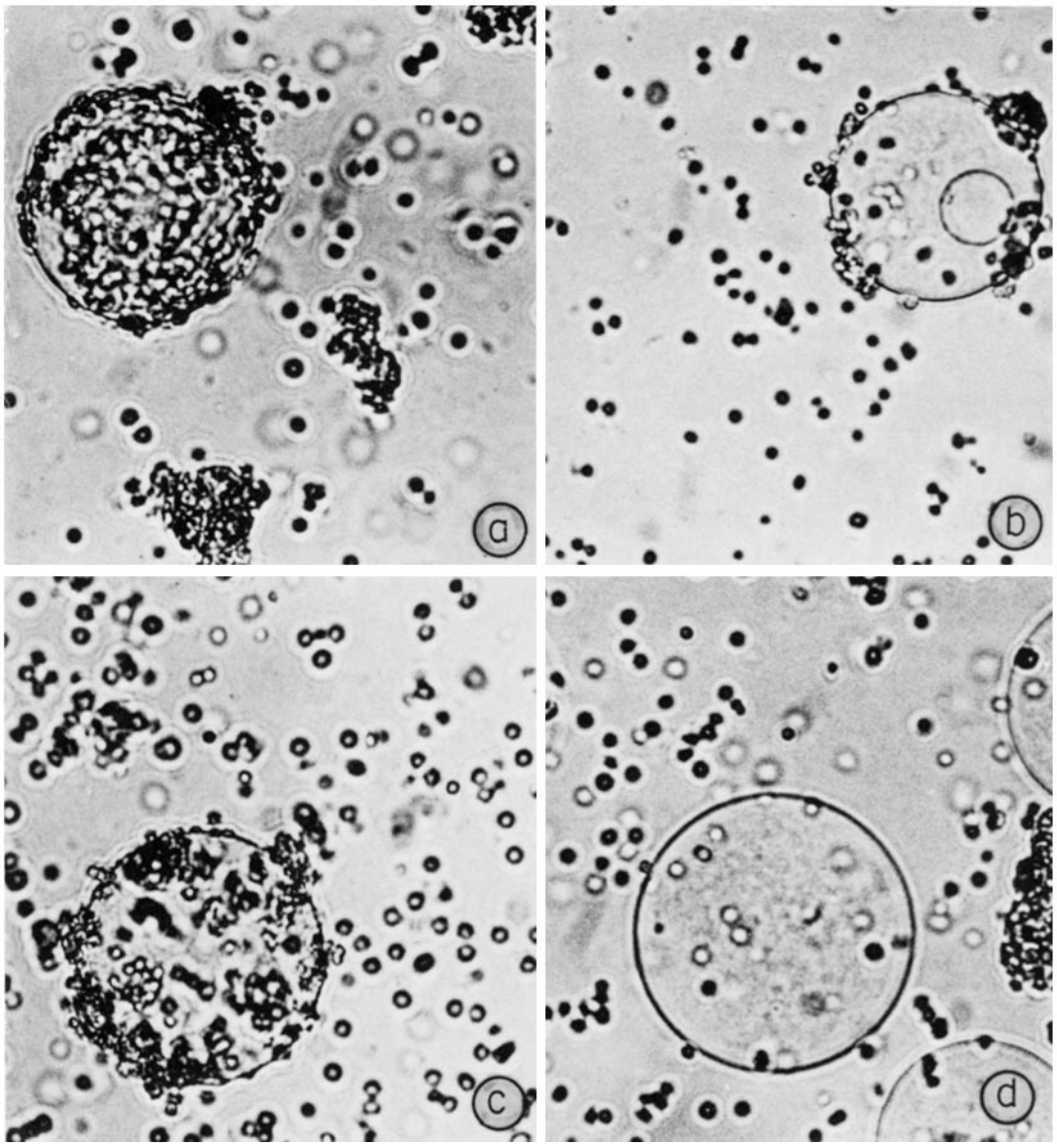

FIG. 1. The binding of lymphoid cells to Con A-arm-Sepharose. In a small plastic petri dish (Falcon Plastics, Div. of Bio Quest, Oxnard, Calif., Catalogue no. 1008, 35 x 10 mm), 2×10^7 cells were mixed with 0.01 ml Con A-arm-Sepharose in 1.0 RPMI with 1% BSA and incubated on a rocking plate (6 cycles/min) at 37°C in a humidified 15% CO_2 and 85% air incubator. Four petri dishes were incubated simultaneously with the same lymphoid cells: (*a*) 37°C; (*b*) 4°C; (*c*) 37°C, 0.2 M D-mannose; and (*d*) BSA-arm-Sepharose, 37°C.

cipitable. SDS-AGE of this material again showed the same two peptides with a lesser amount of the smaller peptide II (mol wt 20,000). On the other hand, the affinity chromatography of detergent solubilized cells revealed additional peptides which were not apparently exposed for Con A binding in the intact cells, indicating that carbohydrate residues of these peptides may not be exposed for Con A binding unless the cells were solubilized.

TABLE I

Binding of ^{125}I-Labeled Surface Proteins

Radioactivity	cpm × 10^6	
	Con A-arm-Sepharose	BSA-arm-Sepharose
Applied	610	490
Bound	5.4	0.8

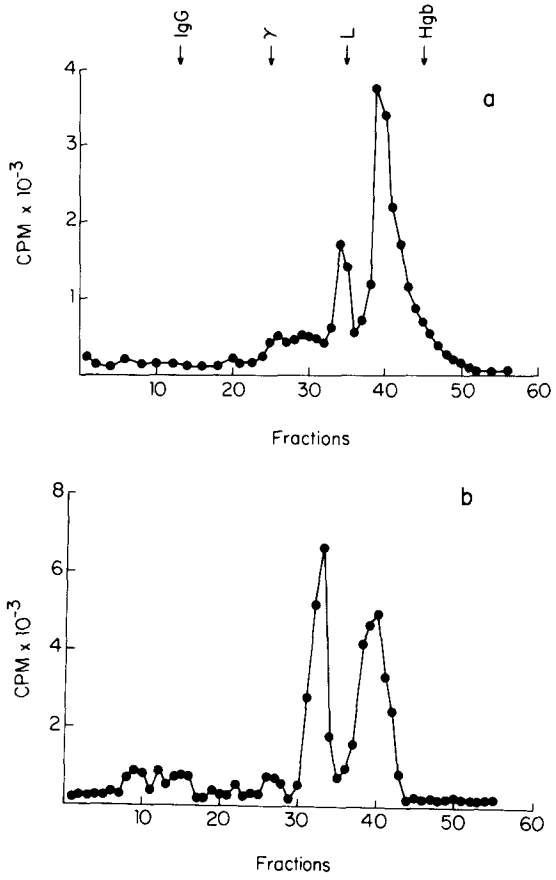

FIG. 2. SDS-acrylamide gel electrophoresis of ^{125}I-labeled surface proteins binding Con A-arm-Sepharose. (*a*) Eluted by 0.2 M mannose; and (*b*) specific serological precipitates with rabbit anti-Con A.

That these two peptides were indeed Con A binding proteins of the intact cells was confirmed by entirely different experimental approaches. ^{125}I-surface-labeled cells were incubated with soluble Con A (i.e. maximum mitogenic doses, 5 μg/ml) at 0°C for 30 min, washed twice with cold PBS to remove free Con A and then lysed with 1% NP-40 TKM. Serological precipitation with rabbit anti-Con A antiserum would be expected to detect ^{125}I-labeled surface proteins complexed with Con A. As shown in Fig. 2 *b*, SDS-AGE of specific serological precipitates revealed the same two peptides as shown from the intact cell binding experiments. Nonspecific serological precipitation with rabbit antikeyhole limpet hemocyanin antiserum failed to reveal such peptides.

Our results demonstrate that the specific Con A binding proteins of chicken lymphoid cells are composed of at least two peptides. In order to characterize the actual structure of Con A receptors on the cell surface, the fraction eluted by 0.2 M mannose was analyzed in Sephadex G-200 column chromatography in 0.1% NP-40 TKM. We found one peak of macromolecules which cochromatographed with the marker, ^{131}I-labeled human IgG (mol wt 160,000) (Fig. 3) and a second peak which was found to be completely dialyzable. Acrylamide gel analysis of the first peak revealed the same two peptides as shown in Fig. 2. Thus, the actual Con A receptor on the cell surface may exist as a noncovalently bonded polymer with a mol wt of about 160,000.

Allan et al. (13) reported that Con A receptors of pig lymphoid cells exhibited two major glycoproteins with mol wt of 33,000 and 27,000 in SDS-AGE analysis. Our studies are unique in demonstrating an actual Con A binding protein (mol wt 160,000) on the surface of intact lymphoid cells. Since the ^{125}I-labeling method by lactoperoxidase label only those proteins with externally accessible tyrosine and histidine residues, it is not known whether the eluted radiolabeled proteins represent all the Con A binding proteins of the plasma membrane. It is

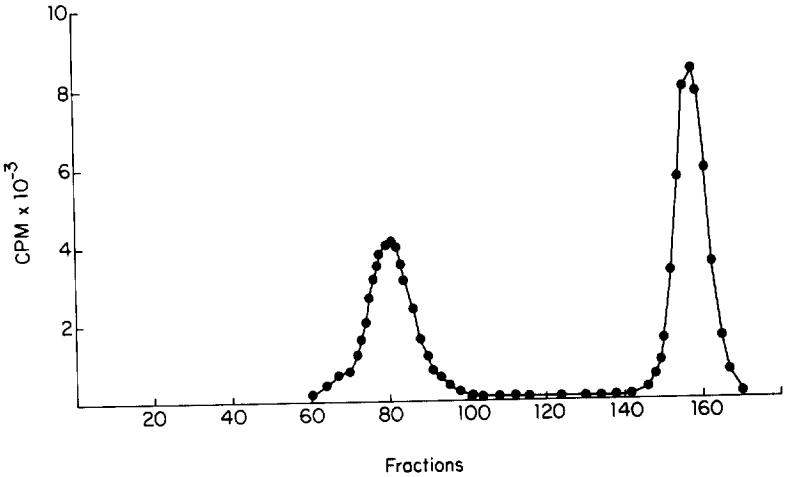

FIG. 3. Filtration on Sephadex G-200 column (1.5 x 90 cm^2) equilibrated with 0.1% NP-40 TKM of the eluted fraction. After a 0.5-ml sample was applied, 0.9-ml fractions were collected at a flow rate of 10 ml/h and counted in an autogamma counter.

quite possible that some unlabeled proteins or labeled proteins with very weak affinity for Con A may be missing in our results. It is, nevertheless, very remarkable to see that Con A binding of the intact cells is attributed to one major protein among many proteins of the cell surface.

SUMMARY

A Concanavalin A binding protein of chicken lymphoid cells was isolated by affinity chromatography of intact lymphoid cells. This protein with a mol wt of 160,000 daltons revealed two peptides (30,000 and 20,000 daltons) in SDS-acrylamide gel electrophoresis.

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