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Characterization of L-CAM, a major cell adhesion molecule from embryonic liver cells

(cell–cell adhesion/monoclonal antibodies/hepatocytes/cleavage protection by calcium)

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ABSTRACT **We have developed a method for purifying L-CAM, the cell adhesion molecule from embryonic chicken liver cells, and have compared its properties with those of N-CAM, the neural cell adhesion molecule. L-CAM was released from membranes with trypsin, purified by a series of chemical techniques, and used to generate monoclonal antibodies which allowed the identification of the intact L-CAM molecule from membranes. The monoclonal antibodies were used to isolate trypsin-released L-CAM in a single step by affinity chromatography. Material purified by either technique was predominantly a component of M_r 81,000 on NaDodSO$_4$/polyacrylamide gel electrophoresis with a pI of 4.0–4.5. Rabbit antibodies to this component and to the M_r 81,000 species that had been further purified on NaDodSO$_4$/polyacrylamide gel electrophoresis displayed all of the activities of anti-L-CAM. Some of the trypsin-released L-CAM bound specifically to lentil lectin, suggesting that L-CAM is a glycoprotein. The apparent molecular weight of material having L-CAM antigenic determinants depended upon the procedures used to extract membranes; this appears to account for the various values reported previously in the literature. Both the rabbit serum antibodies and the monoclonal antibodies detected the M_r 81,000 species on immunoblots of unfractionated trypsin-released material. Immunoblots of whole liver cell membranes with the same antibodies revealed a major M_r 124,000 component, with minor components of M_r 94,000 and 81,000. Active L-CAM derivatives released by trypsin in the presence of EGTA were detected as a species of M_r 40,000. L-CAM derivatives obtained by extraction of membranes with EDTA alone appeared as species of M_r 53,000, 62,000, and 81,000. The combined results suggest that L-CAM on the cell surface is an acidic glycoprotein of M_r 124,000. In the presence of calcium, the molecule can be released from membranes by trypsin as a soluble M_r 81,000 fragment; in the absence of calcium, it is released by either endogenous proteases or by trypsin as a variety of smaller fragments.**

Adhesion among cells of vertebrate tissues plays a key role during development. Two types of cell–cell adhesion, calcium-dependent and calcium-independent, have been identified. Calcium-independent cell–cell adhesion in nervous tissue appears to be mediated by a glycoprotein, neural cell adhesion molecule (N-CAM), distinguished by an unusually large content of sialic acid (1) present in all likelihood as polysialic acid. Molecules involved in calcium-dependent mechanisms are less well-defined. Calcium-dependent systems have been detected in a variety of tissues (2–11), but their relationship to each other is largely unknown. The possibility that most, if not all, of these molecules are identical must be seriously entertained. Because of its presence in a well-differentiated major tissue, the calcium-dependent molecule from liver (2, 3) provides a propitious opportunity to test this idea.

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A variety of approaches have been used to study calcium-dependent cell aggregation in chicken and rat liver. Earlier studies in our laboratory (2) identified material (liver cell adhesion molecule; L-CAM) in EDTA extracts of embryonic chicken liver that neutralized antibodies that blocked liver cell adhesion. Antibodies prepared to the partially purified material were found to prevent colony formation of liver cells in culture. From these studies and those of others (3), it appeared that the embryonic chicken L-CAM activity was associated with a polypeptide of M_r ≈68,000 that was distinct from albumin. Recently, a glycoprotein of M_r 105,000 has been detected in detergent extracts of juvenile rat liver that appears to mediate L-CAM activity (4). The divergence of molecular weights in these studies suggested to us that degradation of a single molecule was possibly taking place, and we therefore attempted to identify L-CAM as an intact species.

To obtain sufficient L-CAM from embryonic chickens, we developed an approach that employed trypsin to release the molecule from membranes. We describe here the isolation of trypsin-released L-CAM, the preparation of monoclonal antibodies to L-CAM, and some properties of the intact L-CAM molecule that are similar to the molecules involved in the calcium-dependent aggregation of embryonal carcinoma cells (5, 6). In contrast to earlier results (2, 3), we found that L-CAM activity on chicken liver cells is associated with a glycoprotein of M_r 124,000. However, the molecule is sensitive to proteolysis and can be released by trypsin or endogenous proteases in a variety of forms, depending upon whether or not calcium is present; this behavior provides an explanation for the apparent discrepancy with the previous molecular weight estimations.

MATERIALS AND METHODS

Liver cells, primary cultures of these cells, and antibodies to liver cells were prepared as described (2). Assays of L-CAM activity (2) and preparation of Fab' fragments (12) also were carried out as published.

Preparation of Membranes (13). Approximately 1,800 livers from 14-day chicken embryos were homogenized (Dounce) in batches, with a total of 1.4 liters of 1.0 mM $NaHCO_3$/0.5 mM $CaCl_2$/2.0 mM iodoacetamide/1.0 mM phenylmethylsulfonyl fluoride (PhMeSO$_2$F), and were diluted to 18 liters with the same buffer. A membrane pellet was collected by centrifugation in a Sorvall SZ-14 rotor with continuous flow accessories, at 7,500 rpm with a flow rate of 235 ml/min.

The pellet was resuspended with a Dounce homogenizer, and a 70% (wt/vol) sucrose solution was added to a final concentration of 48% sucrose. A 1,350-ml discontinuous sucrose gradient with equal volumes of 37%, 41%, and 45% sucrose overlaying the sample was centrifuged 10 hr at 17,000 rpm.

Abbreviations: PhMeSO$_2$F, phenylmethylsulfonyl fluoride; L-CAM, liver cell adhesion molecule; N-CAM, neural cell adhesion molecule.

Material floating above the 45%/48% interface was pooled, diluted 1:1 with cold distilled water, and pelleted by centrifugation at 23,000 × *g* for 40 min. The pellets were resuspended to a total volume of 40 ml with L-CAM assay buffer (137 mM NaCl/4.7 mM KCl/0.6 mM $MgSO_4$/1.2 mM $CaCl_2$/10 mM Hepes, pH 7.4) and frozen at −70°C for later use.

Preparation of Trypsin Extract. Membranes, washed twice in L-CAM assay buffer, were resuspended to a protein concentration of 0.7 mg/ml and were warmed to 37°C. Trypsin (TRL, Worthington) was added to a final concentration of 50 μg/ml. The mixture was incubated for 30 min at 37°C with occasional swirling. The digestion was stopped by addition of $PhMeSO_2F$ (35 mg/ml in ethanol) to a final concentration of 2.0 mM. The digest was centrifuged at 43,000 × *g* for 20 min and the clarified supernatant solution was dialyzed overnight against 10 mM Hepes/1 mM $CaCl_2$, pH 7.4 (HC buffer).

Fractionation of Activity. The dialyzed extract was centrifuged and the clear solution (≈150 ml) was loaded onto a column (2.5 × 1.2 cm) of DEAE-cellulose (DE-52, Whatman) equilibrated in HC buffer. The column was eluted first with a linear gradient of 25 ml each of HC buffer and HC buffer that was 0.3 M in NaCl and then was eluted with 0.5 M NaCl in HC buffer. Fractions (2 ml) containing activity were pooled, dialyzed against 5.0 mM Hepes/0.5 mM $CaCl_2$, pH 7.4, and were lyophilized. Gel filtration of the redissolved material was carried out on a column (75 × 1.7 cm) of Sephadex G-150 (Pharmacia) in L-CAM assay buffer. Fractions (2 ml) containing activity were dialyzed against 5.0 mM Hepes/0.5 mM $CaCl_2$, pH 7.4, and were lyophilized.

The lyophilized material was dissolved in 2 ml of a 1.5% (wt/vol) solution of LKB Ampholines (pH 3.5–10) and was dialyzed against 100 ml of the same solution. Isoelectric focusing (14) then was carried out in a sucrose gradient (10–40%) in a column (15 × 1 cm). Fractions (0.5 ml) were collected by aspiration from the meniscus. Fractions containing active material were pooled, dialyzed into L-CAM assay buffer, and frozen at −20°C.

Preparation of Monoclonal Antibodies. Mice were injected intraperitoneally at 2-week intervals with ≈2 μg of L-CAM in 200 μl of L-CAM assay buffer emulsified with Freund's adjuvant. Three days after the last injection spleens were taken from the mice; the fusion, screening of hybridomas, and production of monoclonal antibodies were performed according to published procedures (15).

Miscellaneous. $NaDodSO_4$/polyacrylamide gel electrophoresis (16), coupling of proteins to Sepharose CL-2B (17), and immuno- or electrophoretic blotting (18) were carried out as described. Protein concentrations were measured by using Coomassie blue staining (19).

RESULTS

Purification of Trypsin-Released L-CAM. Release of L-CAM from embryonic chicken liver cell membranes with EDTA (2) did not give reproducible yields; moreover, once released, the activity was labile. Therefore, we isolated membranes in the presence of iodoacetamide and $PhMeSO_2F$ to block endogenous proteases. L-CAM then could be released reproducibly from washed membranes by proteolysis with trypsin. At 0.05 mg of trypsin per ml and a membrane protein concentration of 0.7 mg/ml, essentially all of the L-CAM activity was released in 30 min.

Once solubilized, the material was fractionated on a column of DEAE-cellulose (Fig. 1*A*) to achieve some separation of the proteins and to remove nucleic acids. Material in the active fractions was dialyzed, lyophilized, and redissolved at 6–7 times the initial concentration with no loss in activity. The redissolved

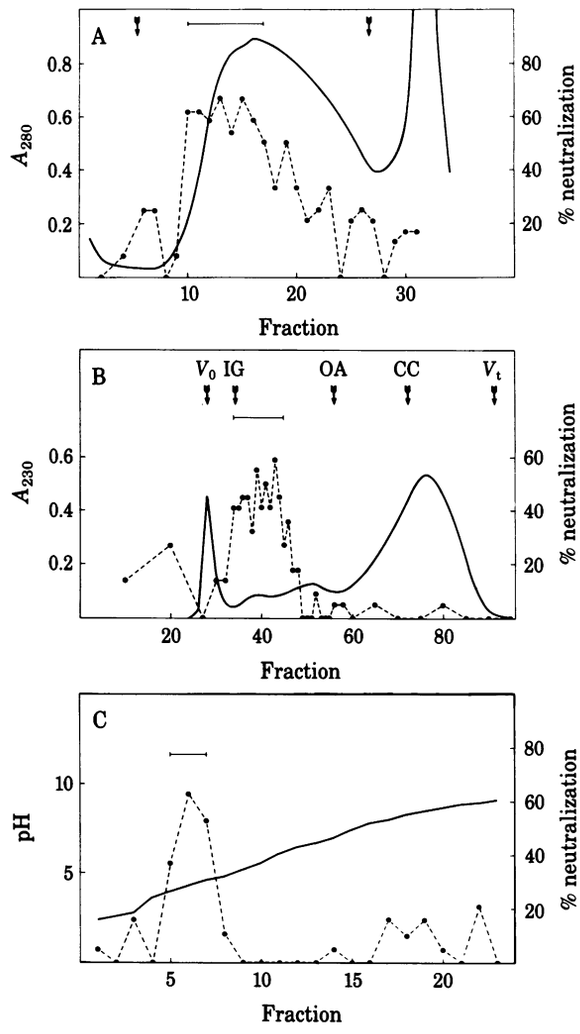

FIG. 1. Purification of trypsin-released L-CAM. (*A*) Chromatography of trpysin-released material on DEAE-cellulose. Proteins were eluted with a linear gradient of 0–0.3 M NaCl (vertical arrows) and then with 0.5 M NaCl. (——) Absorbance at 280 nm; (--●--) ability of aliquots to neutralize the inhibition of liver cell aggregation by antibodies to chicken liver cells. (*B*) Gel filtration of fractions pooled from column shown in *A* (indicated by ⊢—⊣) on Sephadex G-150 in L-CAM assay buffer. (——) Absorbance at 230 nm; (--●--) neutralizing activity. Proteins used for calibration were: IG, IgG (M_r 205,000); OA, ovalbumin (M_r 43,000); and CC, cytochrome *c* (M_r 12,400). (*C*) Isoelectric focusing of fractions pooled from column shown in *B* in Ampholines (pH 3.5–10) in a sucrose gradient (10–40%). (——) pH; (--●--) neutralizing activity. Point to point variations in the activity profiles were observed in all assays and are not significant; the large single peaks of activity were consistently found at the same location in duplicate columns.

material was fractionated further by gel filtration on Sephadex G-150 (Fig. 1*B*); all of the active material migrated in a single region of the column with an estimated average M_r of ≈100,000. Based on $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 2, lane d), the material in the active fractions was heterogeneous but contained a predominant component of M_r 81,000. The material in these fractions was dialyzed and lyophilized to give ≈300 μg from the original 1,800 livers. This material was deployed in one of the two ways: (*i*) for further fractionation by isoelectric focusing or (*ii*) for injection into mice to provide immune spleen cells for monoclonal antibody production.

Isoelectric focusing was carried out in small columns over a pH gradient of 2.5–9.0 (Fig. 1*C*). Yields were generally low but all of the detectable activity appeared in the region of pI = 4.0–

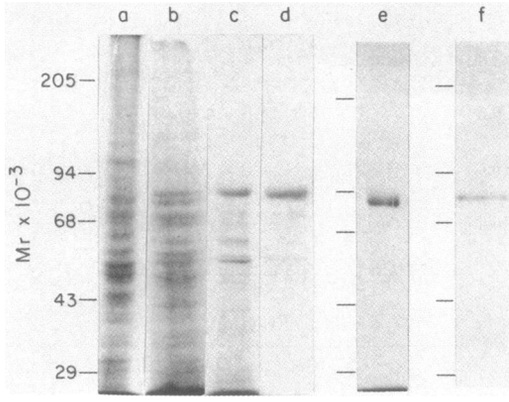

FIG. 2. Purification of trypsin-released L-CAM as assessed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Proteins on slabs of 7.5% acrylamide were detected with Coomassie blue. Lanes: a, liver membranes; b, trypsin-released material; c, poooled fraction from DEAE-cellulose column (Fig. 1*A*); d, pooled fractions from gel filtration (Fig. 1*B*); e, fraction 6 from the isoelectric focusing (Fig. 1*C*); f, trypsin-released L-CAM affinity purified on monoclonal antibody 12G4 attached to Sepharose. Protein standards included myosin (M_r 205,000), phosphorylase a (M_r 94,000), bovine serum albumin (M_r 68,000), ovalbumin (M_r 43,000), and carbonic anhydrase (M_r 29,000).

4.5. On $NaDodSO_4$/polyacrylamide gel electrophoresis, material in these fractions yielded predominantly the M_r 81,000 species with trace amounts (seen only on overloaded gels) of other proteins (Fig. 2, lane e).

The material obtained after isoelectric focusing was used to prepare specific antibodies in rabbits. The Fab' fragments of these antibodies were very effective at blocking cell adhesion (Fig. 3) and were neutralized by liver cell membranes and by purified trypsin-released L-CAM. The Fab' fragments also prevented the organization of liver cells in culture (data not shown) and altered the shape of the cultured cells as described (2). The antibodies obtained in the present studies thus displayed all of the activities of anti-L-CAM.

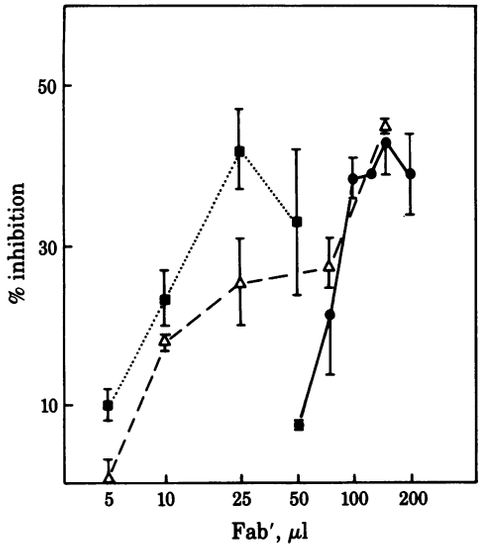

FIG. 3. Inhibition of liver cell aggregation by Fab' fragments of rabbit antibodies to hepatocytes and trypsin-released L-CAM. Abscissa, μl of a solution of Fab' fragments (prepared from antibodies originally at a concentration of 10 mg/ml). Ordinate, % inhibition of cell aggregation (2). (—●—) Antibodies to trypsin-dissociated, embryonic (10 day) chicken hepatocytes; (···■···) antibodies to trypsin-released L-CAM after isoelectric focusing; (—△—) antibodies to the M_r 81,000 component in trypsin-released L-CAM after $NaDodSO_4$/polyacrylamide gel electrophoresis.

To establish more rigorously that the M_r 81,000 material was responsible for the L-CAM antigenic activity, trypsin-released L-CAM after isoelectric focusing (Fig. 1*C* and Fig. 2, lane e) was purified further by preparative $NaDodSO_4$/polyacrylamide gel electrophoresis. Material migrating in the region of M_r 81,000 was cut from the gel and used to immunize rabbits. Antibodies prepared in this fashion were as effective in all assays as those prepared to the material obtained after isoelectric focusing: (*i*) Fab' fragments blocked cell adhesion (Fig. 3); (*ii*) Fab' fragments blocked colony formation in liver cell cultures; and (*iii*) the antibodies detected L-CAM in immunoblots (see below). These results support the notion that the M_r 81,000 material is a trypsin-released L-CAM derivative. Further evidence was provided by the preparation of monoclonal antibodies.

Monoclonal Antibodies to L-CAM. Mouse monoclonal antibodies were prepared by fusing spleen cells from BALB/c mice immunized with trypsin-released L-CAM after gel filtration (Fig. 1*B* and Fig. 2, lane d). Clones were initially selected for their ability to produce antibodies that bound liver cell membranes but not brain cell membranes. Antibodies from liver-specific clones (grown in ascites form) then were attached covalently to Sepharose and selected for their ability to absorb L-CAM activity from trypsin-released material. Of the five positive clones obtained, two were selected for use in further experiments.

Clone 12G4 was the most efficient for affinity purification of L-CAM in that it removed all L-CAM activity from extracts and this activity could be recovered in good yield by elution with 50 mM diethylamine/1 mM $CaCl_2$, pH 11.5 (20). L-CAM activity purified from trypsin extracts of embryonic liver membranes on a 12G4 monoclonal affinity column yielded material that gave a single band of M_r 81,000 on $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 2, lane f).

Clone 7C5 gave the best visualization of L-CAM in immunoblotting experiments. Immunoblots were used to detect L-CAM under various conditions and to compare the rabbit and monoclonal antibodies (Fig. 4). In immunoblots of L-CAM released by trypsin in the presence of calcium, all antibodies detected predominantly a M_r 81,000 species. When immunoblots were prepared with membranes dissolved directly in $NaDodSO_4$/polyacrylamide gel electrophoresis sample buffer, all antibodies detected a predominant species of M_r 124,000 and

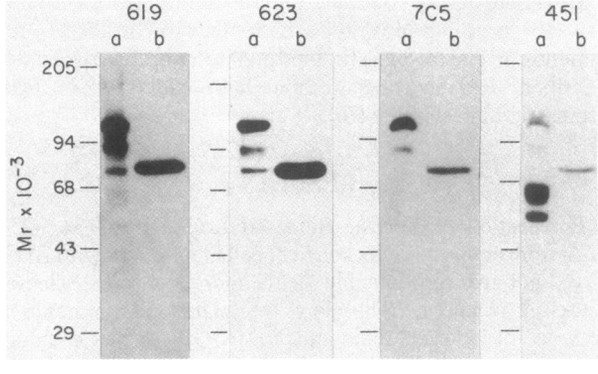

FIG. 4. Use of immunoblots to detect L-CAM. Material was separated on 8.5% polyacrylamide gels and transferred to nitrocellulose. Components were visualized by addition of antibodies, then ^{125}I-labeled *Staphylococcus aureus* protein A, followed by autoradiography. Lane a, whole liver membranes; lane b, trypsin-released material; 619, rabbit antibody to trypsin-released L-CAM after isoelectric focusing; 623, rabbit antibody to the M_r 81,000 component after $NaDodSO_4$/polyacrylamide gel electrophoresis; 7C5, mouse monoclonal antibody 7C5; 451, rabbit anti-L-CAM prepared previously (2). Protein standards are described in the legend to Fig. 2.

smaller amounts of components at M_r 94,000 and 81,000. These results confirm those obtained by affinity chromatography of trypsin-released material when using the monoclonal antibody and suggested that the molecule on the cell surface is a component of M_r 124,000; the species of M_r 94,000 and particularly that of M_r 81,000 appear to arise from this molecule by proteolysis.

We also prepared immunoblots by using the rabbit antibody to L-CAM prepared in the earlier work from this laboratory (2). This antibody detected predominantly the M_r 81,000 component in trypsin-released material and detected the M_r 124,000 material as well as a variety of other species in extracts of whole membranes (Fig. 4).

Influence of Calcium on L-CAM. In earlier studies (2, 3), L-CAM activity appeared to be associated with a component of $M_r \approx 68{,}000$. Studies from this laboratory (2) used EDTA to release L-CAM from membranes that, unlike those used in the present work, were not isolated in the presence of iodoacetamide and $PhMeSO_2F$. Therefore, we prepared membranes by both procedures and made immunoblots of EDTA extracts of these membranes (Fig. 5, lanes a and b) using our antibodies. The antibodies detected species at M_r 81,000, 62,000, and 53,000 in both extracts; these were in much lower yield than the amount of the M_r 81,000 species released by trypsin in the presence of calcium (Fig. 5, lanes e and f). The antibody used in the earlier study (2) detected these same components (not shown) as well as material at M_r 68,000; this antibody is known to have antialbumin activity.

When membranes were treated with trypsin in the absence of calcium (1 mM EGTA), activity was released but again the yield was decreased markedly and the components detected (Fig. 5, lanes c and d) were smaller than the M_r 81,000 species; the residual membranes were not active and no L-CAM was detected on immunoblots of residual membranes. The major L-CAM component released (M_r 40,000) was not detected by any monoclonal antibody but only by the rabbit antibodies.

From the combined results we conclude that the previously observed release of L-CAM in 50 mM EDTA was due to the action of endogeneous proteases and that L-CAM is protected from extensive proteolysis by calcium.

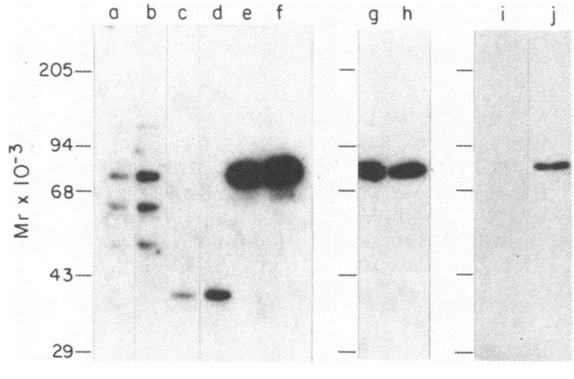

FIG. 5. Forms of L-CAM released by various procedures as detected on immunoblots with rabbit antibody 623 (see Fig. 4). Membranes were prepared with (lanes a, c, and e) and without (lanes b, d, and f) $PhMeSO_2F$ and iodoacetamide. Lanes: a and b, material released with 50 mM EDTA (2); c and d, material released with trypsin in 1 mM EGTA; e and f, material released with trypsin in 1.2 mM Ca^{2+}. Material used in lane f was fractionated on lentil lectin-Sepharose to give bound (lane g) and unbound (lane h) components. Material used in lane h was reapplied to fresh lentil lectin-Sepharose to give bound (lane i) and unbound (lane j) components. Samples were run in 7.5% polyacrylamide gels; protein standards are described in the legend to Fig. 2. Exposure of autoradiograms that detected components in lanes a–d resulted in overexposure of lanes e and f, but it provided some estimate of the relative amount of L-CAM derivatives and allowed detection of material of M_r 68,000 in lanes e and f.

Binding to Lectins. To test for the presence of carbohydrate on L-CAM, we determined the ability of lectins bound to solid supports to remove L-CAM from unfractionated trypsin-released material. A portion of the trypsin-released L-CAM bound to lentil lectin but did not bind to wheat germ agglutinin. The material bound to the lentil lectin was eluted with glucose. The eluted material was detected on $NaDodSO_4$/polyacrylamide gel electrophoresis by staining with Coomassie blue and by anti-L-CAM antibodies after transfer to nitrocellulose (Fig. 5, lane g). In both cases, a predominant band of M_r 81,000 was seen. However, not all of the trypsin-released L-CAM bound to the lectin (Fig. 5, lane h), even when subjected to a fresh lectin support (Fig. 5, lanes i and j), suggesting that some molecules may not be glycosylated or may have different sugar moieties.

DISCUSSION

Liver cells have molecules that mediate cell–cell interactions (2) distinct from those that are involved in cell–substrate adhesion (21), although both systems appear to involve cell-surface glycoproteins. At least one type of liver cell–cell adhesion is calcium dependent; derivatives of the cell-surface proteins involved have been released by digestion with papain (4) and trypsin, the use of detergents (3, 4) or butanol (4), and extraction with EGTA and EDTA (2, 4). However, the specific molecular species involved have not been clearly identified and components ranging in size from M_r 50,000 to 150,000 have been described.

The results we present here suggest that the adhesion activity is probably mediated by a single molecule, L-CAM, and that the multiple species detected in earlier studies, including our own, probably arose by proteolysis of this component. Our studies indicate that L-CAM is an acidic glycoprotein that appears on the cell surface as an integral membrane protein of at least M_r 124,000. Trypsin, in the presence of calcium, releases L-CAM from membranes as M_r 81,000 polypeptide, but, in the absence of calcium, L-CAM appears to be degraded by trypsin, and only relatively small amounts of a M_r 40,000 fragment are detected. The membrane themselves appear to have protease activity that in the presence of EDTA cleaves L-CAM to a series of fragments of M_r 81,000, 63,000, and 52,000.

The properties of chicken L-CAM isolated as described here agree with those reported earlier (2–4), with the exception of the estimated molecular weight. The earlier studies that used EDTA extraction (2) had indicated a species of M_r 68,000; this result was supported by other studies that used sodium deoxycholate to release L-CAM from the chicken liver membranes (3) and EGTA to release a M_r 68,000 component from rat liver membranes (4). A calcium-dependent liver cell adhesion molecule of M_r 105,000 has been detected recently (4) in detergent and butanol extracts of rat hepatocytes and species ranging in size from M_r 50,000 to 100,000 were released by papain. In some of these studies, the identification of L-CAM was probably masked by the large amounts of easily iodinated material at M_r 68,000 and by the degradation of L-CAM by intrinsic proteases. In support of this hypothesis, a component of apparent M_r 68,000 was detected on trypsin-released material by our antibodies when autoradiograms were overexposed (see Fig. 5, lanes e and f). We cannot as yet rule out the possibility that cell–cell adhesion involving L-CAM includes more than one molecule and different approaches and antibodies may be detecting different components of this system. Specific studies similar to those used for N-CAM (22) will be required to settle this issue.

L-CAM appears to differ from N-CAM, as expected from the fact that L-CAM mediates calcium-dependent adhesion, whereas N-CAM mediates calcium-independent adhesion. Consistent with this notion, the structure of L-CAM, unlike N-CAM, appears to be sensitive to the presence of calcium, as indicated by its protection from proteolysis in the presence of this ion. Moreover, N-CAM contains an unusually large amount of sialic acid in a unique form that causes it to appear as a broad band on $NaDodSO_4$/polyacrylamide gel electrophoresis (1); in contrast, L-CAM migrates on $NaDodSO_4$/polyacrylamide gel electrophoresis as a relatively sharp band and thus appears to lack sialic acid in this form. Alteration in the sialic acid content of N-CAM appears to be important in the transition from the embryonic to the adult forms of the molecule (23, 24). We have detected L-CAM activity in adult tissue but have not yet identified the molecular species responsible for the activity. Despite the differences in their properties, the polypeptide chains of L-CAM and N-CAM are comparable in size and it remains possible that detailed analysis will reveal some evolutionary similarities.

In addition to N-CAM, brain cells also appear to have a calcium-dependent (T/Ca) cell aggregation system (9–11). Direct assays by using our antibodies and purified L-CAM indicate that L-CAM is not the molecule responsible for this activity. Similarly, we have detected no L-CAM in immunoblots of fibroblasts, which have been shown to contain a molecule that is involved in calcium-dependent cell–cell aggregation (7).

L-CAM has some features that are similar to the descriptions of the calcium-dependent cell aggregation molecules seen in embryonal carcinoma cells and early mouse embryos (5, 6). Hyafil *et al.* (5) described a molecule, uvomorulin, that is released from embryonal carcinoma cells by trypsin in the presence of calcium as a M_r 84,000 glycoprotein. In the absence of calcium, the molecule is degraded further by trypsin and no longer binds to a monoclonal antibody. These features of the trypsin-released molecules are comparable to those of trypsin-released L-CAM. Yoshida and Takeichi (6) have described a molecule on teratocarcinoma cells that appears as a species of M_r 140,000 but is not released from the cells by trypsin in the presence of calcium. In the absence of calcium, trypsin releases from the cells a M_r 34,000 fragment. The production of this fragment resembles the generation of the M_r 40,000 fragment of L-CAM under similar conditions (Fig. 5). Hyafil *et al.* and Yoshida and Takeichi appear to be describing closely related or identical molecules that could be comparable to L-CAM. There is a possibility that all of these molecules are similar or identical. Direct comparison of chicken L-CAM with uvomorulin has as yet not been possible. Nevertheless, the similarities of this molecule to chicken L-CAM raise the possibility that L-CAM may play a more general role in development than the mediation of interaction among liver cells as suggested for N-CAM by recent studies (25). Further studies must be undertaken which use the antibodies described here to examine the appearance of L-CAM in early embryos and the distribution of the molecule in other tissues.

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