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# Linear organization of the liver cell adhesion molecule L-CAM

**(surface orientation/N-linked oligosaccharides/phosphoamino acids/radiochemical sequence)**

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**ABSTRACT** **A linear model of the liver cell adhesion molecule L-CAM from embryonic chickens is proposed in terms of its orientation on the cell surface, the number, type, and distribution of carbohydrate moieties, and sites of phosphorylation. L-CAM is isolated from cell membranes as a glycoprotein of $M_r$ = 124,000. A soluble fragment (Ft1) of $M_r$ = 81,000 can be released from cells by digestion with trypsin in the presence of calcium. Radiochemical amino acid sequence analyses indicated that both polypeptides have the same sequence for the first 10 amino acids, suggesting that fragment Ft1 contains the amino terminus of the L-CAM molecule and that the carboxyl-terminal portion of the peptide chain is associated with the cell. Digestions with endoglycosidase H and endoglycosidase F indicated that Ft1 has all of the N-linked carbohydrate groups associated with the larger species, including one high mannose oligosaccharide and three complex oligosaccharides. When hepatocytes were grown in the presence of $^{32}PO_4$, $^{32}P$ was detected in phosphoserine and phosphothreonine residues of intact L-CAM, but little or no $^{32}P$ was detected in Ft1, suggesting that L-CAM is phosphorylated in the carboxyl-terminal region. On CNBr cleavage, the bulk of the $^{32}P$ was detected in a single fragment of $M_r$ = 20,000. The overall features of the L-CAM molecule incorporated in the model provide a basis for correlating its structure with its cell–cell binding activity and for detailed comparisons with similar molecules described in mammalian species.**

Cell–cell adhesion has long been recognized as a primary process in early development (1). Only recently, however, have some of the molecules mediating these interactions in vertebrates been identified. Two glycoproteins, designated N-CAM (neural cell adhesion molecule) and L-CAM (liver cell adhesion molecule) are important in cell–cell interactions in tissues of embryonic chickens (2); both molecules appear in very early embryos and persist in adult tissues but they are distinct chemical entities and show no immunological crossreactivity (3–8). A molecular model of N-CAM has been formulated in terms of its binding domain, the location of its unusual sialic acid residues, and its orientation on the cell surface (8). In addition, many details of the homophilic (N-CAM to N-CAM), calcium-independent binding have been described (9). N-CAM is involved in nerve–nerve interactions and is found in all central and peripheral neurons (3, 4).

L-CAM, originally detected in liver cells, is found in nearly all epithelial cells (5). Antibodies to L-CAM perturb hepatocyte aggregation and liver colony formation *in vitro* (6). In early embryogenesis, L-CAM and N-CAM appear in specific patterns, suggesting a key role for these molecules in morphogenesis (7).

The chemical structure of L-CAM has been less extensively explored than that of N-CAM. L-CAM is a glycoprotein ($M_r$ = 124,000) found on cell surfaces, but it lacks the large amount of sialic acid found on N-CAM, and its binding requires calcium. An antigenically active L-CAM derivative can be released from the cell surface as a fragment ($M_r$ = 81,000) by proteolysis with trypsin in the presence of calcium (6); we designate this fragment Ft1. As indicated previously (6), L-CAM closely resembles molecules described in a number of mammalian systems. Uvomorulin (10), a murine glycoprotein involved in the compaction of early mouse blastomeres, shows many of the features of L-CAM, although it has recently been suggested that uvomorulin is not an integral membrane protein (11). Takeichi and co-workers have identified a similar molecule in embryonal carcinoma cells (12) and have shown that antibodies to the molecule inhibit the calcium-dependent aggregation of murine hepatocytes (13). Recent studies have also described a molecule in human mammary carcinoma cells with features and cellular distributions similar to those of L-CAM, and antibodies to this molecule inhibit the compaction of mouse blastomeres (14). The collective results from all of these reports suggest that these molecules may be mammalian equivalents of L-CAM.

To delineate the role of L-CAM in mediating cell–cell adhesion and to provide a basis for detailed comparisons to its presumed mammalian equivalents as well as to N-CAM, we have been carrying out detailed structural studies. We describe here the number and distribution of N-linked carbohydrate moieties, the nature and location of the sites of phosphorylation, and the probable orientation of the molecule on the cell surface.

## MATERIALS AND METHODS

**Crude Membrane Preparation.** Six hundred livers from 14-day embryos were homogenized in 500 ml of 1 mM $NaHCO_3$/0.5 mM $CaCl_2$/2 mM iodoacetamide (Sigma)/1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$) (Eastman). Step gradients consisting of 12 ml of 48% sucrose overlaid with 50 ml of sample were centrifuged at 35,000 rpm for 30 min in a Beckman Ti45 rotor. The supernatant was carefully decanted and membranes floating as a loose layer on top of the sucrose were collected. The membranes were resuspended, washed twice with L-CAM assay buffer [g/liter: 8, NaCl; 0.35, KCl; 0.16, $MgSO_4 \cdot 7H_2O$; 0.18, $CaCl_2 \cdot 2H_2O$; 2.4, Hepes (pH 7.4)], and stored at −70°C.

**Affinity Purification of Ft1.** Crude membranes at a protein concentration of 2.5 mg/ml in L-CAM assay buffer were incubated with 150 μg of trypsin (Worthington, 2× crystallized) per ml for 30 min at 37°C, with occasional swirling. The digestion was stopped by adding 1/100 vol of 35 mg of $PhMeSO_2F$ per ml in ethanol and the mixture was centrifuged for 20 min at 19,000 rpm in a Sorvall SS-34 rotor. The supernatant was shaken with monoclonal antibody 12G4 (6)

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Abbreviations: N-CAM, neural cell adhesion molecule; L-CAM, liver cell adhesion molecule; $PhMeSO_2F$, phenylmethylsulfonyl fluoride.

coupled to Sepharose CL-2B (Pharmacia) and the $M_r$ = 81,000 fragment was eluted with 50 mM diethylamine, pH 11.5/1 mM $CaCl_2$. The eluate was neutralized with 1/10 vol of 1 M Hepes (pH 7.0), dialyzed against water, lyophilized, redissolved in water, and clarified by centrifugation.

**Preparation of Intrinsically Labeled Proteins.** Embryonic hepatocytes were plated at $1 \times 10^7$ per 60-mm culture dish (Costar, Cambridge, MA) (6). They were allowed to attach to the dish for 3 hr in Dulbecco's minimum essential medium/10% fetal calf serum and then for 3 hr in medium lacking an amino acid or phosphate. In experiments using tunicamycin, the drug was added at this point to 1 μg/ml and maintained at this concentration throughout the labeling period. Radiolabeled material was added to the medium and the cultures were incubated for 16–18 hr. Unless otherwise indicated, 1 mCi (1 Ci = 37 GBq) of isotope was added per dish.

To prepare intact L-CAM from these cultures, cells were washed on the dish with 0.15 M NaCl/1 mM EDTA/10 mM Tris·HCl, pH 7.2, and then 0.5 ml of boiling $NaDodSO_4$ buffer was added (0.15 M NaCl/25 mM EDTA/20 mM $Na_2HPO_4$, pH 7.4/1% $NaDodSO_4$). Cells were scraped off the dish and boiled for 2 min; then 2 ml of the same buffer, containing 1.25% Nonidet P-40 instead of $NaDodSO_4$, was added. After centrifugation, the supernatant was used for immunoprecipitation of the intact L-CAM with polyclonal rabbit antibody (6).

To prepare the trypsin fragment Ft1, cells were rinsed with L-CAM assay buffer and then incubated for 30 min at room temperature with 100 μg of trypsin (Worthington, 2× recrystallized) dissolved in 2 ml of L-CAM assay buffer. The reaction was stopped by the addition of 20 μl of a solution of $PhMeSO_2F$ (35 mg/ml) in ethanol. After centrifugation, Ft1 was immunoprecipitated from the trypsin-released material. The purity of the isolated components was checked by analytical $NaDodSO_4$/polyacrylamide gel electrophoresis (15). For automatic amino-terminal sequence determination (16), intact L-CAM and the Ft1 fragment were each labeled with [$^3$H]valine, [$^3$H]leucine, and [$^3$H]isoleucine.

**Glycosidase Digestions.** For endoglycosidase H digestion, aqueous samples were prepared by adding $NaDodSO_4$ to a final concentration of 1%, Tris·HCl (pH 7.4) to 1 mM, and dithiothreitol to 50 mM. The sample was then incubated in a boiling water bath for 3 min and cooled to room temperature. The sample was diluted 1:1 with 0.3 M sodium citrate (pH 5.5). Ten milliunits of enzyme (Miles, lot 13) was added to the sample, and the reaction mixture was incubated at 37°C (17).

For endoglycosidase F digestion, a sample of the Ft1 fragment was adjusted to 50 mM dithiothreitol/2% Nonidet P-40/2 mM $PhMeSO_2F$ and treated with enzyme (a gift from John Elder and Steve Alexander) as published (18). The tritiated intact L-CAM was treated directly in gel sample buffer (0.05 M Tris pH 7.0/2% $NaDodSO_4$/5% 2-mercaptoethanol) with Nonidet P-40 added to 2% final concentration.

Samples for neuraminidase digestion were adjusted to 50 mM sodium acetate, pH 5.0/0.2 mM EDTA/2 mM $CaCl_2$. Five milliunits of neuraminidase (*Vibrio cholerae*, Calbiochem) was added in 5 μl, and the reaction mixture was incubated at 37°C.

**Phosphoamino Acid Analysis.** Hepatocytes were grown in 60-mm dishes and labeled with 3 mCi of $^{32}PO_4$ for 18 hr. L-CAM was immunoprecipitated from a $NaDodSO_4$ extract of these cells (see above). After washing, the immunoprecipitates were fractionated on a $NaDodSO_4$/polyacrylamide gel and portions of the gel corresponding to $M_r$ = 124,000 were cut out and treated with 0.1 mg of proteinase K per ml in 25 mM ammonium bicarbonate for 6 hr at 37°C. After several lyophilizations, the released peptides were hydrolyzed for 2 hr in 6 M HCl at 110°C. The samples were dried and dissolved in several microliters of a solution of phosphotyrosine, phosphoserine, and phosphothreonine in water. The samples were separated by two-dimensional thin-layer electrophoresis as described by Hunter and Sefton (19). The thin-layer plates were exposed for autoradiography at −70°C for 48 hr using Kodak SB-5 film.

**Preparation of CNBr Phosphopeptides.** $^{32}PO_4$-labeled L-CAM was prepared as described above. The material from two 60-mm dishes of hepatocytes was eluted from the protein A-Sepharose with 0.5 ml of 70% aqueous formic acid, brought to 10% (wt/vol) CNBr, and digested for 4.25 hr at ambient temperature. CNBr and formic acid were removed by lyophilization, and the sample was resolved on a 17% acrylamide gel and visualized by autoradiography.

## RESULTS

Intact L-CAM can be prepared from cells or plasma membrane-enriched material as a protein of $M_r$ = 124,000 (Fig. 1, lane 1). A soluble fragment (Ft1) of $M_r$ = 81,000 can be released from liver membranes or hepatocytes (lane 2) by treatment with trypsin in the presence of calcium and isolated in milligram quantities by chromatography on affinity supports containing monoclonal antibodies to L-CAM.

**Carbohydrate Moieties.** Some properties of the sugar moieties were revealed by treatment of Ft1 with glycosidases (Fig. 1, lanes 3–6). Neuraminidase altered the mobility only slightly (lane 6). Similarly endoglycosidase H caused a small but significant decrease in apparent molecular weight (lane 5); this result suggests that there is a small amount of N-linked oligosaccharide of the high mannose type (20). In contrast, endoglycosidase F (18) caused a substantial decrease in apparent molecular weight (lane 4), suggesting that the bulk of the N-linked sugar is present as oligosaccharides of the complex type. When cultured hepatocytes were labeled in the presence of tunicamycin, which prevents the attachment of N-linked oligosaccharides to glycoproteins, an L-CAM species of $M_r$ = 110,000 was immunoprecipitated. This material comigrated with L-CAM that had been digested exhaustively with endoglycosidase F (lanes 7 and 8), confirming that endoglycosidase F removed all of the N-linked oligosaccharides.

To estimate the number of N-linked oligosaccharides, Ft1

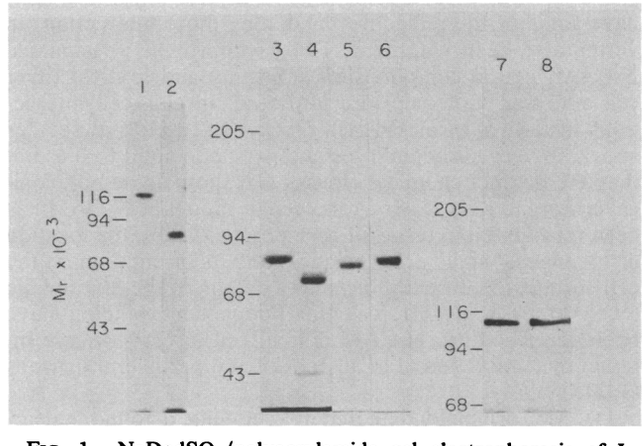


FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of L-CAM. Lanes 1 and 2, L-CAM and Ft1, respectively. Lanes 3–6, Ft1 before (lane 3) and after treatment with endoglycosidase F (lane 4), endoglycosidase H (lane 5), and neuraminidase (lane 6). Lanes 7 and 8, L-CAM after treatment with endoglycosidase F (lane 7) and from tunicamycin-treated hepatocytes (lane 8). Lanes 1 and 2 (7.5% acrylamide) and lanes 7 and 8 (5% acrylamide) are autoradiographs of [$^3$H]leucine-labeled material. Lanes 3–6 (6.5% acrylamide) are Coomassie blue stained. Protein standards included myosin ($M_r$ = 205,000), β-galactosidase ($M_r$ = 116,000), phosphorylase b ($M_r$ = 94,000), bovine serum albumin ($M_r$ = 68,000), and ovalbumin ($M_r$ = 43,000).

was treated with serial dilutions of endoglycosidase F and the products were separated by NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 2*A*). Decreasing amounts of the enzyme caused smaller changes in the apparent molecular weight and a total of five evenly spaced components could be detected across the gel. Assuming (18) that the largest component is fully glycosylated Ft1, the smallest is totally deglycosylated Ft1, and the N-linked oligosaccharides are of approximately equal size, these results suggest that there are at least four N-linked oligosaccharides attached to Ft1. The decrease in apparent molecular weight caused by endoglycosidase H is comparable to the difference in molecular weight between the undigested Ft1 fragment and the largest presumed intermediate after endoglycosidase F digestion, suggesting that of the four N-linked oligosaccharides, one is of the high mannose type and three are of the complex type. This procedure was also applied to undigested intrinsically labeled L-CAM, (Fig. 2*B*). A comparable pattern of five closely spaced bands was observed, indicating that the intact L-CAM molecule also has four N-linked oligosaccharide side chains, all of which are present in Ft1.

**Phosphate Groups.** Incubation of embryonic chicken hepatocytes in culture with $^{32}PO_4$ resulted in incorporation of significant amounts of $^{32}P$ into intact L-CAM as detected by immunoprecipitation and autoradiography (Fig. 3*A*, lane 2). Immunoprecipitation of similar material with serum from unimmunized rabbits (lane 1) yielded small amounts of high molecular weight material at the top of the gel, but no $^{32}P$ in the region where L-CAM migrated. To test for the appearance of $^{32}P$ in fragment Ft1, hepatocytes cultured in $^{32}PO_4$ and [$^3$H]leucine were digested with trypsin and the released material was immunoprecipitated with anti-L-CAM. No $^{32}P$-labeled Ft1 was detected (lane 5), even though the $^3$H label indicated that the fragment was present (lane 7). A more quantitative estimate of the relative labeling of intact L-CAM and Ft1 with $^{32}PO_4$ was made by preparing both components from cultures labeled with $^{32}PO_4$ and [$^3$H]leucine, isolating the L-CAM and Ft1 by immunoprecipitation and NaDodSO$_4$/polyacrylamide gel electrophoresis, and assaying the samples for $^{32}P$ and $^3$H (Table 1). Essentially no $^{32}P$ was detected in a Ft1 sample that contained 820 cpm of $^3$H. In contrast, L-CAM contained significant $^{32}P$. Assuming that the amount of $^3$H reflected the amount of L-CAM or Ft1 present, phosphate was at least 100 times more prevalent in the intact molecule.

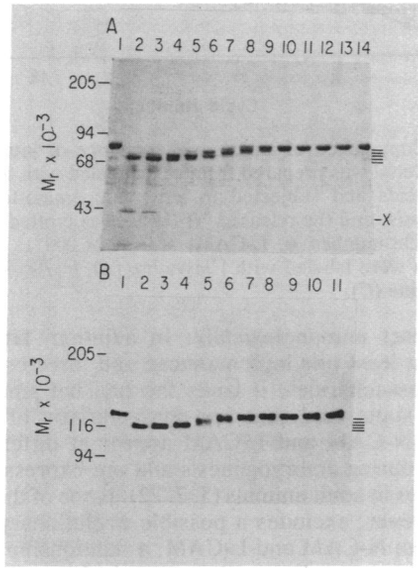


FIG. 2. Digestion of Ft1 and L-CAM with endoglycosidase F. (*A*) Digestion of affinity-purified Ft1. Lane 1, untreated material; lane 2, 48-hr digestion with undiluted enzyme; lane 3, 24-hr digestion; lanes 4–14, 24-hr digestion with 1:3 serial dilutions of enzyme. The five closely spaced lines on the right of the panel indicate the positions of the five discrete bands. X indicates a polypeptide present in the enzyme solution (6.5% acrylamide gel). (*B*) Digestion of [$^3$H]leucine-labeled L-CAM. Lane 1, untreated material; lane 2, 24-hr digestion with undiluted enzyme; lanes 3–11, 24-hr digestion with 1:3 serial dilutions of enzyme. Autoradiograph of a 7.5% acrylamide gel in which the dye front was run off the gel slab. Standards were as described in the legend to Fig. 1.

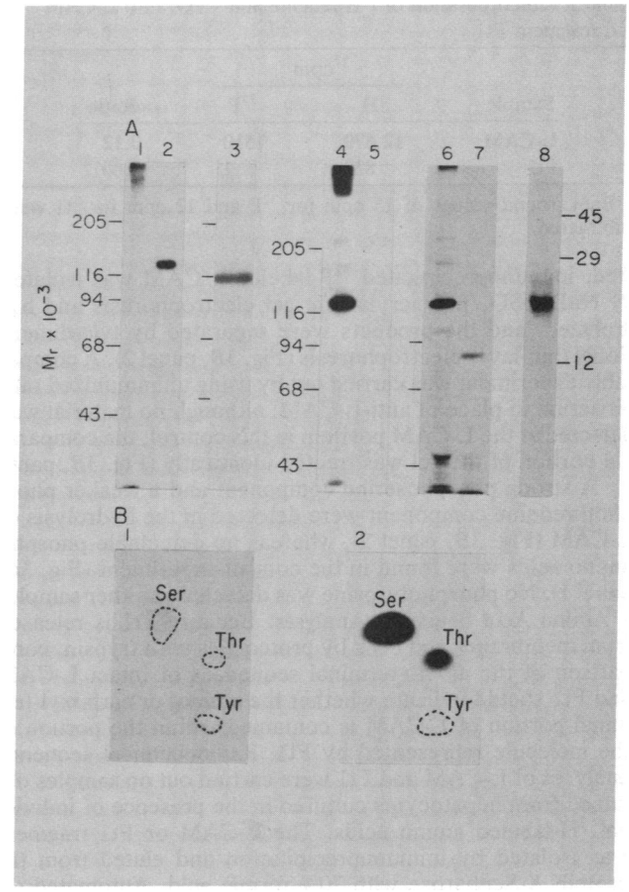


FIG. 3. Electrophoretic analysis of the L-CAM phosphorylation site. (*A*) Control (lane 1) and immunoprecipitate of L-CAM (lanes 2 and 4), L-CAM treated with endoglycosidase F (lane 3) and Ft1 (lane 5), all from cells labeled with $^{32}PO_4$, and [$^3$H]leucine-labeled L-CAM (lane 6) and Ft1 (lane 7); $^{32}$P-labeled CNBr fragment of L-CAM (lane 8). Lanes 1–7, 7.5% acrylamide gels; lane 8, 17% acrylamide gel. All lanes are shown as autoradiograms. (*B*) Phosphoamino acids in L-CAM. Two-dimensional thin-layer electrophoresis of hydrolyzed $^{32}$P-labeled L-CAM isolated from polyacrylamide gels. Panel 1, the region of the gel corresponding to $M_r$ = 124,000 after immunoprecipitation with serum from an unimmunized rabbit (see *A*, lane 1); panel 2, after immunoprecipitation with rabbit 623 serum (see *A*, lane 2). The positions of the phosphoamino acid standards are marked with dotted lines.

To describe the nature of the $^{32}P$ on L-CAM, the molecule was immunoprecipitated from cells cultured in $^{32}PO_4$ and then treated with endoglycosidase F. Subsequent NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 3*A*, lane 3) indicated that the N-linked oligosaccharides had been removed and that the $^{32}P$ was still associated with the polypeptide. This result is consistent with the results showing that all N-linked sugars are on Ft1, and the phosphate is on only the $M_r$ = 124,000 species. CNBr digestion of $^{32}P$-labeled intact L-CAM yielded a single major radioactive peptide ($M_r$ = 20,000) detected on polyacrylamide gels (lane 8).

To determine which amino acid residues were phosphoryl-

Table 1. Incorporation of [$^3$H]leucine and $^{32}PO_4$ into L-CAM and fragment Ft1

| Sample | cpm $^3$H | cpm $^{32}$P | Ratio |
|---|---|---|---|
| L-CAM | 12,670 | 1510 | 0.12 |
| Ft1 | 820 | 1 | 0.0012 |

Background values of 15 cpm for $^{32}$P and 12 cpm for $^3$H were subtracted.

ated, immunoprecipitated $^{32}$P-labeled L-CAM was isolated by NaDodSO$_4$/polyacrylamide gel electrophoresis and hydrolyzed, and the products were separated by two-dimensional thin-layer electrophoresis (Fig. 3*B*, panel 2). A comparable experiment was carried out by using unimmunized rabbit serum in place of anti-L-CAM; although no material was detected at the L-CAM position in this control, the comparable portion of the gel was treated identically (Fig. 3*B*, panel 1). A strong phosphoserine component and a weaker phosphothreonine component were detected in the hydrolysis of L-CAM (Fig. 3*B*, panel 2), whereas no detectable phospho amino acids were found in the control experiment (Fig. 3*B*, panel 1). No phosphotyrosine was detected in either sample.

**Amino Acid Sequence Analyses.** Because Ft1 is released from membranes and cells by proteolysis with trypsin, comparison of the amino-terminal sequences of intact L-CAM and Ft1 should indicate whether the amino- or carboxyl-terminal portion of L-CAM is contained within the portion of the molecule represented by Ft1. Radiochemical sequence analyses of L-CAM and Ft1 were carried out on samples obtained from hepatocytes cultured in the presence of individual $^3$H-labeled amino acids. The L-CAM or Ft1 fragment was isolated by immunoprecipitation and eluted from the protein A-Sepharose with 70% formic acid. Automated sequence analysis of both L-CAM and Ft1 yielded a single peak at position 3 for valine (Fig. 4*A*), and a single peak at position 10 for leucine (Fig. 4*C*). Isoleucine (Fig. 4*B*) appeared at positions 4 and 7 in both samples. The sequence of the Ft1 fragment was verified by sequence analysis of 2 mg of unlabeled material. These results indicate the L-CAM and Ft1 have the same amino-terminal sequence and that release of Ft1 from hepatocytes is coincident with a proteolytic cleavage located in the carboxyl-terminal portion of the polypeptide.

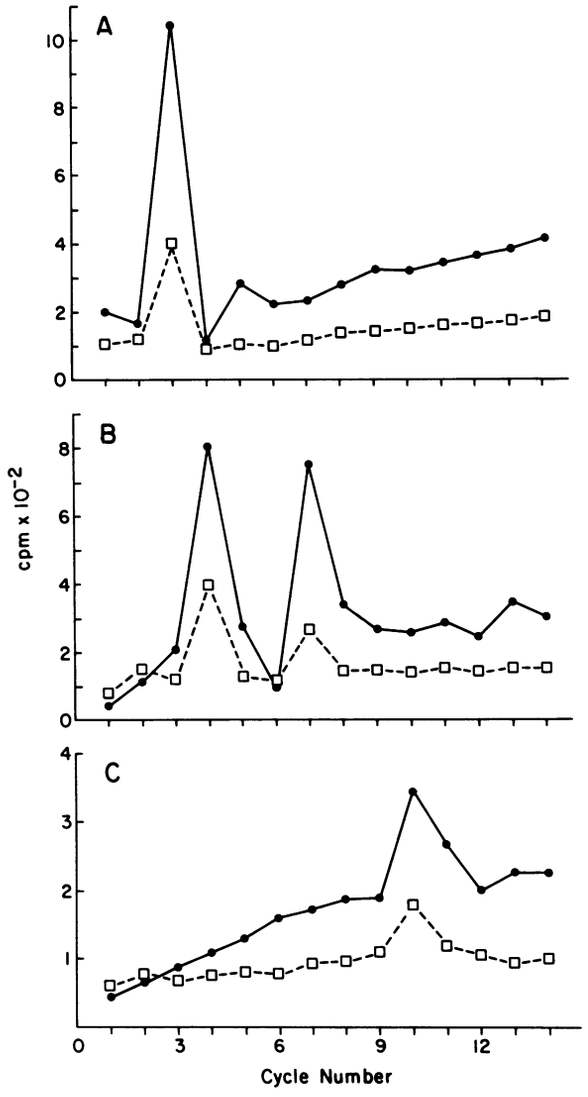


FIG. 4. Comparison of amino acid sequence of intact L-CAM and Ft1. Material was prepared from cells labeled with three different amino acids and subjected to automatic amino-terminal sequence analysis, and the released $^3$H (cpm) was plotted against sequencing cycle number. ●, L-CAM, $M_r$ = 124,000. □, Ft1, $M_r$ = 81,000. Cells were labeled with [$^3$H]valine (*A*), [$^3$H]isoleucine (*B*), and [$^3$H]leucine (*C*).

## DISCUSSION

Earlier studies defined the role of L-CAM in liver cell adhesion (6) and identified the molecule on cell surfaces (5) as a glycoprotein of $M_r$ = 124,000 (6). The L-CAM activity was released from cells with trypsin in the presence of calcium as a component of $M_r$ = 81,000 (6) which we designate here Ft1. We have now extended the description of the L-CAM molecule to include the location of phosphoamino acids in the carboxyl-terminal region, the presence of four N-linked oligosaccharides in the region represented by the fragment Ft1, and the site of proteolysis that produces Ft1. These features are summarized in Fig. 5.

The topography of L-CAM resembles that of N-CAM; for example, upon proteolysis both L-CAM and N-CAM release a fragment from the cell surface representing about two-thirds of the molecule. In both cases, the fragments include the amino-terminal portion of the polypeptide chain, the bulk of the carbohydrate, and the antigenic sites recognized by antibodies that block cell adhesion. Both CAMs also have phosphoserine and phosphothreonine residues in the carboxyl-terminal third of their peptides (see ref. 21). In detail, however, the molecules are clearly different. The polypeptides (and fragments produced from them) differ in size; the molecules have different amino-terminal sequences and do not crossreact immunologically. In addition, although L-CAM has at least one high mannose and three complex N-linked oligosaccharides, it lacks the unusual amounts and linkage of sialic acid that are characteristic of N-CAM. Moreover, N-CAM and L-CAM appear at different times and places during embryogenesis and are expressed on different tissues in adult animals (5, 7, 22). None of these differences, however, excludes a possible evolutionary relationship between N-CAM and L-CAM, a relationship that must be assessed by complete sequence analysis.

A question sharpened by the present studies concerns the manner in which the carboxyl-terminal portion of L-CAM is associated with the cell surface. Such information could be essential to understanding the mechanism of L-CAM-mediated cell adhesion and the influence of such adhesion on the cells involved. Because detergent or proteases were required to solubilize the $M_r$ = 124,000 species, we suggested (6) that L-CAM may be an intrinsic membrane protein. However, Peyrieras *et al.* (11) have suggested recently that uvomorulin, a molecule that is similar to L-CAM and is detected in early mouse embryos, is not an integral membrane protein.

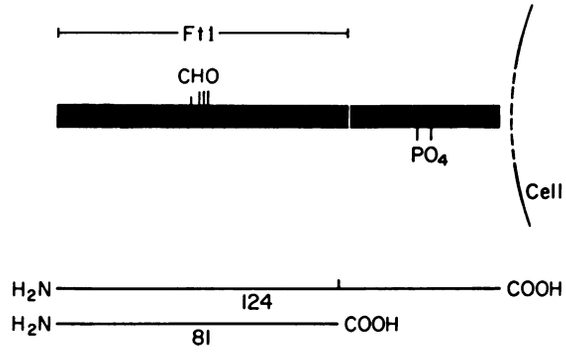


FIG. 5. Schematic drawing summarizing structural features of the L-CAM molecule. Ft1 is the amino-terminal $M_r$ = 81,000 peptide of L-CAM. It contains all four N-linked oligosaccharides (CHO): one (short vertical bar) susceptible to digestion with endoglycosidase F and endoglycosidase H and the other three (long vertical bars) susceptible only to endoglycosidase F. L-CAM is phosphorylated ($PO_4$) in the carboxyl-terminal third of the molecule, on both serine and threonine residues, suggesting a minimum of two sites (two vertical bars). The carboxyl-terminal region is shown associated with the cell membrane; the dotted lines indicate that the nature of that association is not definitively established. Molecular weights are shown as $M_r \times 10^{-3}$.

This conclusion was based on studies of uvomorulin in embryonal carcinoma cells: the molecule could be solubilized by EDTA solutions; its electrophoretic mobility when tested in anionic and cationic detergents did not differ from its mobility in neutral detergents; and it was not found in the detergent phase when aqueous solutions of the molecule in Triton X-114 were placed at temperatures (above 30°C) at which the detergent forms a separate phase.

In contrast, we have not been able to extract significant amounts of the $M_r$ = 124,000 component of L-CAM from liver cell membranes or cultured hepatocytes with EDTA solutions, although species of lower molecular weight corresponding to known fragments of L-CAM can be detected in such extracts with antibodies to L-CAM (6), supporting the notion that L-CAM is an integral membrane protein. Moreover, the fact that L-CAM, but not the trypsin-released fragment, is phosphorylated is consistent with this conclusion, although kinases that phosphorylate serine or threonine residues on the extracellular portion of proteins have been described (23). Preliminary measurements of the relative electrophoretic mobility of L-CAM in neutral, anionic, and cationic detergents have been equivocal, and L-CAM is detected in both the aqueous phase and the detergent-enriched phase when solutions in Triton X-114 are allowed to undergo a phase transition. A definitive conclusion will probably require a new experimental approach.

The data presented have allowed us to construct a linear model of the L-CAM molecule (Fig. 5) that incorporates a number of its important structural features. These features should permit even more detailed comparisons with the cell adhesion molecules detected in mouse embryos (10), embryonal carcinoma cells (12), and human mammary tumors (14) that closely resemble L-CAM. All of the results we have described can be obtained by using radiochemical approaches, allowing comparison of L-CAM structure even with those molecular systems in which the amount of available material is severely limited.

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