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# Collagen Binding Proteins Derived from the Embryonic Fibroblast Cell Surface Recognize Arginine-Glycine-Aspartic Acid

Roy C. Ogle<sup>1</sup> and Charles D. Little<sup>2</sup>

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Several cell surface proteins ( $M_r = 120,000$ ,  $90,000$ ,  $63,000$  and  $47,000$ ) apparently integral to embryonic fibroblast plasma membranes were extracted with detergent and isolated by collagen affinity chromatography. Certain of these proteins ( $M_r = 120,000$ ,  $90,000$  and  $47,000$ ) were specifically eluted from collagen affinity columns by synthetic peptides containing the amino acid sequence arginyl-glycyl-aspartic acid (RGD). These data show that a number of collagen binding proteins exist on the embryonic fibroblast cell surface. Some of the proteins may be collagen receptors binding to RGD sequences in the collagen molecule while at least one of the proteins ( $M_r = 63,000$ ) recognizes features other than RGD.

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**KEY WORDS:** collagen receptor; RGD-peptides; integrins; embryonic cell surface.

## INTRODUCTION

The adhesive interaction of cells with the extracellular matrix (ECM) influences fundamental cellular properties such as morphology, proliferation, migration and differentiation (Hay, 1984; Kleinman *et al.*, 1981; Yamada, 1983). Collagen, the most abundant component of the extracellular matrix, forms the scaffold upon which tissues are organized. Cells are able to attach directly to native collagen, presumably via specific receptors (Linsenmayer *et al.*, 1978; Schor and Court, 1979; Mauch *et al.*, 1986; Aumailley and Timpl, 1986). Indeed, saturable binding of collagen by fibroblastic cells has been observed (Goldberg, 1979; Goldberg, 1982; Goldberg and Burgeson, 1982) and membrane-bound glycoproteins with

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affinity for collagen have been isolated from a variety of cell types (Chiang and Kang, 1982; Mollenhauer and von der Mark, 1983; Kurkinen *et al.*, 1984; Ogle and Little, 1985, 1986; Rubin *et al.*, 1986; Nagata and Yamada, 1986; Chandrasekhar *et al.*, 1986; Sugrue, 1987; Dedhar *et al.*, 1987). Although some of these collagen binding proteins have been localized to the endoplasmic reticulum (Hughes *et al.*, 1987; Saga *et al.*, 1987), where they presumably function in collagen biosynthesis, there is evidence that others are present on the plasma membrane, where they may mediate cell adhesion to collagen (Mollenhauer and von der Mark, 1983; Ogle and Little, 1985, 1986; Rubin *et al.*, 1986; Dedhar *et al.*, 1987).

The discovery of the Arg-Gly-Asp (RGD) peptide and its role in cell surface-ECM binding was a major advance in the understanding of substrate adhesion (Pierschbacher and Ruoslahti, 1984). The RGD receptors comprise a superfamily of translation products termed the Integrins (Hynes, 1987; Buck and Horwitz, 1987; Ruoslahti and Pierschbacher, 1987). In some instances, receptors bind RGD-containing proteins with high selectivity, whereas in other instances a single, apparently "promiscuous" receptor complex may recognize the tripeptide ligand in a variety of different polypeptides. Further, the polypeptides comprising the RGD receptor complex(es) from different cell sources appear quite heterogeneous (Ruoslahti and Pierschbacher, 1987; Hynes, 1987; Buck and Horwitz, 1987).

A key feature in RGD-receptor binding is its rather weak association constant (approximately  $10^{-6}$  M, Akiyama *et al.*, 1986), which also raises the possibility that variable numbers of binding sites or other mechanisms distinguish transient from stable interactions. Since collagen is relatively rich in RGD sequences, we (Ogle and Little, 1985, 1986; Ogle, 1986) and others (Dedhar *et al.*, 1987) have begun to evaluate whether RGD may mediate collagen-cell surface adhesions.

There is good evidence that in the embryo, cell surface-collagen interactions are important in cell migration and morphogenesis (Harris, 1983; Yamada, 1983; Hay, 1984; Markwald *et al.*, 1984). These interactions are presumably mediated by specific receptors. Our original analysis of collagen binding proteins on the surface of embryonic fibroblasts identified a set of polypeptides that bound to collagen I affinity columns (Ogle and Little, 1985, 1986; Ogle, 1986). Two of the proteins had remarkably similar properties (judged by SDS-polyacrylamide gels under reducing and non-reducing conditions) to the RGD-dependent fibronectin-receptor proteins under study by a number of laboratories (Akiyama and Yamada, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987, Buck and Horwitz, 1987).

To examine whether some of the collagen binding proteins are members of the RGD recognition system, we tested GRGDS and RGDS peptides for their ability to disrupt the affinity for collagen I, on the part of embryonic cell surface proteins. The results show that certain of these proteins ( $M_r = 120, 90$  and  $47$  K) were eluted from the columns by synthetic peptides containing the RGD sequence, while at least one protein ( $M_r = 63$  K) recognizes features in collagen other than RGD. This work suggests that embryonic mesenchymal cells may have more than one means of adhering to collagen substrates.

## MATERIALS AND METHODS

### Materials

Iodine-125 (16.7 mCi/mg) was used for radioiodination, glycanase (N-glycosidase F, peptide-N<sup>4</sup>-[N-acetyl-beta-glucosaminyl] asparagine amidase) as purchased from Genzyme, (Boston, MA). Glycyl-L-arginyl-glycyl-L-aspartyl-L-serine (GRGDS) and glycyl-L-arginyl-glycyl-L-glutamyl-L-serine (GRGES) were prepared at the University of Virginia Peptide Synthesis Facility. Human plasma fibronectin was provided by Dr Gary Balian, University of Virginia.

### Cell Culture

Chicken heart fibroblasts (CHF) were prepared from minced hearts dissected from 9-day embryos by treatment with 0.1% trypsin, 0.002 M ethylenediaminetetraacetic acid (EDTA) in Ca<sup>++</sup> and Mg<sup>++</sup>-free Hank's balanced salt solution. CHF were plated and grown in high glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 µg/ml gentamicin and 0.002 M glutamine in an atmosphere of 10% CO<sub>2</sub>. Cells used for experiments were in the third and fourth passage.

### Collagen Purification

Type I collagen was purified from adult chicken skin and tendons of 17-day embryos by neutral salt purification (Miller and Rhodes, 1982) with and without pepsin treatment (1 mg/ml). Two cycles of neutral pH salt precipitation were required to remove all contaminating type III collagen. Purity was assessed by SDS polyacrylamide gel electrophoresis.

### Iodination of Chick Heart Fibroblasts

Cultures of CHF were detached from dishes by treatment with phosphate buffered saline containing 5 mM EDTA (PBS-EDTA). The cells were washed twice with PBS containing 0.5 mM MgCl<sub>2</sub>, 0.9 mM CaCl<sub>2</sub>, resuspended at a concentration of 10<sup>7</sup> cells/ml and iodinated by the lactoperoxidase-glucose oxidase method as described (Schneider *et al.*, 1982).

### Preparation of Membranes

Plasma membranes were prepared from CHF released by PBS-EDTA treatment as described above. For preparative procedures, plasma membrane fractions were obtained from the 32%/40% interface of a discontinuous sucrose gradient (Cates and Holland, 1978). The membranes from 10<sup>7</sup> cells were washed with 10 mM triethanolamine (TEA), pH 7.2, 2 mM CaCl<sub>2</sub>, 1 mM phenylmethylsulfonyl fluoride (PMSF) containing 1 M urea (Schwartz-Mann, Ultrapure). Next, the membranes were washed in the same buffer except 0.5 M NaCl was

substituted for the 1 M urea. The NaCl was removed by washing in 1 mM TEA, pH 7.2, 1 mM CaCl<sub>2</sub>. The remaining intrinsic membrane proteins were solubilized in 1% (33 mM) octylglucoside (Baron and Thompson, 1975), 1 mM TEA, pH 7.2, 1 mM CaCl<sub>2</sub>; this last buffer will be referred to as the solubilization buffer.

### Binding Studies

Initial binding studies were accomplished as follows. Type I collagen, human plasma fibronectin, and bovine serum albumin were coupled to cyanogen bromide activated Sepharose at a concentration of 2 mg/ml. Protein-conjugated Sepharose beads (12.5  $\mu$ l packed volume) were suspended with 250  $\mu$ l of solubilization buffer containing extracts of radioiodinated plasma membranes, then mixed by end-over-end rotation for 1–3 hours. The beads were collected by centrifugation, washed vigorously with a 40-fold excess (500  $\mu$ l) of the solubilization buffer, centrifuged again and washed with a second 40-fold excess of solubilization buffer. The beads with bound material were then boiled in SDS sample buffer and transferred to new tubes. After a final centrifugation (10,000g, 5 min) to pellet the beads, the sample was applied to an SDS-polyacrylamide slab gel for electrophoresis.

For chromatographic isolation of CBP, octylglucoside extracts of membranes were applied to 1.0 ml bed volume columns of collagen-Sepharose equilibrated in the solubilization buffer, then recycled 5 times at a flow rate of 4 ml per hour. Then the column was washed with 25 bed volumes of solubilization buffer, after which the detectable radioactivity (<sup>125</sup>I) dropped to background levels. A solution of 0.5 M NaCl in solubilization buffer was applied to the column, and 0.2 ml fractions were collected and monitored for <sup>125</sup>I radioactivity. The peak fractions were collected, and dialyzed against H<sub>2</sub>O to remove the octylglucoside. The resulting insoluble pellet was collected by centrifugation.

To test the effects of synthetic peptides on the binding of CBP to collagen, octylglucoside extracts of <sup>125</sup>I-surface labeled whole cells were applied to 200  $\mu$ l columns of Type I collagen-Sepharose, fibronectin-Sepharose or albumin-Sepharose as described above. After washing to remove unbound material the columns were treated with 1 ml of 1 mg/ml solution of synthetic peptides in solubilization buffer by recycling for 15 minutes. This eluate was dialyzed versus 0.1 mM phenylmethylsulfonyl fluoride, lyophilized, and subjected to SDS-PAGE. Control studies were conducted in which GRGES (1 mg/ml) or GARGND (1 mg/ml) were cycled over the column for 15 minutes prior to elution with either GRGDS or RGDS. Proteins in the various eluates were resolved by SDS electrophoresis.

### Antibodies

Collagen binding proteins, twice purified by affinity chromatography, were mixed with Freund's complete adjuvant, and injected into a rabbit. Three weeks later the rabbit was boosted with CBP in incomplete adjuvant, then bled 10 days later. The rabbit was boosted thereafter every 30 days, and bled every 14 days.

Whole mounted embryonic chicken heart fibroblasts were immunolabeled with affinity purified CBP antibodies according to previously used methods (Little and Chen, 1982).

### Other Methods

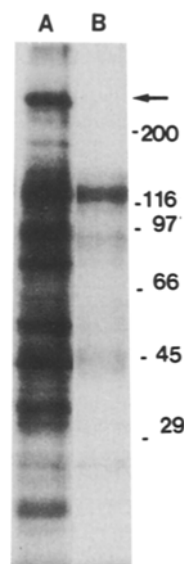
Electrophoresis was performed as described (Laemmli, 1970) and proteins visualized with Coomassie Blue R-250 or by autoradiography of dried gels exposed to Kodak XAR-5 film employing Dupont Cronex lighting plus intensifying screens. Molecular weight standards were myosin ( $M_r = 116,000$ ), phosphorylase B ( $M_r = 97,400$ ), bovine serum albumin ( $M_r = 66,000$ ), ovalbumin ( $M_r = 45,000$ ) and carbonic anhydrase ( $M_r = 29,000$ ).

## RESULTS

### Surface Labeling and Cell Membrane Purification

Cell surface proteins on live CHF cells, released from culture dishes by PBS/EDTA treatment, were labeled by lactoperoxidase catalyzed iodination (cells had greater than 95% viability after labeling as assessed by replating efficiency). Plasma membrane fractions purified from cell homogenates were washed with buffers containing urea and NaCl to remove loosely adherent proteins, which might complicate analysis of putative integral membrane receptors (see Methods).

Figure 1, lane A shows material extracted with 1 M NaCl, whereas lane B shows integral membrane proteins derived from washed membranes, which were



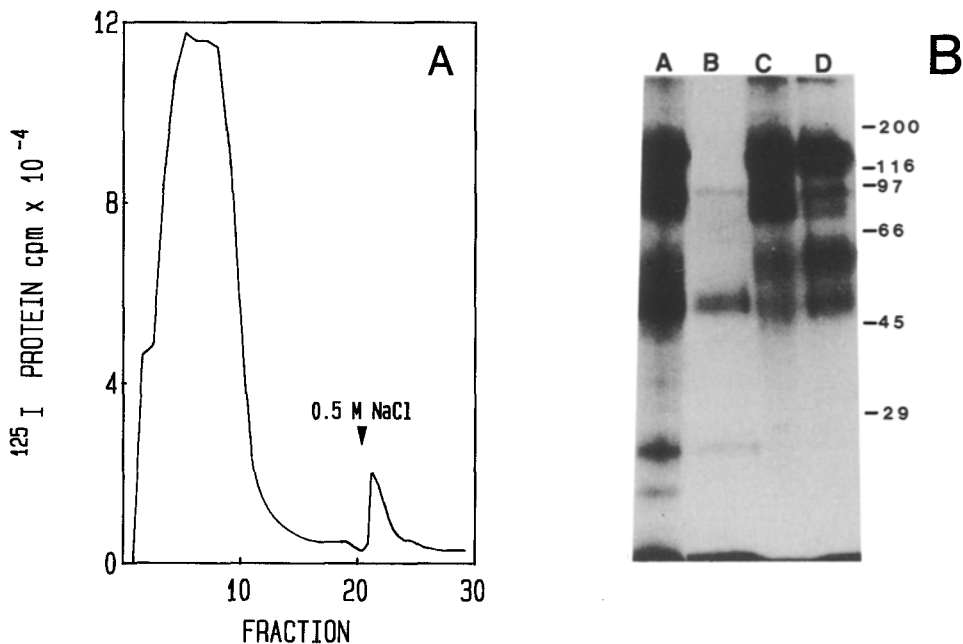
**Fig. 1.** Autoradiogram of SDS-PAGE of  $^{125}\text{I}$ -labeled polypeptides extracted from purified plasma membranes of chick heart fibroblasts as described in Methods. Samples were subjected to electrophoresis on 5 to 15% acrylamide gels under reducing conditions. Lane A shows the 1.0 M NaCl extract of a plasma membrane preparation, the arrow indicates the migration position of fibronectin. The membranes were washed to remove residual NaCl, then detergent solubilized. Lane B shows a sample of the octylglucoside extract. Equal counts were loaded in each lane.

solubilized in 1% octylglucoside (solubilization buffer, see Methods). The sequential urea, and NaCl treatment was effective in removing extrinsic polypeptides such as fibronectin (arrowhead). Polypeptides of Mr = 120, 90 and 47 K were the predominant radiolabeled constituents of washed, detergent solubilized membrane extracts (Fig. 1B). Although the polypeptide composition observed in Fig. 1B was qualitatively constant from experiment to experiment, there may be partial extraction of similar or identical species (judged by common electrophoretic mobility) in the washes. This could reflect true structural differences or limited removal of integral membrane proteins associated with lipids in the form of micelles, as occurs for viral glycoproteins (Fries and Helenius, 1979). We have not distinguished these possibilities in our initial characterization.

The sequential membrane washing treatments yielded 38, 13, and 11% of non-dialyzable iodinated proteins, from the urea, NaCl, and octylglucoside washes, respectively. The remaining iodinated material was not extractable.

### Purification of Collagen Binding Proteins by Collagen Affinity Chromatography

Native type I collagen affinity chromatography was employed to purify collagen binding proteins for preliminary biochemical characterization and for the

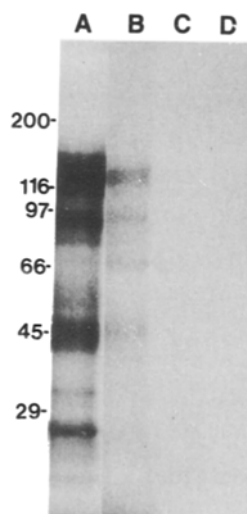


**Fig. 2.** Purification of collagen binding protein by collagen I-Sepharose affinity chromatography. Membranes radiolabeled, purified, and washed as described in Methods were extracted in octylglucoside, then passed over native collagen I-Sepharose (A). Chromatographic fractions were pooled and analyzed by SDS-polyacrylamide gel electrophoresis (B). The washed radioiodinated membrane constituents prior to loading on the column are shown in lane A. A sample of material from the large unbound peak, lane B. Material eluted by 0.5 M NaCl, lane C. Fractions from the small peak were pooled and dialyzed against water to remove the detergent. The resulting precipitate was collected by centrifugation, resolubilized in octylglucoside, then electrophoresed, lane D.

production of antibodies (Fig. 2). Detergent extracts of washed membranes were applied to a column of native type I collagen-Sepharose (Fig. 2A and 2B, lane a). Material eluted with 0.5 M NaCl consisted of polypeptides ( $M_r = 120, 90, 63$  and  $47$  K) which constituted about 5% of the protein in the membrane extract (Fig. 2B, lane c). These polypeptides were designated collagen binding proteins (CBP) 1, 2, 3 and 4 respectively. Following NaCl elution, this material was dialyzed against 1 mM TEA, pH 7.2, 0.1 mM PMSF. The relatively hydrophobic nature of the CBP is shown by the fact that after the dialysis step, the detergent-solubilized membrane-polypeptides formed a precipitate (Fig. 2B, lane d). If columns were washed with 1 M NaCl subsequent to the 0.5 M wash, a smaller peak of qualitatively similar collagen binding proteins was eluted (not shown).

### Elution of CBP with RGD Peptides

Collagen binding proteins bound to native collagen type I-Sepharose were tested for their susceptibility to elution by the synthetic peptide GRGDS. In this experiment, equal amounts of surface labeled  $^{125}\text{I}$  radioactivity from whole cells, not purified membranes, were incubated with native collagen I, fibronectin and bovine serum albumin-Sepharose (Fig. 3, lane a). Following exhaustive washing with solubilization buffer, 1 mg/ml of GRGDS in 1% octylglucoside was applied to each affinity column. No detectable iodinated material was eluted from fibronectin-Sepharose or albumin-Sepharose by GRGDS (Fig. 3, lanes c and d). Polypeptides of  $M_r = 120, 90, 70$  and  $47$  K were eluted from collagen-Sepharose (Fig. 3, lane b) while the 63 kDa protein, CBP 3, was not eluted from the column by GRGDS. The 70 kDa polypeptide was not observed consistently. Identical results were obtained eluting the column with the peptide RGDS. Longer exposures of Lane a to X-ray film revealed bands with mobilities in the range of 200–500 kDa, which eluted from collagen-Sepharose with 0.5 M NaCl, data not shown. In the case of fibronectin-Sepharose, two bands at approximately 70 and



**Fig. 3.** Autoradiogram of SDS-PAGE analysis of  $^{125}\text{I}$ -labeled proteins bound and eluted from collagen-Sepharose by treatment with the synthetic peptide GRGDS. Surface labelled ( $^{125}\text{I}$ ) cells were extracted in solubilization buffer (see Methods), lane A. This extract was passed over columns of Sepharose conjugated with either native collagen I, fibronectin or bovine serum albumin. Each column was washed exhaustively with solubilization buffer and treated with GRGDS in this same buffer at a concentration of 1 mg/ml. Lane B shows proteins of  $M_r = 120,000, 90,000, 70,000$  and  $47,000$  were isolated on collagen-Sepharose, but not fibronectin-Sepharose, lane C; or bovine serum albumin-Sepharose, lane D.

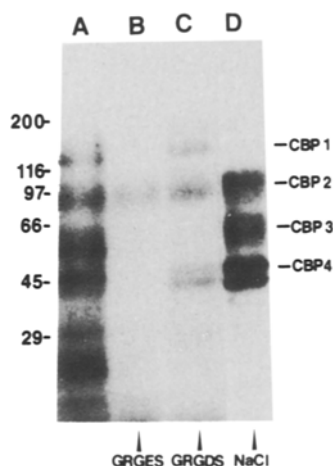


205 kDa were eluted by 0.5 M NaCl (Ogle, 1986). The proportion of counts bound to collagen-Sepharose that could be eluted by GRGDS was 75–80% of the counts released by stripping the beads with SDS sample buffer at 100°C.

CBP 1, 2, 3 and 4 were present in all collagen-affinity preparations, but the relative abundance of the polypeptides varied, similar to the observations of others (Kurkinen *et al.*, 1984). This was thought to be due to heterogeneity in these early passage embryonic cells rather than proteolysis. Native collagen types II, III, IV and V were also used as absorbents and found to bind membrane proteins of similar electrophoretic mobility, as has been observed in other laboratories (Kurkinen *et al.*, 1984).

### Specificity of CBP Elution by RGD Peptides

The specificity of elution of CBP from collagen-Sepharose by the RGD peptide was examined further by treatment with peptides of similar sequence and charge. When columns were washed with a 1 mg/ml solution of GRGES (E = glutamic acid) a small amount of CBP 2 but no CBP 1 or CBP 4 were eluted (Fig. 4, lane b). Following GRGES elution, the columns were washed with the peptide GRGDS, CBP 1 was completely eluted by this treatment, as were detectable amounts CBP 2 and 4 (Fig. 3, lane c). No detectable CBP 3 was displaced by treatment of the column with GRGDS. Subsequent treatment of the column with 4 M NaCl yielded considerable CBP 2, 3 and 4 (Fig. 4, lane d). Thus, none of the synthetic peptides were effective in eluting CBP 3 from collagen, including the synthetic peptide GARGND (data not shown), a sequence similar to GRGDS, which occurs in the interstitial collagens (Bornstein and Traub, 1979; Bernard *et al.*, 1983; Fuller and Boedtger, 1983).



**Fig. 4.** Autoradiogram of SDS-PAGE analysis of  $^{125}\text{I}$ -labeled proteins specifically eluted from collagen-Sepharose by treatment with an RGD containing peptide. Iodinated cell extracts were prepared as described in the preceding legend and passed over columns containing collagen I-Sepharose, lane A. Following exhaustive washing, the column was sequentially eluted with buffer containing 1 mg/ml GRGES, lane B, and GRGDS, lane C. Remaining bound material was stripped from the column with 4 M NaCl, lane D. A small amount of material of migrating  $M_r = 90,000$  (CBP 2) was eluted by GRGES, but no other CBPs were detected. Treatment with GRGDS eluted CBP 1, 2 and 4. The absence of CBP 1 in the wash, lane D, revealed that prior treatment with GRGDS had removed all bound CBP 1.

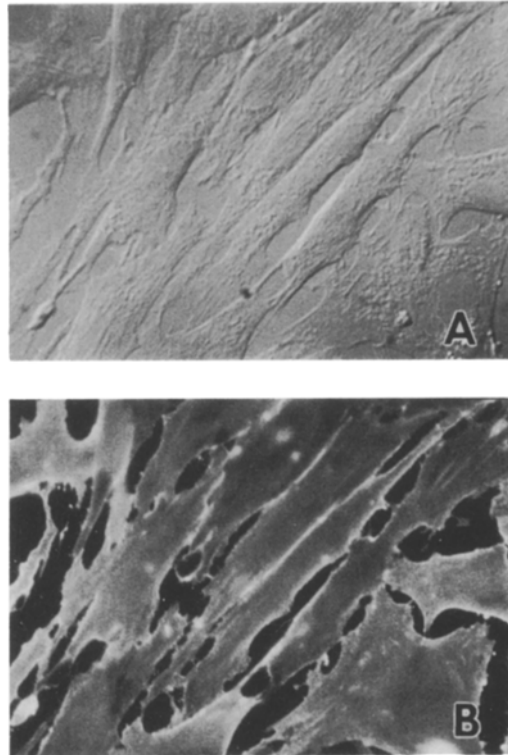
### Presence of N-linked Carbohydrate

Iodinated CBPs were treated with the enzyme N-glycanase which cleaves N-linked oligosaccharides but does not digest protein or carbohydrates. This

treatment resulted in a marked decrease in the apparent molecular weight of CBP 1, 4. The banding region corresponding to CBP 2 is altered by N-glycanase treatment but unambiguous interpretation is not possible, due to shift of CBP 1 bands into the same molecular weight region (data not shown). CBP 3 has not, as yet, been shown to contain oligosaccharides.

### Cell Surface Immunolabeling of CBP

Affinity purified rabbit antibodies raised against CBP were used to immunolabel whole-mounted primary embryonic chicken heart fibroblasts (Fig. 5A). The staining appeared as a uniform, fine granular distribution over the entire surface of fixed, non-permeabilized cells (Fig. 5B).



**Fig. 5.** A primary culture of lightly-fixed, whole-mounted embryonic chicken heart fibroblasts are shown using differential interference contrast microscopy, A. The corresponding immunofluorescence image (B) shows a fine granular pattern over the entire surface of the cells after labeling with affinity-purified CBP-antibodies raised against a preparation similar to that shown in Fig. 4B, lane D.

## DISCUSSION

Based upon the following criteria: (1) susceptibility to iodination on intact cells, (2) purification from membranes washed free of extrinsic proteins, (3) presence of N-linked carbohydrate (on CBP 1, 4 and possibly 2), and (4) detergent requirement for solubilization, we conclude that CBP are cell surface-associated integral membrane proteins. Collagen binding appears to be a property of these proteins. Moreover, antibodies which immunoprecipitated all of the CBP (Ogle and Little, 1986), will also label the cell surface (Fig. 5), and co-distribute with collagen antibodies in cryosections of chicken embryos (Ogle and Little, 1986). Taken together, these results suggest that CBP may exhibit binding activity on the cell surface, and thus, function as collagen receptors.

Immunolabeling results, electrophoretic behavior and RGD recognition, suggest that CBP 1 and 2 are similar to the  $M_r = 140,000$  protein complex variously termed CSAT (Neff *et al.*, 1982), JG22 antigen (Greve and Gottlieb, 1982) and integrin (Tamkun *et al.*, 1986). Avian integrin binds to fibronectin by RGD recognition (Horwitz *et al.*, 1985; Akiyama *et al.*, 1986) with a binding constant of approximately  $10^{-6}$  M. In contrast, Goldberg (1979) reported a binding constant of  $10^{-9}$  M for collagen binding to the cell surface. In the experiment depicted in Fig. 2, no significant amount of 140 kDa protein was bound by fibronectin or eluted by RGDS, whereas a significant amount of CBP 1, 2 and 4 were bound by and eluted from collagen type I. Perhaps this significantly higher binding constant is a consequence of multiple RGD sequences present in the collagen molecule; there are eight such sequences per native chicken collagen type I molecule. This would account for the fact that under the conditions used in this study, collagen-Sepharose but not fibronectin-Sepharose, would bind significant amounts of  $^{125}$ I-labeled polypeptides. Alternatively, it is possible that the RGD sequence within fibronectin may be masked when coupled to Sepharose beads; since the cell binding domain, not the intact fibronectin molecule is normally used as the affinity ligand (Pytela *et al.*, 1985).

Work by other groups has shown that antibodies to rat hepatocyte membranes which block hepatocyte attachment to type I collagen were directed primarily against a  $M_r = 130,000$  protein (Rubin *et al.*, 1986), possibly a rat homolog of the chicken CBP 1. The recognition sequences within collagen I recognized by hepatocyte and chicken epithelial collagen receptors remain to be identified (Rubin *et al.*, 1986; Sugrue, 1987).

CBP 2 is of similar size to a protein implicated in substrate adhesion (Hsieh and Sueoka, 1980) and may be related to CBP 1. When the N-linked carbohydrates are removed, the slowest migrating band, presumably CBP 1, migrates at approximately the same position as undigested CBP 2, under both reducing and non-reducing conditions (not shown). However, the shift of CBP 1 after N-glycanase treatment to the 90,000 dalton banding region, makes it difficult to interpret the effects of N-glycanase treatment on CBP 2. Alternatively, it is possible that CBP 2 ( $M_r = 90,000$ ) is an integrin  $\beta$  subunit. In this regard it is worth noting that the  $\beta$  chain of the integrin complex, which has been cloned and sequenced, has an estimated molecular weight attributable to amino acid

composition of Mr = 89,000 (Tamkun *et al.*, 1986).

To the best of our knowledge CBP 3, Mr = 63,000, does not appear to be identical to any previously described protein. The results showed that CBP 3 is not susceptible to RGD elution. Although we do not know what features on the collagen molecular CBP 3 recognizes, the fact that significant amounts remain on the column after washing with 1 M NaCl suggests that the interaction is especially stable. Perhaps CBP 3 is a collagen "specific" receptor.

The Mr = 47,000 protein (CBP 4) is probably distinct from avian analogues of the murine collagen binding protein termed colligin (Kurkinen *et al.*, 1984; Nagata and Yamada, 1986), since these polypeptides were localized to the ER with no detectable presence at the cell surface (Hughes *et al.*, 1987; Saga *et al.*, 1987).

The adhesion of cells to collagen is a complex process. Collagen binding protein 1, or a complex of CBP 1, 2 and 4, may allow cells to attach to collagen and possibly other ligands by RGD recognition. Other collagen receptors may use polypeptides such as CBP 3, which appear to recognize features other than RGD. Laminin is an example of an ECM component which interacts with multiple cell surface receptors, including integrins (Horwitz *et al.*, 1985; Gehlsen *et al.*, 1988) and other laminin specific receptors of Mr = 67,000 (Terranova *et al.*, 1983; Brown *et al.*, 1983; Lesot *et al.*, 1983; Graf *et al.*, 1987). Thus, cells may express a single promiscuous receptor-complex which interacts with multiple ECM-ligands, such as integrin; and also express multiple receptors for a single ECM polypeptide. Most likely, embryonic cells have several distinct ligand-specific receptors that permit various behaviors. In the case of collagen it is possible that one class of receptors might be involved in migration or modulation of cell shape, whereas cell surface adhesion during fibrillogenesis (Trelstad, 1984) or traction-driven morphogenesis (Harris, 1983) would employ other receptors. Expression of multiple receptors for collagen would permit a variety of cellular responses to this ubiquitous ECM ligand.

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