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Purification of an Arg-Gly-Asp selective matrix receptor from brain synaptic plasma membranes

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Brain synaptic plasma membranes specifically associated with matrix protein monolayers containing the Arg-Gly-Asp sequence recognized by integrin-type adhesion receptors. Experiments using fibronectin affinity chromatography to identify the synaptosomal receptors responsible for this interaction led to the purification of a 55 kDa Arg-Gly-Asp recognition protein that is labelled by antibodies against the $\alpha_5\beta_1$ integrin.

INTRODUCTION

Synapses in the central nervous system are sites of communication and anatomical connectivity. Constituent molecules involved in the latter likely include members of the adhesion receptor classes responsible for forming and maintaining junctional contacts between many types of cells (McDonald, 1989; Akiyama *et al.*, 1990). Two such classes of particular interest are: (*i*) the immunoglobulin superfamily and (*ii*) the integrin family of transmembrane matrix receptors which mediate a broad spectrum of cell–cell and cell–substrate interactions (Albelda & Buck, 1990). Neural cell-adhesion molecules ('NCAMs') of the immunoglobulin class are enriched in synapses (Persohn *et al.*, 1989), though they are known to be expressed on the plasma membrane throughout the neuron (Pollerberg *et al.*, 1987). In contrast, little has been established about the localization of integrins in the brain. Fibronectin binds to brain synaptic membrane fractions (Bahr *et al.*, 1991*a*) and antibodies against the $\alpha_5\beta_1$ integrin (the mammalian fibronectin receptor) or the $\alpha_v\beta_3$ integrin (vitronectin receptor) label different proteins that are highly concentrated in these fractions (Bahr *et al.*, 1991*a*,*b*). It is noteworthy that these proteins are smaller than known integrins. Evidence implicating integrin-like receptors in synaptic function was obtained in two studies showing that peptides which block a subclass of integrins (Ruoslahti & Pierschbacher, 1987) disrupt the stabilization of synaptic potentiation in hippocampal slices (Staubli *et al.*, 1990; Xiao *et al.*, 1991).

The above results raise the possibilities (*i*) that synaptic membranes bind to matrix proteins, and (*ii*) do so via one or more unusual integrin-like adhesion receptors. The present experiments explored these points. Adhesion between synaptosomal membranes and various matrix proteins was tested by seeding the former on to monolayers of the latter. The possibility that any interactions were dependent upon the membranes recognizing the Arg-Gly-Asp (RGD) sequence to which some integrins bind was tested by using matrix proteins lacking the sequence or by including RGD-containing peptides in the assays to competitively block the binding site. A second series of studies was then carried out to identify synaptosomal membrane receptor(s) for fibronectin or related matrix constituents. Solubilized membranes were applied to fibronectin affinity columns, and the material selectively displaced by RGD-containing peptide was collected and analysed. Two proteins appearing as a 55/51 kDa doublet recognized by antibodies against the $\alpha_5\beta_1$ integrin were obtained; additional experiments confirmed that this pair of molecules binds to fibronectin. The results provide evidence that brain synaptic membranes contain integrin-like matrix recognition receptors of unusual molecular mass. The proteins identified are thus candidates for elements contributing to the formation, maintenance and modification of synaptic connections.

EXPERIMENTAL

Materials

Polyclonal antibodies (goat anti-gp140) to the Chinese-hamster ovarian fibronectin receptor (Pytela *et al.*, 1985; Schreiner *et al.*, 1989; Schwarz *et al.*, 1989) were generally provided by Dr. R. L. Juliano (Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, U.S.A.). The peptide Arg-Gly-Asp-Ser (RGDS) was purchased from Bachem (Torrance, CA, USA). Falcon 3912 MicroTest III flexible microtitre plates [poly(vinyl chloride)] were obtained from Becton Dickinson Labware (Oxnard, CA, U.S.A.). Precast acrylamide gradient mini-gels, nitrocellulose paper, and alkaline phosphatase-conjugated antibodies to rabbit, mouse or goat IgGs were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP), human plasma fibronectin and its 45 kDa α-chymotryptic fragment, and fibronectin–agarose were from Calbiochem Corp. (San Diego, CA, U.S.A.). Leupeptin and polyclonal antibodies against the $\alpha_v\beta_3$ vitronectin receptor from human placenta were obtained from Chemico International (Temecula, CA, U.S.A.). Quantigold protein assay reagent was obtained from Diversified Biotech (Newton Centre, MA, U.S.A.). Gly-Arg-Gly-Glu-Ser-Pro (GRGESP), Gly-penicillaminyl-Gly-Arg-Gly-Asp-Ser-Pro-Cys-Ala (GPenGRGDSPCA), human vitronectin, and a monoclonal antibody against the human α_v integrin subunit were purchased from Telios Pharmaceuticals (San Diego, CA, U.S.A.). Mouse laminin, polyclonal antibodies (Anti-keratin) against fetal-bovine keratin, alkaline phosphatase-conjugated antibodies to guinea pig IgG, a monoclonal antibody (anti-synaptophysin) against synaptophysin from rat synaptosomes, and n-octyl β-D-glucopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other materials were from usual commercial sources, unless otherwise noted.

Abbreviations used: Anti-gp140, antibodies to the Chinese-hamster ovarian fibronectin receptor; *F55*, 55 kDa polypeptide(s) specifically eluted from fibronectin-agarose; GPenGRGDSPCA, Gly-penicillaminyl-Gly-Arg-Gly-Asp-Ser-Pro-Cys-Ala; GRGDSP, Gly-Arg-Gly-Asp-Ser-Pro; GRGESP, Gly-Arg-Gly-Glu-Ser-Pro; RGD, Arg-Gly-Asp; RGDS, Arg-Gly-Asp-Ser; SPM, synaptic plasma membrane.

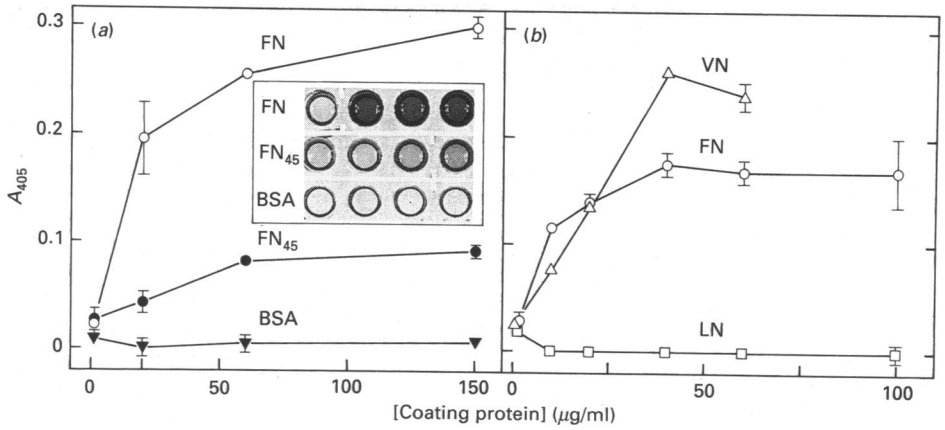

Fig. 1. Synaptosomal membrane adhesion to matrix protein monolayers

Fibronectin (FN), fibronectin fragment (FN_{45}), vitronectin (VN), laminin (LN), or BSA were immobilized (1–15 μg/well) for 12 h at 23 °C as described in the Experimental section. Adhesion of 15 μg of whole brain SPM protein to each coated well was determined by an anti-keratin e.l.i.s.a.; the enzyme activity is expressed as A_{405}. Results are means ± 1 S.D. of triplicate determinations. The inset shows the immunoreactivity of adhered SPMs in wells coated with an increasing concentration (from left to right: 0, 20, 60 and 150 μg/ml) of the noted protein. Control wells coated with protein but incubated without SPMs had a background A_{405} range of 0.044–0.066, which was substracted from respective data. Similar results were obtained from at least three other SPM preparations.

Synaptic-plasma-membrane isolation

Synaptic plasma membranes (SPMs) were prepared from adult male rats (Sprague–Dawley) as previously described (Bahr *et al.*, 1991*b*). Briefly stated, this involved differential-sedimentation-velocity pelleting, buoyant-density centrifugation in Percoll gradients, and hypo-osmotic lysis. The SPMs were suspended at 2–4 mg of protein/ml in Buffer A consisting of 35 mM-Tris, pH 7.4, 0.05 mM-EDTA and broad-spectrum proteinase inhibitors [antipain, 20 μg/ml; aprotinin, 2 μg/ml; calpain inhibitor I, 40 μg/ml; leupeptin, 2 μg/ml; pepstatin A, 2 μg/ml; fresh phenylmethane sulphonyl fluoride, 35 μg/ml; *N*-tosyl-L-phenylalanyl-chloromethane ('TPCK'), 20 μg/ml] divided into aliquots, and stored at −70 °C. The protein content was determined using the Bradford (1976) assay with BSA as standard.

Micro-well adhesion assay

Protein immobilization. Vitronectin, laminin, BSA and fibronectin (or its 45 kDa proteolytic fragment) were immobilized on microtitre plates as previously described (Tomaselli *et al.*, 1987). In brief, pure protein was diluted in plate adhesion buffer (PAB; composed of Buffer A with 150 mM-NaCl, 1 mM-$CaCl_2$, 1 mM-$MgCl_2$ and 0.02% NaN_3) to a final concentration of 10–150 μg/ml, added to wells of a poly(vinyl chloride) plate, allowed to bind for 12–16 h at 23 °C, and then 3% (w/v) non-fat dry milk was incubated with the pure protein for 2 h at 37 °C to block unoccupied sites on the surface of the microwells. Before milk blocking, control plates were washed with PAB and the extent of surface coating was determined using the nanogram-sensitive Quantigold colorimetric protein assay. The colorimetric analyses of the different proteins coated could be compared, since each protein exhibited a similar abosrbance-change-versus-protein-concentration relationship.

SPM adhesion. After four 3 min washes with PAB at 23 °C, adhesion was initiated by adding SPMs (15 μg of protein in 0.1 ml of PAB), prepared from whole brain, forebrain or hippocampus, to each well. The plate was incubated for 12 h at 4 °C in the absence or presence of potential adhesion inhibitors, after which three rapid washes with 0.2 ml of ice-cold PAB were used to remove unbound SPMs by aspiration. Bound membranes were fixed to the plate overnight with 3% (w/v) paraformaldehyde in PAB at 4 °C in order to avoid loss of adhered SPMs due to extensive washing steps in subsequent procedures. In early work, bound SPMs were determined with an e.l.i.s.a. method utilizing an antibody against the structural protein keratin, which we have shown to react with a proteoglycan concentrated in rat brain SPMs (Capaldi *et al.*, 1991). Later work used an antibody to the synaptic-vesicle marker synaptophysin, which is found in virtually all nerve terminals in brain (Navone *et al.*, 1986). The e.l.i.s.a. procedure entailed (1) thoroughly washing the fixed plate, (2) blocking the wells with 3% (w/v) non-fat dry milk and (3) incubating with antibody diluted 1:150 in Tris-buffered saline (30 mM-Tris/200 mM-NaCl), pH 7.4, with 2% (w/v) milk at 4 °C for 12–16 h. Secondary-antibody incubation and colour development utilized alkaline phosphatase-conjugated antibodies and the *p*-nitrophenyl phosphate substrate system. Quantitative immunoreactivity was determined by measuring the A_{405} using a microplate reader (Molecular Devices $Thermo_{max.}$). The background immunoreactivity obtained from similarly treated control wells containing no SPM protein was subtracted from all data.

Affinity chromatography

Hippocampal SPMs at 0.8 mg of protein/ml were solubilized in ice-cold PAB with either 150 mM-n-octyl β-D-glucopyranoside or 1% (w/v) Triton X-100. Each membrane suspension was homogenized in an etched-glass Potter–Elvehjem tissue grinder for 30 s at high speed, then placed on ice for 1 h. The particulate matter was removed by centrifugation at 50 400 *g* for 25 min at 4 °C, after which the supernatant was divided into portions and stored at −70 °C. The human plasma fibronectin–agarose column was equilibrated at 4 °C in PAB with the respective detergent (30 mM-n-octyl β-D-glucopyranoside or 0.1% Triton X-100), which solubilized the SPMs before loading. A portion of solubilized membranes was thawed, diluted with an equal volume of ice-cold PAB, and applied to a 1 cm × 4 cm or 1.5 cm × 7 cm column over a 1 h period at 4 °C. The column was subsequently washed with 5–10 column vol. of the respective equilibration buffer, after which solutions with GRGESP (control peptide) and then with GRGDSP were applied to the column. Individual or pooled fractions of the eluant were concentrated to appropriate

volumes with Centricon-10 concentrators (Amicon, Danvers, MA, U.S.A.) before electrophoresis and immunoblotting steps.

Electrophoresis and immunoblotting

For Western-blot analysis, samples were treated with 2.5% (w/v) SDS in the absence or presence of 50 mM-dithiothreitol at 100 °C for 5 min, then subjected to PAGE (Laemmli, 1970). Linear acrylamide gradient [3–17 or 4–15 (w/v)] gels were used to separate proteins, after which the proteins were either silver-stained (Merril *et al.*, 1981) or transferred to nitrocellulose (0.2 μm pore size) as described by Burnette (1981) for 6–12 h. Incubation of the nitrocellulose with anti-gp140 (diluted 1:3000) or with polyclonal (1:1000) or monoclonal (1:300) antibodies against the $\alpha_v\beta_3$ integrin in Tris-buffered saline, pH 7.4, with 0.1% (v/v) Tween-20 and 1.5% (w/v) non-fat dry milk, was carried out at 4 °C with agitation for 12–16 h. Secondary-antibody incubation and colour development utilized alkaline phosphatase-conjugated antibodies and the 5-bromo-4-chloro-indol-3-yl phosphate and Nitroblue Tetrazolium substrate system. Calibration of gels and immunoblots using prestained protein molecular-mass standards allowed size determination for pertinent species.

RESULTS

To screen for adhesion molecules in synaptic contacts, we used a simple test in which lysed SPMs were seeded on to microtitre plates coated with a particular matrix component. As a control, protein analyses of coated microwells were carried out. Vitronectin exhibited moderate surface coating, whereas all other proteins tested had good coating efficiencies (Table 1). As Fig. 1 shows, synaptic membrane fractions adhere to plates coated with fibronectin or vitronectin, but not to those covered with a laminin monolayer. The membrane adhesion is dependent upon the concentration of matrix protein during coating and is saturable. Vitronectin monolayers appear to allow the adherence of more SPMs at higher coating concentrations than do monolayers of fibronectin. This was unexpected, since vitronectin coats plate surfaces four or five times less efficiently than does fibronectin. Similar results were obtained when the adhesion response was measured by using one of two antibodies: (*i*) anti-keratin, which recognizes a high-molecular-mass (400–600 kDa) heparin-binding keratan sulphate proteoglycan that displays tissue-specific localization to brain synaptic membranes (Capaldi *et al.*, 1991) or (*ii*) anti-synaptophysin, which reacts with a universal synaptic-vesicle marker that is shuttled between vesicular and plasma membranes via exocytotic–endocytotic cycles (Lowe *et al.*, 1988; Valtorta *et al.*, 1988, 1989; Torri-Tarelli *et al.*, 1990) and reacts intensely with a 38 kDa protein in immunoblots of the lysed synaptosomal membranes used here (results not shown). Interestingly, hippocampal SPMs consistently displayed 40–50% more fibronectin attachment/μg of membrane protein over that displayed by SPMs prepared from whole brain tissue. The anti-keratin e.l.i.s.a. activity from the adhered SPMs accounted for 45.5 ± 1.4% (mean ± S.D.; $n = 5$) and 29.9 ± 3.0% ($n = 4$) of that exhibited by the total SPMs from hippocampus and whole brain respectively that were applied to the microwells and subsequently fixed (without prior washing steps) and assayed as described in the Experimental section.

Two lines of evidence indicate that the adhesion response involves RGD-selective receptors: (*i*) Fig. 1(*a*) shows that synaptic membranes do not attach well to a gelatin-binding fibronectin fragment lacking the RGD sequence (FN_{45}; Pierschbacher *et al.*, 1981), even though the fragment appears to coat surfaces better than fibronectin itself does (Table 1); and (*ii*) RGD-containing peptides (RGDS and GRGDSP) that block the integrin fibronectin-binding site cause about half of the membranes to remain in solution above the fibronectin monolayer (Table 2). This loss is equal to the extent to which integrin attachment to fibronectin monolayers is blocked by RGD peptide (Pytela *et al.*, 1985). It is not surprising that a significant amount of SPM adhesion still remains under the above two conditions, since RGD-independent interactions exist between fibronectin and other extracellular components, including proteoglycans (Hakomori *et al.*, 1984; Ruoslahti, 1988, 1989; McDonald, 1989). Table 2 also shows that 1.5 mM-GRGDSP is more effective at inhibiting fibronectin attachment than 3 mM-RGDS; this is similar to the relative potencies reported by Pierschbacher & Ruoslahti (1984). Note that RGD peptides are only partial antagonists (Ruoslahti & Pierschbacher, 1987) and hence are effective at relatively high concentrations. A control peptide lacking the complete RGD sequence (GRGESP) did not decrease the attachment of synaptic membranes. The SPM–fibronectin association is apparently via a ligand-specific receptor, since the vitronectin-specific peptide inhibitor GPenGRGDSPCA, which is cyclized via a disulphide bridge between the penicillamine (Pen) and the half-cystine, was only weakly effective at blocking

Table 1. Surface coating of microtitre plates with extracellular-matrix proteins and BSA

Poly(vinyl chloride) microwells were incubated with the indicated concentrations of either BSA, fibronectin (FN), fibronectin fragment (FN_{45}), vitronectin (VN) or laminin (LN) for 14 h at 23 °C as described in the Experimental section. The wells were subsequently washed and treated with 100 μl of Quantigold protein assay reagent for 20 min. The A_{650} increased as a result of protein coating and is expressed as the average value for duplicate wells minus the background absorbance of the microtitre plate. Control wells without protein had an A_{650} range of −0.003 to 0.003 after subtracting background absorbance.

Protein	Concn. (μg/ml)...	Surface coating (A_{650}) 40	100	200
BSA		0.153	0.170	0.185
FN		0.060	0.087	0.095
FN_{45}		0.107	0.140	0.159
VN		0.011	0.022	0.021
LN		0.023	0.074	0.166

Table 2. Effect of RGD-containing peptides on SPM–fibronectin adhesion

Forebrain SPM samples (15 μg of protein) and the indicated concentration of various RGD-related peptides were incubated in microtitre wells previously coated with fibronectin (100 μg/ml). E.l.i.s.a.-type immunoreactivity (see Fig. 1) was expressed as a percentage of control (no peptide present) ± 1 S.D. The number of determinations (n) is shown in parentheses.

Peptide	SPM adhesion [% of control (n)]
None	100.0 ± 6.7 (10)
RGDS	
0.1 mM	98.3 ± 6.2 (4)
3 mM	75.6 ± 2.5* (3)
10 mM	51.7 ± 2.2* (6)
GRGDSP (1.5 mM)	52.7 ± 6.8* (9)
GRGESP (3 mM)	111.8 ± 3.9 (6)
GPenGRGDSPCA (1.5 mM)	88.0 ± 8.2 (4)

* Two-tailed t test comparing with no-peptide data: $P < 0.001$.

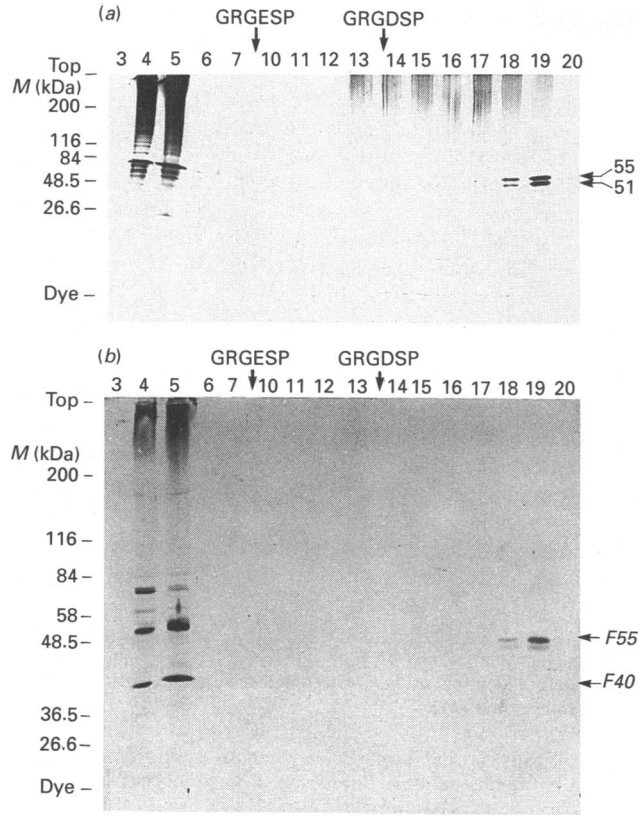

Fig. 2. Affinity chromatography of solubilized SPMs on fibronectin-agarose: analysis by SDS/PAGE for silver staining (*a*) and anti-gp140 immunoblotting (*b*)

Hippocampal SPMs (0.7 mg of protein) were solubilized with n-octyl β-D-glucopyranoside and applied to an equilibrated fibronectin–agarose column (3 ml bed volume) as described in the Experimental section. The column was subsequently washed and 2.5 mM-GRGESP or -GRGDSP was added to the elution buffer where indicated by arrows for one column volume. Fractions (1 ml) were collected, and portions of each were analysed by SDS/PAGE under reducing conditions, after which the gels were either silver-stained or electroblotted to nitrocellulose for Western-blot analysis (see the Experimental section). The top of the gel, the dye front, and molecular-mass (*M*) standards from 26.6 to 200 kDa are shown. The molecular masses of relevant bands are shown on the right with arrows. Similar results were obtained with four other SPM preparations from forebrain.

SPM–fibronectin adhesion (Table 2); this peptide did block SPM–vitronectin attachments by 30% (results not shown).

In an effort to identify the proteins responsible for adhesion, we subjected detergent-solubilized hippocampal synaptic membranes to affinity chromatography on fibronectin–agarose. After the column was washed thoroughly, sequential column volumes of solutions with GRGESP (control peptide) and then GRGDSP were applied to the column. The eluted proteins were separated by SDS/PAGE and the resultant gels stained with silver; a typical result is shown in Fig. 2(*a*). No bound proteins were displaced by control peptide, whereas a doublet consisting of 55 and 51 kDa polypeptides was eluted by the RGD-containing peptide (lanes 17–19). These polypeptides are not interlinked by disulphide bonds in their native form, since the absence of reducing agents had no effect on their electrophoretic mobilities. As shown in the immunoblot in Fig. 2(*b*), antibodies against the $\alpha_5\beta_1$ fibronectin receptor (anti-gp140) react intensely with the 55 kDa polypeptide (previously named '*F55*'; Bahr *et al.*, 1991*a*) and less intensely with its 51 kDa counterpart. Antibodies against

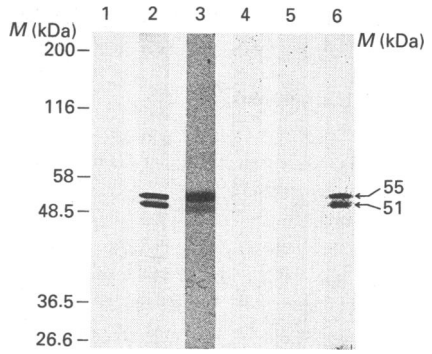

Fig. 3. Isolated *F55* re-applied to fresh fibronectin–agarose

Forebrain SPMs (3.5 of protein) were solubilized with Triton X-100 and applied to an equilibrated fibronectin–agarose column (12 ml bed volume) as described in the Experimental section. The column was washed thoroughly, then sequentially eluted with 3 mM-GRGESP (lane 1) and 3 mM-GRGDSP (lanes 2 and 3). *F55* was eluted with the RGD-containing peptide, and the peak fractions were pooled, diluted 3-fold with equilibration buffer and applied to an unused fibronectin–agarose column (2.5 ml bed volume). Fractions were collected containing the breakthrough volume (lane 4), subsequent washes, and each of two 2.5 ml elutions with 2.5 mM-GRGESP (lane 5) then with 2.5 mM-GRGDSP (lane 6). Appropriate fractions were pooled, concentrated and subjected to SDS/PAGE and silver-staining, except for lane 3, which was immunoblotted after electrophoresis and stained with anti-gp140. Electrophoretic positions of molecular-mass (*M*) standards are shown on the left. Similar results were obtained with two separate preparations of *F55* isolated from solubilized SPMs.

either the $\alpha_v\beta_3$ vitronectin receptor or the α_v integrin subunit did not recognize either polypeptide. Proteins not interacting with fibronectin or those over the binding capacity of the column material were evident in lanes 4 and 5 of Fig. 2. In these breakthrough lanes, anti-gp140 clearly labelled a single band at 55 kDa (*F55*), a 40 kDa species (*F40*), and a less abundant 80 kDa band, the first two of which have been previously identified with the same antibody (Bahr *et al.*, 1991*a*). Additional immunoreactivity not seen in previous SPM preparations was evident, probably because of non-specific secondary-antibody reactivity towards the heavy load of high-molecular-mass proteins in lanes 4 and 5. The anti-gp140 antibodies did not label any antigens in the molecular-mass range of *F55* and *F40* in tissue homogenates from rat lung, liver, spleen, kidney, adrenal gland, heart or intestinal lining (results not shown).

In order to determine whether the *F55* species interacts independently with immobilized fibronectin, GRGDSP-eluted *F55* (Fig. 3, lanes 2 and 3) from affinity-chromatographic isolation was concentrated and re-applied to a fresh fibronectin–agarose column. Fig. 3 shows that all of the re-applied *F55* bound to the new column material, since silver-stained breakthrough fractions were 'clean' (lane 4). Similarly, no silver-stained bands were evident when the column was eluted with up to three bed vol. of GRGESP solution (lane 5). The *F55* doublet was then specifically eluted with GRGDSP (lane 6). This 'RGD' recovery of *F55* was unchanged in the absence of prior elution of the column with GRGESP control peptide. Double silver staining of the polyacrylamide gels revealed that the *F55* protein isolated initially was accompanied by faint bands of 75, 41, and 26 kDa (lane 2); these were not evident in the GRGDSP-eluted *F55* from the fresh affinity column (lane 6).

DISCUSSION

The present studies provide evidence that RGD recognition proteins are present in synapses and have identified a possible

receptor of this type. Synaptic membranes adhered to matrix proteins containing the RGD sequence and were displaced by peptides incorporating the sequence. SPM attachment to vitronectin appeared to be more efficient than to other extracellular matrix molecules, suggesting that the membranes possess receptors of high affinity and/or density for vitronectin. Tests for a specific marker (synaptophysin) confirmed that synaptic components were involved in the adhesion response. These findings complement previously obtained results showing that fibronectin binds to SPMs in an RGDS-displaceable fashion (Bahr *et al.*, 1991*a*). The present experiments also describe a candidate for an adhesion receptor contributing to the binding of the membranes to the matrix proteins. This 55 kDa protein, *F55*, binds to fibronectin in an RGD-dependent fashion and is recognized by antibodies against the mammalian fibronectin receptor ($\alpha_5\beta_1$ integrin), but not by antibodies to the $\alpha_v\beta_3$ vitronectin receptor. *F55* is also greatly (20–40-fold) concentrated in SPMs (Bahr *et al.*, 1991*a*) and is undetectable in many non-neural tissues. However, although these points are suggestive, there is as yet no direct evidence linking the protein to the observed membrane–fibronectin interaction.

The $\alpha_5\beta_1$ integrin receptor is found in many types of cells, but is composed of subunit proteins that are considerably larger (Schreiner *et al.*, 1989; Akiyama *et al.*, 1990) than the synaptosomal membrane *F55* polypeptides specifically eluted from the fibronectin column. Matrix receptors of small size have been well characterized for fibronectin (Aplin *et al.*, 1981), laminin (Clegg *et al.*, 1988; Mafune *et al.*, 1990; Tandon *et al.*, 1991), collagen (Mollenhauer & von der Mark, 1983) and elastin (Mecham *et al.*, 1989), but these do not appear to utilize the RGD recognition site and hence are not likely to be related to *F55*. The possibility also exists that *F55* is a cleavage product resulting from proteolysis occurring during tissue preparation and SPM purification. That this can occur is suggested by the observation that a 51 kDa species weakly recognized by antibodies to the $\alpha_5\beta_1$ integrin appears along with *F55* only after affinity chromatography. This suggests that the chromatographic conditions promote digestion of the larger polypeptide to a species that is 10% smaller and significantly less antigenic. It is also the case that at least one integrin ($\alpha_{IIb}\beta_{IIIa}$) can be cleaved by CNBr to produce either a 54 or 40 kDa fragment containing an RGD-binding domain similar ($> 80\%$) in amino acid sequence to a region of the α_5 subunit (D'Souza *et al.*, 1990). This is of particular interest in the present context because the antibodies against the $\alpha_5\beta_1$ fibronectin receptor that detect the 55 kDa protein also react with a 40 kDa polypeptide in SPMs (Bahr *et al.*, 1991*a*). Specific proteinases may exist, then, that are resistant to the broad-spectrum inhibitors used in the present experiments and that yield the *F55* antigens under discussion.

The contributions of integrin-like receptors in SPMs to synaptic functioning is unclear. Prolonged infusion of RGD-containing peptides into slices of adult hippocampus does not produce any evident perturbations of baseline physiology (Staubli *et al.*, 1990; Xiao *et al.*, 1991), suggesting that RGD-selective receptors are not chiefly responsible for maintenance of connections, at least in the short term. Slices exposed to the RGD-containing peptides do exhibit one interesting difference from those treated with control peptides, namely a much decreased capacity for sustaining long-term potentiation of synaptic responses (Staubli *et al.*, 1990; Xiao *et al.*, 1991). These observations raise the possibility that RGD-selective matrix receptors anchor changes in the configuration of the synapse (Lynch *et al.*, 1991). Whether the matrix recognition molecule described here is involved will require isolation of its endogenous ligands and infusion of their receptor-binding regions into hippocampal slices. Interestingly, however, $\alpha_5\beta_1$ integrins in resting human T-cells become fully functional as adhesion molecules subsequent to an activation event (Matsuyama *et al.*, 1989; Shimizu *et al.*, 1990). Perhaps, then, the Ca^{2+}-driven events leading to expression and stabilization of long-term potentiation include the activation of latent adhesion receptors of which *F55* may be an example.

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