Hexabrachions are extracellular proteins expressed in certain tissues and at specific points in development. cDNA sequencing has revealed that they contain a region of repeats that are similar to the type III homology units of fibronectin. The corresponding region of fibronectin contains heparin- and DNA-binding domains. We have compared the heparin and DNA binding of hexabrachion secreted by the human glioblastoma cell line U87MG to that of fibronectin. Both proteins bound to heparin-agarose in low salt (0.05 M NaCl) buffers. Using linear salt gradients, hexabrachion was eluted from heparin prior to fibronectin. The addition of 5 mM CaCl₂ decreased the affinity of both proteins for heparin, but it had a greater effect upon the binding of fibronectin. Free heparin but not chondroitin sulfate inhibited the binding of both proteins to heparin-agarose. In addition, hexabrachion bound to DNA as fibronectin does, and this binding could be inhibited by heparin but not by chondroitin sulfate. Unlike fibronectin, hexabrachion did not bind to gelatin when samples containing both proteins were passed over gelatin-agarose, also indicating that there was no interaction between hexabrachion and fibronectin. In contrast to hexabrachion isolated from brain, the protein secreted by the human glioblastoma cell line U87MG does not bear the HNK-1 epitope which is on a carbohydrate that can mediate interactions between cells.

Hexabrachion is a descriptive name given to a large hexameric extracellular glycoprotein or a family of proteins (Erickson and Inglesias, 1984). It has a restricted tissue distribution and expression that depends on stage of development and is influenced by certain disease processes (Mackie et al., 1988; Chiquet-Ehrismann et al., 1986). It appears in rotary-shadowed electron micrographs as a six-armed structure with each arm believed to be a polypeptide chain of approximately 240 kDa. This glycoprotein has been described in chicken as myotendinous antigen (Chiquet and Fambrough, 1984, a and b), cytotactin (Grumet et al., 1985) and tenascin (Chiquet-Ehrismann et al., 1986), in mouse as J1 glycoprotein (Kruse et al., 1985), and in human as glioma mesenchymal extracellular matrix antigen (Bourdon et al., 1983), hexabrachion (Erickson and Inglesias, 1984), and gp 150/225 (Gulcher et al., 1986). Partial amino acid sequences of hexabrachion deduced from cDNA clones (Jones et al., 1988; Pearson et al., 1988; Gulcher et al., 1989) contain three different structural domains, two of which are composed of multiple repeating units. At the amino terminus, there is a series of high cysteine motifs such as those found in epidermal growth factor motifs. Following these is a series of 8 (in a smaller form) or 15 (in a larger form) repeats similar to the type III homology units of fibronectin. At the carboxyl terminus of the known sequence, there is a domain similar to the β and γ chains of fibrinogen. There is one gene for the human hexabrachion(s) and the difference between the various forms appears to be due to alternate splicing (Gulcher et al., 1989).

Hexabrachion isolated from brain (cytotactin, J1, and gp225) bears the HNK-1 epitope, a carbohydrate epitope that is also present on a number of proteins which have been shown to mediate adhesion between cells. Among them are the neural cell adhesion molecule, the neuron-glial cell adhesion molecule (Kruse et al., 1984), and the myelin-associated glycoprotein (McGarry et al., 1983). There are reasons to believe that the HNK-1 carbohydrate can by itself mediate adhesion between cells (Kunemund et al., 1988). However, not only does the hexabrachion contain the HNK-1 epitope, it has been shown to contain an RGD sequence through which it binds to fibroblasts (Friedlander et al., 1988). It also mediates adhesion between astrocytes and neurons in vitro (Gruet et al., 1985; Kruse et al., 1986). We looked for interactions of hexabrachions with heparin because of the striking sequence similarity of repeats in hexabrachion to the repeats in fibronectin that contain the high affinity heparin binding site. Here we report that hexabrachion secreted by a human glioblastoma cell line U87MG binds to heparin.

Diverse classes of proteins bind to heparin (Farooqui, 1980). Among others are growth factors, serum protease inhibitors, lipoproteins, and extracellular matrix components, some of which are also present in serum. A number of these proteins can be distinguished on the basis of the nature of their interactions with heparin. Many growth factors bind avidly to heparin requiring salt concentrations in excess of 0.5 M for elution while the binding of most other proteins is blocked at lower concentrations. The heparin binding of thrombospondin, von Willebrand factor, plasma histidine-rich glycoprotein, and human serum amyloid P is either Ca²⁺-dependent or -enhanced. We have compared the heparin binding properties of hexabrachion secreted by the human glioblastoma cell line U87MG to the heparin binding characteristics of fibronectin. We also looked for binding of hexabrachion to DNA because fibronectin binds to DNA through sites in the type III homology units that have their counterparts in the hexabrachion.

**MATERIALS AND METHODS**

**Cell Culture**—The human glioblastoma cell line U87MG (ATCC HTB 14) was grown in Dulbecco's Modified Eagle's Medium with 10% fetal calf serum. Medium was collected from cells in log growth.
Hexabrachion and other secreted and serum proteins were precipitated by the addition of ammonium sulfate to 40% saturation.

Affinity Chromatography—Heparin-agarose, gelatin-agarose, and double-stranded DNA-cellulose were purchased from Sigma. Samples were applied to heparin-agarose and DNA-cellulose columns at room temperature in the presence of 0.05 M NaCl, 0.5 mM EDTA, 10 mM triethanolamine, pH 7.5, except where noted. Bound proteins were eluted with increasing concentrations of NaCl. To test the specificity of binding to heparin-agarose and DNA-cellulose, 1.0 mg/ml solutions of heparin (porcine intestinal mucosa, Sigma), chondroitin sulfate A (bovine trachea, Sigma), chondroitin sulfate B (porcine mucosa, Calbiochem), and chondroitin sulfate C (shark cartilage, Calbiochem) were dialyzed against the starting buffer. The glycosaminoglycan solutions were then mixed with equal volumes of buffer or a sample and held for 1 h before application to heparin-agarose or DNA-cellulose. Dialysis reduced the concentrations of the glycans stocks 10-20-fold as estimated by a Safranin O assay (Lammi and Tammi, 1988) making the final concentrations used for the inhibition studies 25-50 µg/ml. Gelatin affinity chromatography was done in the presence of 0.1 M NaCl, 0.5 mM EDTA, 0.02 M triethanolamine, pH 7.5. Bound proteins were eluted from gelatin with 2 M urea. All unbound and eluted column fractions were dialyzed and lyophilized before sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE).

Electrophoresis and Blotting—SDS-PAGE was done in 0.75-mm vertical slab gels using the buffer and detergent conditions of Laemmli (1970). The running gels contained 4.8% acrylamide (acrylamide/bisacrylamide, 40:1.5). Gels were stained with silver (Merrill et al., 1984), or the proteins were electrophoretically transferred to nitrocellulose (BA 85, Schleicher and Schuell) in 20% methanol, 20 mM Tris, pH 7.5. The nitrocellulose blots were blocked by incubation with 1.5% bovine serum albumin in Tris-buffered saline containing 0.15 M NaCl, 0.05 M Tris, pH 7.6. All further washes and incubations were done with Tris-buffered saline containing 0.05% Tween 20.

Antigens were detected on the blots after 1-h incubations with primary antibody and peroxidase-conjugated secondary antibody followed in each case by a minimum of three washes. The rat antihexabrachion polyclonal antibody was prepared using protein isolated from human brain (Gulcher et al., 1986). The HNK-1 epitope was detected with a supernatant from a mouse hybridoma line (ATCC V. Lightner of Duke University). Antibodies to fibronectin and hexabrachion were detected using an arrowhead monoclonal antibody and an anti-human fibronectin antiserum (3). Hexabrachion is marked with an arrowhead and myelin-associated glycoprotein with an asterisk. It is obvious that there is no detectable HNK-1 epitope on the hexabrachion secreted by U87MG and there is no fibronectin among the brain proteins.

RESULTS

When grown in vitro with 10% fetal calf serum, the human glioblastoma cell line U87MG secreted hexabrachion into the medium as has been described for a number of glial tumor cell lines (Bourdon et al., 1983). Following ammonium sulfate precipitation and Western blotting, our polyclonal rat sera reacted with an 180-kDa and a 240-kDa protein isolated from U87MG media (B) were separated by SDS-PAGE and transferred on to nitrocellulose membranes. Strips were stained using: antibody to hexabrachion (1), monoclonal antibody HNK-1 (2), or an anti-human fibronectin antiserum (3). Hexabrachion is marked with an arrowhead and myelin-associated glycoprotein with an asterisk. It is obvious that there is no detectable HNK-1 epitope on the hexabrachion secreted by U87MG and there is no fibronectin among the brain proteins.

![Fig. 1. Antibody staining of hexabrachion from brain and the human glioblastoma cell line U87MG. An ammonium sulfate precipitate from soluble brain protein (A) and proteins precipitated from U87MG media (B) were separated by SDS-PAGE and transferred on to nitrocellulose membranes. Strips were stained using: antibody to hexabrachion (1), monoclonal antibody HNK-1 (2), or an anti-human fibronectin antiserum (3). Hexabrachion is marked with an arrowhead and myelin-associated glycoprotein with an asterisk. It is obvious that there is no detectable HNK-1 epitope on the hexabrachion secreted by U87MG and there is no fibronectin among the brain proteins.](image)

1 The abbreviations used are: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; RGD, arginine, glycine, aspartic acid.

Medium from U87MG in a low salt buffer (0.05 M NaCl, 0.01 M triethanolamine, pH 7.5, 0.5 mM EDTA) was passed over heparin-agarose affinity columns to look for the binding of hexabrachion to heparin. Most medium proteins (>75%) were not retained by the affinity matrix but step elution with 0.1, 0.2, 0.4, and 1.4 M NaCl released distinct sets of proteins visible in silver-stained gels (see Fig. 2). The majority of the bound proteins were eluted by the first three steps. Concentrations above 0.4 M NaCl did not release additional proteins. Antibody-stained Western blots indicated that most of the hexabrachion and fibronectin was bound to and eluted from heparin-agarose. Nearly all of both proteins eluted with the 0.4 M NaCl step and could be identified as distinct bands on the silver-stained gels. In some experiments, parts of the fibronectin and hexabrachion were released from heparin-agarose with 0.2 M salt. When the unbound proteins were applied to fresh heparin columns, an additional small amount of hexabrachion and fibronectin bound to heparin-agarose, but the bulk of proteins that were unbound initially, remained unbound. Rechromatography of bound and eluted hexabrachion and fibronectin resulted in their binding to heparin-agarose and elution at the salt concentrations that were effective initially.

Passage of media in 0.1 M NaCl over gelatin-agarose resulted in the binding of fibronectin. Subsequent elution of the gelatin columns with 2 M urea yielded nearly pure fibronectin.
Binding of Hexabrachions to Heparin and DNA

A

FIG. 2. Heparin chromatography of U87MG media proteins. Proteins precipitated from culture media were applied to heparin-agarose in low salt buffer, and bound proteins were eluted with the indicated molarities of NaCl. Original (ori), unbound (unb), and eluted fractions were separated by SDS-PAGE, and the gels were silver-stained (A) or were blotted and stained using anti-hexabrachion (B) or anti-fibronectin (C) sera. The location of hexabrachion (single arrowhead) and fibronectin (double arrowhead) in the silver-stained gels is marked. Both hexabrachion and fibronectin bound to heparin-agarose and were eluted with 0.2 and 0.4 M NaCl.

B

FIG. 3. Specificity of glycosaminoglycan binding. Media proteins were applied to heparin-agarose in the presence of buffer only (Buf), buffer plus heparin (Hep), or buffer plus chondroitin sulfate A (CS). Bound proteins were then eluted with 1.4 M NaCl. Blots of the original (o) and the respective unbound (u) and eluted (e) samples were stained for hexabrachion (A) and fibronectin (B). Heparin, but not chondroitin sulfate, inhibited binding of both hexabrachion and fibronectin to heparin-agarose. The lanes with eluted proteins appear narrower due to the presence of the added glycosaminoglycan in the adjacent unbound samples.

C

but no hexabrachion. The results were the same whether 5 mM EDTA or CaCl₂ was included. The fact that no hexabrachion was retained with fibronectin on the gelatin columns indicates that there is no interaction between hexabrachion and fibronectin. The failure of hexabrachion to bind to gelatin is consistent with the fact that the gelatin binding site of fibronectin has been mapped to a region which includes type II homology units. Hexabrachion does not contain domains that have sequences similar to the type II homology units of fibronectin.

The binding of hexabrachion to heparin could be inhibited by soluble heparin but not by chondroitin sulfates A, B, or C (Fig. 3), which indicates that there is some specificity in this interaction. In fact, less hexabrachion and fibronectin were unbound in the presence of chondroitin sulfates A and C than in buffer only (not shown).

We compared the relative heparin affinities of hexabrachion and fibronectin by eluting the proteins from heparin-agarose with a linear gradient of salt. Fig. 4 shows that hexabrachion eluted ahead of fibronectin. The difference in salt concentrations for peak elution of hexabrachion versus fibronectin was 0.1 M, approximately 0.2 versus 0.3 M.

The binding of intact fibronectin to heparin is inhibited by calcium ions (Siri et al., 1986). We compared the binding of hexabrachion and fibronectin to heparin in the presence of 5 mM CaCl₂ to the binding in buffers containing 5 mM EDTA (see Fig. 5). While the two proteins bound to heparin-agarose in 0.05 M NaCl regardless of whether calcium or chelator was included in the solution, they both had less affinity for heparin in the presence of calcium. In buffers containing 5 mM EDTA,

FIG. 4. Elution of heparin-bound media proteins with a linear NaCl gradient. The sample in lane 1 is a pool of the first 10 fractions which eluted from heparin-agarose with a linear salt gradient. Lanes 2-8 are consecutive pools of three fractions each. The blots are stained for hexabrachion (A) and fibronectin (B). Peak elution of hexabrachion was in pool 3, while fibronectin was released less readily with maximal elution in pool 5, representing a NaCl difference of 0.1 M.

FIG. 5. Effect of EDTA and CaCl₂ on heparin binding. Heparin-agarose chromatography of media samples was done as before with the addition of either 5 mM EDTA (A, C) or 5 mM CaCl₂ (B, D). Blots of eluted samples were stained for hexabrachion (A, B) or fibronectin (C, D). Fibronectin and fibronectin eluted from heparin-agarose at lower salt concentrations in the presence of CaCl₂. Fibronectin showed a greater decrease in heparin affinity than did hexabrachion.
fibronectin was again found to have a greater affinity for heparin than did hexabrachion although peaks of both proteins eluted in the step gradients at 0.4 M NaCl. When the divalent cation was included, fibronectin eluted from heparinagarose at 0.1 and 0.2 M salt and hexabrachion at 0.2 M. While hexabrachion binds more avidly than fibronectin when CaCl₂ is introduced, fibronectin has a greater affinity than hexabrachion for heparin in the presence of EDTA. However, these differences are small.

Proteolytic fragments of fibronectin that bind to heparin and contain type III homology units also have affinity for DNA. Therefore, we looked to see whether the hexabrachion binds to DNA-cellulose (Fig. 6). Indeed, while nearly all proteins in the conditioned media were retarded by DNA, both hexabrachion and fibronectin bound. Both proteins eluted at the same concentrations of NaCl; however, the DNA affinity of hexabrachion seemed less than that of fibronectin. Hexabrachion and fibronectin were among the few proteins that bound to the DNA-cellulose, and, if Fig. 6 is compared to Fig. 2, it would appear that this matrix may provide a more effective means of purifying hexabrachions than does heparinagarose. The proteins probably bind to DNA through the same sites that bind to heparin for when media proteins were applied to DNA-cellulose in the presence of dialyzed glycans, the binding of both fibronectin and hexabrachion was inhibited by heparin, but chondroitin sulfate A had no effect (data not shown). The apparent equivalency of DNA and heparin is consistent with the effectiveness of heparin affinity media in the purification of enzymes of nucleic acid metabolism (Farooqui, 1980).

**DISCUSSION**

While there are subtle differences, the binding of hexabrachion to heparin and DNA is similar to the binding of fibronectin to these same molecules. Both proteins are eluted from heparin-agarose between 0.2 and 0.3 M NaCl, unlike the heparin-binding growth factors which require concentrations in excess of 0.5 M for elution. The heparin binding of both hexabrachion and fibronectin is decreased in the presence of Ca²⁺ in contrast to the binding of several human serum components (e.g. plasma histidine-rich glycoprotein, serum amyloid P, and von Willebrand factor) whose binding is calcium-dependent. Intact hexabrachion and fibronectin may bind to DNA through the same sites that bind to heparin for we have found that heparin but not chondroitin sulfate inhibits the binding of both proteins to DNA-cellulose. Previous studies have not shown inhibition of fibronectin binding to DNA by heparin (McMaster and Zardi, 1982). However, it has been shown that DNA weakly inhibits the binding of heparin to fibronectin-gelatin complexes (Ruoslahti et al., 1979). Because of the similarities between fibronectin and hexabrachion in both primary structure and binding to heparin and DNA, the binding site(s) in hexabrachion are also likely to be within the region of type III homology units.

Proteolytic cleavage of fibronectin has revealed that there are three distinct binding sites for heparin and DNA within the type III homology units which can be distinguished by their relative affinities and calcium sensitivities (Hayashi and Yarmada, 1982). Peptide mapping of specifically tailored fusion proteins will be required to determine whether the binding properties of hexabrachion are attributable to interaction through one or more sites. No difference was seen between the 240-kDa and 190-kDa hexabrachions in any of the binding experiments with heparin, DNA, or gelatin. The two proteins are encoded by two different mRNAs formed through alternative splicing of one primary transcript. The larger polypeptide contains seven additional type III homology units. Hence, the heparin- and DNA-binding regions are within the common parts of these two polypeptides.

It is not likely that a unique amino acid sequence, such as the RGD cell binding site, will be identified that confers heparin binding. The fact that there are multiple mechanisms of heparin binding is indicated by the vast differences in conditions allowing for the binding/elution of the various proteins. However, there is little doubt that the binding sites will contain positively charged residues, appropriately spaced to align with charges on the heparin polymers. Synthetic peptides representing positively charged regions of laminin and fibronectin which are expected to bind to heparin have been shown to bind heparin and to promote cell adhesion (Charonis et al., 1988; McCarthy et al., 1988). However, a point which must be considered in the analysis of interactions of peptides with heparin or other ligands is that the reactivity of the peptides is not always an indication of the reaction of the intact proteins from which they are derived. Fibronectin provides an example of this. Following proteolysis, four different heparin-binding sites have been identified differing in their relative affinities and calcium sensitivities. The heparin affinity of intact fibronectin is comparable to that of high affinity site on a carboxyl-terminal peptide but unlike this site, the heparin binding of the intact protein is inhibited by calcium just as one of the low affinity amino-terminal peptides.

Although fibronectin has been shown to bind to heparin, its natural ligand in the pericellular matrix is likely to be high sulfated heparan sulfate. Others have shown that intact fibronectin at physiologic ionic strength only binds to heparinagarose but not to heparan sulfate, dermatan sulfate, or to chondroitin sulfate A and C. However, at low ionic strength, fibronectin binds not only to heparin but also to highly sulfated heparan sulfate (not to less sulfated forms) (Sekiguchi et al., 1983). Ogamo et al. (1985) also demonstrated that a

![FIG. 6. DNA-cellulose chromatography of U87MG media.](image-url)
Binding of Hexabrachions to Heparin and DNA

higher degree of sulfation and greater molecular size results in greater affinity for fibronectin. Because hexabrachion and fibronectin showed similar binding to heparin-agarose in our studies, we believe it is likely that hexabrachion also binds to heparan sulfate and in a manner similar to fibronectin. However, this has yet to be demonstrated.

The suggestion of Hoffman and Edelman (1987) that a chondroitin sulfate proteoglycan may be a natural ligand for the hexabrachion in chicken brain is not inconsistent with our results that chondroitin sulfate is ineffective in blocking binding to heparin-agarose. They found that chondroitinase ABC digestion caused an apparent increase in interaction between the protein and the proteoglycan which could be explained by a greater accessibility of heparan sulfate on the proteoglycan following removal of the chondroitin chains.

Two groups have reported an interaction between hexabrachion and fibronectin (Hoffman and Edelman, 1987; Chiquet-Ehrismann et al., 1988). We were unable to detect any such intermolecular binding when we passed media containing both proteins over gelatin-agarose columns. Most of the fibronectin bound to the gelatin, but no hexabrachion was recovered from the columns even after subsequent passage of the same samples over the fibronectin-heparin-agarose complexes. This apparent discrepancy concerning the binding of hexabrachion to fibronectin may be related to differences in the hexabrachions and/or to differences in the methods used. The other groups used chicken hexabrachion, we used human. They could also have been working with different forms of hexabrachion as multiple forms exist. Alternatively, our results may be due to the possibility that fibronectin binds gelatin and hexabrachion through the same binding site and has higher affinity for gelatin than it has for hexabrachion.

The presence or absence of HNK-1 carbohydrate has no effect on binding to heparin. Hexabrachion secreted by this human glioblastoma cell line does not carry the HNK-1 epitope but we have previously demonstrated that the HNK-1 epitope is present on the protein in extracts from brain. Both forms bind to heparin-agarose. Hoffman et al. (1988) also found that the HNK-1 carbohydrate had no effect on cytotactin-proteoglycan interaction. It is of interest here that the HNK-1 carbohydrate has been shown to mediate adhesion between cells (Kunemund et al., 1988).

Preparation of fusion proteins containing specific portions of the hexabrachion molecule may make it possible to determine which portion(s) of the sequence are crucial for binding to heparin and DNA. The presence of a binding site in an alternatively spliced region could serve to regulate the function of the molecule. The heparin-binding site is likely to grant the multimeric hexabrachion the ability to bridge between cells that contain heparan sulfate proteoglycans on their surfaces and between these cells and extracellular matrix. Hence, it is likely that the hexabrachion may serve as an adhesion molecule if not for anything else than the heparin affinity. However, the hexabrachion from brain could mediate adhesion through three different mechanisms, the RGD sequence, the HNK-1 epitope, and the heparin-binding.

Acknowledgment—We would like to thank Concepcion Roman-Blanco for expert technical assistance.

REFERENCES

Bourdon, M. A., Wikstrand, C. J., Forthmayr, H., Matthews, T. J., and Bigner, D. D. (1983) Cancer Res. 43, 2706-2805
Charonis, A. S., Skubitz, A. P. N., Kollaros, G. G., Reger, L. A., Dege, J., Vogel, A. M., Wohlueter, R., and Furcht, L. T. (1988) J. Cell Biol. 107, 1253-1260
Chiquet, M., and Fambrough, D. M. (1984a) J. Cell Biol. 98, 1926-1936
Chiquet, M., and Fambrough, D. M. (1984b) J. Cell Biol. 98, 1937-1946
Chiquet-Ehrismann, R., Mackie, E. J., Pearson, C. A., and Sakakura, T. (1986) Cell 47, 131-139
Chiquet-Ehrismann, R., Kalla, P., Pearson, C. A., Beck, K., and Chiquet, M. (1988) Cell 53, 383-390
Erickson, H. P., and Inglesias, J. L. (1984) Nature 311, 267-269
Farooqui, A. A. (1980) J. Chromatogr. 184, 335-345
Friedlander, D. R., Hoffman, S., and Edelman, G. M. (1988) J. Cell Biol. 107, 2292-2340
Grumet, M., Hoffman, S., Crossin, K. L., and Edelman, G. M. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8075-8079
Gulcher, J. R., Marton, L. S., and Stefansson, K. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2113-2122
Gulcher, J. R., Nies, D. E., Marton, L. S., and Stefansson, K. A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1588-1592
Hayashi, M., and Yamada, K. M. (1982) J. Biol. Chem. 257, 5263-5267
Hoffman, S., and Edelman, G. M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2523-2527
Hoffman, S., Crossin, K. L., and Edelman, G. M. (1988) J. Cell Biol. 106, 519-532
Jones, F. S., Burgoon, M. P., Hoffman, S., Crossin, K. L., Cunningham, B. A., and Edelman, G. M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2186-2190
Kruse, J., Mailhammer, R., Wernicke, H., Faisans, A., Sommer, I., Goridis, C., and Schizcerz, M. (1984) Nature 311, 153-155
Kruse, J., Keilhauer, G., Faisans, A., Timpl, R., and Schachner, M. (1985) Nature 316, 146-148
Kunemund, V., Jungfalwa, F. B., Fischer, G., Chou, D. K. H., Keilhauer, G., and Schachner, M. (1988) J. Cell Biol. 106, 213-223
Laemmli, U. K. (1970) Nature 227, 680-685
Lamm, M., and Tammi, M. (1988) Anal. Biochem. 168, 352-357
Mackie, E. J., Halfter, W., and Leverani, D. (1988) J. Cell Biol. 107, 2757-2767
McCarthy, J. B., Chelberg, M. K., Mickelson, D. J., and Furcht, L. T. (1988) Biochemistry 27, 1380-1388
McCarty, R. C., Helfand, S. L., Quaries, R. H., and Roder, J. C. (1983) Nature 306, 376-378
McMaster, G. K., and Zardi, L. (1982) Biochem. Biophys. Res. Commun. 107, 609-617
Merrick, C. R., Goldman, D., and Van Keuren, M. L. (1984) Methods Enzymol. 104, 441-447
Ogamo, A., Nagai, A., and Nagasawa, K. (1985) Biochim. Biophys. Acta 841, 30-41
Pearson, C. A., Pearson, D., Shibahara, S., Hofsteenge, J., and Chiquet-Ehrissmann, R. (1988) EMBO J. 7, 2977-2982
Ruoslahti, E., Pekkala, A., and Engvall, E. (1979) FEBS Lett. 107, 51-54
Sekiguchi, K., Ishikomori, S., Funahashi, M., Matsutomo, I., and Seno, N. (1983) J. Biol. Chem. 258, 14359-14365
Siri, A., Balza, E., Carnemolin, B., Castellani, P., Boris, L., and Zardi, L. (1988) Eur. J. Biochem. 154, 533-538

2 L. S. Marton, J. R. Gulcher, and K. Stefansson, unpublished observations.