The EMBO Journal vol.14 no.8 pp.1674–1679, 1995

Protein H—a bacterial surface protein with affinity for both immunoglobulin and fibronectin type III domains

Inga-Maria Frick, Kathryn L.Crossin[1], Gerald M.Edelman[1] and Lars Björck[2]

Department of Medical and Physiological Chemistry, Section for Molecular Pathogenesis, Lund University, PO Box 94, S-221 00 Lund, Sweden and [1]Department of Neurobiology, Scripps Research Institute, La Jolla, California 92037, USA

[2]Corresponding author

Communicated by S.Normark

Several bacterial species express surface proteins with affinity for the constant region (Fc) of immunoglobulin (Ig) G. The biological consequences of the interaction with IgG are poorly understood but it has been demonstrated that genes encoding different IgG Fc-binding proteins have undergone convergent evolution, suggesting that these surface molecules are connected with essential microbial functions. One of the molecules, protein H, is present in some strains of *Streptococcus pyogenes*, the most significant streptococcal species in clinical medicine. In contrast to other Ig-binding bacterial proteins tested, protein H was found to interact also with the neural cell adhesion molecule (N-CAM), a eukaryotic cell surface glycoprotein mediating homo- and heterophilic cell–cell interactions. The affinity for the interaction between protein H and N-CAM was 1.6×10^8/M and the binding site on protein H was mapped to the NH_2-terminal 80 amino acid residues. N-CAM and IgG are both members of the Ig superfamily and analogous to N-CAM, IgG binds to the NH_2-terminal part of protein H. However, the binding sites for the two proteins were found to be separate, an unexpected result which was explained by the observation that the fibronectin type III (FNIII) domains and not the Ig-like domains of N-CAM are responsible for the interaction with protein H. Thus, the binding of N-CAM to protein H was blocked with fibronectin but not with IgG. Moreover, apart from fibronectin itself and N-CAM, fragments of fibronectin and the matrix protein cytotactin/tenascin containing FNIII domains also showed affinity for protein H. Ig and FNIII domains are two of the most widely spread building blocks in eukaryotic proteins. The demonstration that a bacterial surface protein has developed separate binding sites for these domains emphasizes the power of microbial adaptation.

Key words: evolution/fibronectin/immunoglobulin/streptococci

Introduction

Several bacterial proteins with affinity for the constant (Fc) region of immunoglobulin (Ig) G antibodies have been identified and characterized (reviewed in Boyle, 1990; Kehoe, 1994). Among these, protein A of *Staphylococcus aureus* (Forsgren and Sjöquist, 1966) and protein G of group C and G streptococci (Björck and Kronvall, 1984; Reis *et al.*, 1984) are the best known. Many strains of *Streptococcus pyogenes*, the most important streptococcal species in clinical medicine, also have IgG Fc-binding activity (Kronvall, 1973). The primary structures of some of the molecules responsible for this IgG Fc-binding activity have been determined (Heath and Cleary, 1989; Gomi *et al.*, 1990; Raeder *et al.*, 1992; Stenberg *et al.*, 1992; Retnoningrum *et al.*, 1993) and sequence comparisons reveal extensive heterogeneity in the NH_2-terminal regions. Nevertheless, this group of proteins has similar overall structure. They are elongated rod-like molecules with repeated domains and a common COOH-terminal cell wall-associated region, and they belong to the M protein family (Fischetti, 1989; Kehoe, 1994). M proteins are regarded as major virulence determinants due to their anti-phagocytic property, whereas the role for IgG Fc-binding bacterial proteins in pathogenicity and virulence remains unclear. However, studies on the structure and evolution of the IgG Fc-binding regions of protein A, protein G and one of the *S.pyogenes* M-like proteins called protein H (Åkesson *et al.*, 1990), revealed that the three proteins have evolved convergently; despite a lack of sequence homology in their binding regions they all interact with the same region of IgG Fc (Frick *et al.*, 1992). These findings suggest that IgG Fc-binding proteins participate in essential microbial functions adding selective advantages to the bacteria.

The neural cell adhesion molecule (N-CAM) is a large transmembrane glycoprotein and a member of the Ig superfamily (for references see Edelman and Crossin, 1991). The NH_2-terminal extracellular part consists of five homologous Ig-like domains (Cunningham *et al.*, 1987) followed by two fibronectin type III (FNIII) domains (Hemperly *et al.*, 1986b). Different isoforms of N-CAM are generated by alternative splicing (Hemperly *et al.*, 1986a), but the extracellular region is invariant in the three major forms. N-CAM mediates homo- and heterophilic cell–cell interactions and is an important morphoregulatory molecule, especially in the central nervous system. Inactivation of the N-CAM gene in mice reduces the size of the olfactory bulb and affects spatial learning (Cremer *et al.*, 1994).

The presence of Ig-like domains in N-CAM originally stimulated this study, in which a possible interaction between N-CAM and various Ig-binding bacterial proteins was investigated. Only protein H, the IgG Fc-binding protein of *S.pyogenes*, was found to have affinity for N-CAM. Further analysis of the interaction between these two proteins, schematically depicted in Figure 1, demonstrated that the FNIII domains and not the Ig-like domains

© Oxford University Press

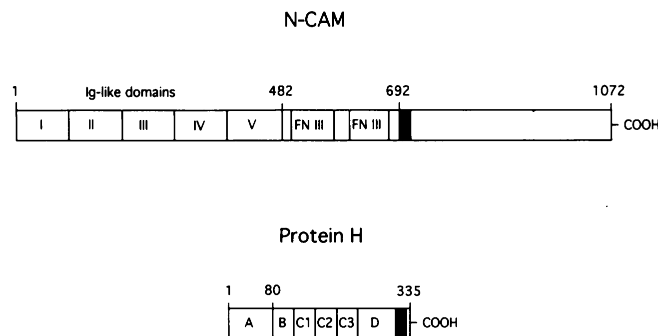


Fig. 1. Schematic representation of N-CAM and streptococcal protein H. The model of N-CAM according to Cunningham *et al.* (1987) shows the extracellular region with its five homologous Ig-like domains (I-V) followed by the FNIII-like domains. In protein H, the IgG Fc-binding is in the A–B domains, which are followed by three albumin-binding homologous C repeats (Frick *et al.*, 1994). Membrane spanning regions are shaded. Numbers in the figure refer to amino acid residue positions.

were responsible for the binding of protein H. Subsequent studies on the interaction between the ubiquitous FNIII domain (Bork and Doolittle, 1992) and protein H revealed that protein H could interact also with the FNIII domains from other cell and substrate adhesion proteins.

Results

Streptococcal protein H has affinity for N-CAM

Bacterial Ig-binding proteins, including staphylococcal protein A, streptococcal proteins G and H, and protein L from the anaerobic bacterial species *Peptostreptococcus magnus*, were applied in slots to PVDF membranes and probed with ^{125}I-labeled embryonic N-CAM and IgG, respectively. As expected all bacterial proteins reacted with IgG, whereas only protein H showed affinity for N-CAM (Figure 2A). Adult N-CAM contains less sialic acid than the embryonic form (Rothbard *et al.*, 1982) but when used as the probe the same result was obtained with adult N-CAM (not shown). Various N-CAM preparations, both adult and embryonic, were now applied to PVDF filters and tested against ^{125}I-labeled streptococcal IgG Fc-binding proteins (proteins G and H). Again, only protein H was reactive and there was no significant difference between the various N-CAM preparations. In the subsequent studies on the interaction with protein H, embryonic N-CAM was used.

As shown in Figure 2B, [^{125}I]N-CAM was bound to protein H immobilized on Sepharose and 75% of the bound material could be eluted from the column with KSCN. In contrast, 90% of the [^{125}I]N-CAM applied to a protein G column passed through the column. SDS–PAGE demonstrated that the material bound and eluted from protein H-Sepharose was identical to the run-through material from protein G–Sepharose (Figure 2B, right). The interaction between N-CAM and protein H was also measured quantitatively and the affinity constant was determined to 1.6×10^8/M in Scatchard plots (Figure 3). Plots were linear, suggesting only one kind of interaction between the two proteins.

Protein H has separate binding sites for IgGFc and N-CAM

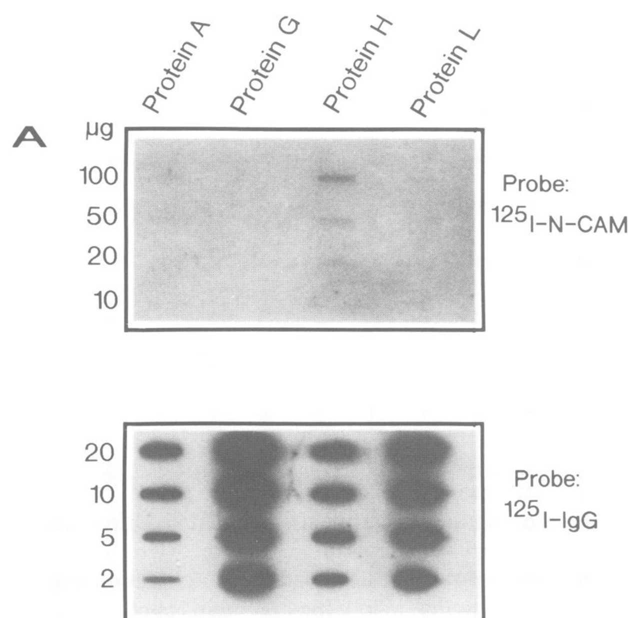
The IgG Fc-binding region of protein H is located in the NH_2-terminal AB region (see Figure 1), and DNA fragments corresponding to AB and A have been expressed to produce these peptides in *Escherichia coli* (Frick *et al.*, 1994). Slot binding experiments revealed no difference in binding activity for protein H and the two fragments (Figure 4A, right) suggesting that the A domain represents the N-CAM-binding region. Intact protein H and fragments AB and A were also tested in a competitive binding assay. In these experiments protein H–Sepharose was incubated with ^{125}I-labeled N-CAM and various amounts of unlabeled protein H, fragment AB or fragment A. Fragment AB blocked the binding of N-CAM to protein H almost as efficiently as intact protein H, whereas about 300-fold more of fragment A was needed to obtain 50% inhibition (Figure 4A, left). Protein H is known to form multimers in solution with higher affinity for IgG than the monomeric form (Åkerström *et al.*, 1992). Recent work has demonstrated that the B domain participates in the multimerization of protein H in solution (I.-M.Frick and L.Björck, unpublished) which could explain why fragment A appears to have higher affinity for N-CAM in relation to protein H and the AB fragment when bound to a membrane.

To map the binding site on protein H and N-CAM further, we performed additional inhibition studies. Despite the observation that both N-CAM and IgG bind to the NH_2-terminal region of protein H and that both contain Ig domains, large molar excess of IgG could not block the binding of N-CAM to protein H (Figure 4B). Furthermore, a synthetic peptide (25 amino acid residues long) constructed from a sequence in the B domain involved in IgG Fc-binding (Frick *et al.*, 1994) did not interfere with N-CAM binding to protein H (not shown). These results indicate that IgG Fc and N-CAM interact with different sites in protein H.

Protein H interacts with fibronectin type III domains

The findings described above suggested that regions other than the Ig-like domains of N-CAM were responsible for the interaction with protein H. This notion was also supported by the observation that ICAM-1, another cell adhesion molecule and member of the Ig superfamily (Dustin *et al.*, 1986; Staunton *et al.*, 1988) showed no affinity for protein H (I.-M.Frick, T.Springer and L.Björck, unpublished). We therefore focused on the possibility that the FNIII domains of N-CAM (see Figure 1) may interact with protein H.

Human plasma fibronectin (for a fibronectin review see



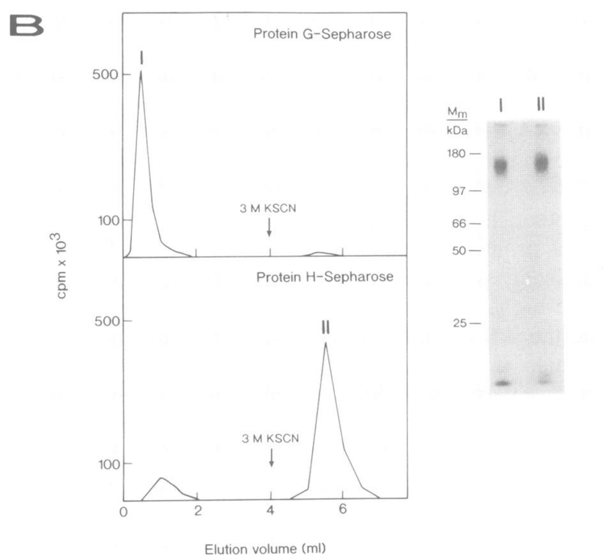


Fig. 2. Binding of N-CAM to Ig-binding bacterial proteins. (**A**) Various amounts of proteins A, G, H and L were applied in slots to PVDF membranes and probed with ^{125}I-labeled N-CAM. ^{125}I-labeled IgG was used as a positive control. Filters were incubated with 2×10^5 c.p.m./ml (10–30 ng protein/ml) for 3 h and autoradiographed for 6 days. (**B**) ^{125}I-labeled N-CAM was applied to columns of protein G− or protein H−Sepharose. Bound radioactivity was eluted with 3 M KSCN. The radioactivity of fractions was determined. Fractions I and II were also analysed by SDS−PAGE (right).

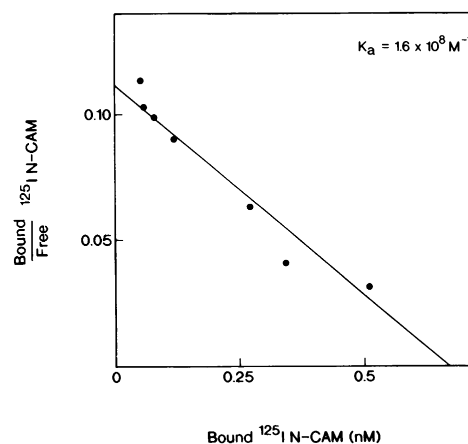


Fig. 3. Scatchard plot for the reaction between ^{125}I-labeled N-CAM and immobilized protein H. Protein H coupled to Sepharose 4B was mixed with a constant amount of [^{125}I]N-CAM and different amounts of unlabeled N-CAM. The radioactivity of bound N-CAM was measured and concentrations of bound and free N-CAM were calculated and plotted.

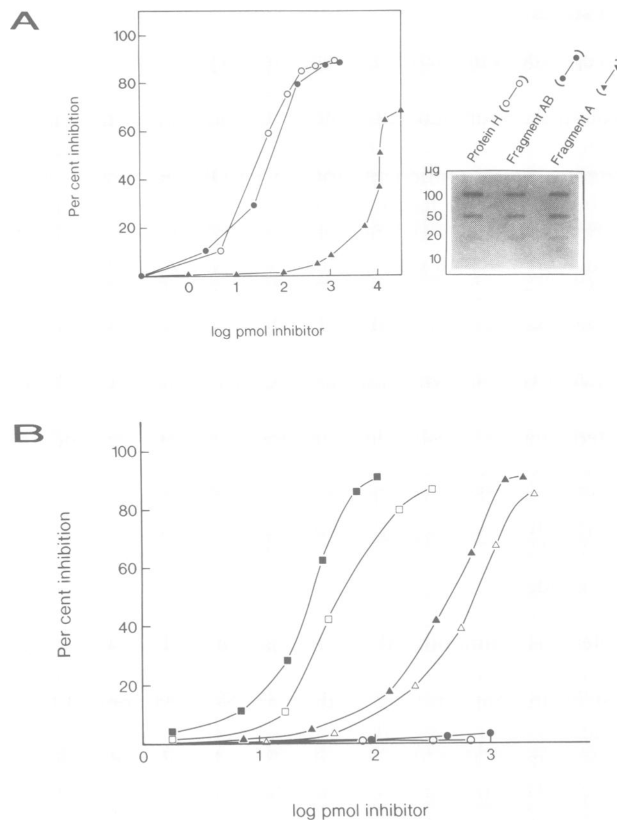


Fig. 4. (**A**) Mapping of the N-CAM-binding region of protein H. The binding of ^{125}I-labeled N-CAM to protein H−Sepharose (left) was inhibited with different amounts of unlabeled protein H (○) or with fragments AB (●) and A (▲) of protein H. The proteins were also applied to a PVDF filter (right) which was incubated with ^{125}I-labeled N-CAM (2×10^5 c.p.m./ml) for 3 h and autoradiographed for 6 days. (**B**) Identification of the protein H-interacting region of N-CAM. Binding of ^{125}I-labeled N-CAM to protein H−Sepharose was inhibited with different amounts of unlabeled IgG (○), fibronectin (■), a 120 kDa fibronectin fragment (□) and two cytotactin/tenascin fragments, FN1 (▲) and FN3 (△). The cytotactin/tenascin fragments were expressed as fusion proteins with glutathione S-transferase (GST). GST alone was also tested (●).

Hynes, 1990) contains 15 FNIII domains and was found to block the binding of N-CAM to protein H. Equally efficient in blocking this interaction was a 120 kDa fibronectin fragment comprising 11 FNIII domains (Figure 4B). Inhibition of binding was also obtained with two additional FNIII-containing fragments from the extracellular matrix protein cytotactin/tenascin (FN1 and FN3) expressed in fusion proteins together with glutathione S-transferase (GST; Prieto *et al.*, 1992). Analogous to

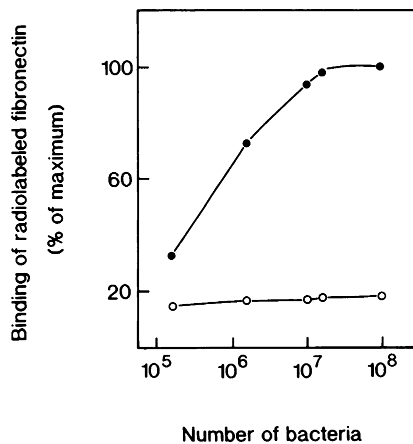


Fig. 5. Binding of fibronectin to AP1 streptococci. The binding of ^{125}I-labeled fibronectin to AP1 streptococci (●) and cysteine proteinase-treated AP1 streptococci (○) at different bacterial concentrations was measured. 10^4 c.p.m. corresponding to ~2 ng was added to the bacteria. Maximum binding varied between 15 and 20% of added radioactivity.

fibronectin and the 120 kDa fragment of fibronectin, these fusion proteins could inhibit the interaction of N-CAM with protein H, although the affinity for protein H was lower (FN1 and FN3 contains only four and two complete FNIII domains, respectively). The GST portion of FN1 and FN3 as well as IgG showed no inhibitory activity (Figure 4B). This was also true for an Arg-Gly-Asp (RGD) peptide, despite the fact that very large quantities (up to 600 nmol) of the peptide were used in the competitive binding assay. Taken together, the results demonstrate that protein H interacts with FNIII domains and that the binding is independent of the RGD sequence.

Fibronectin was also tested in direct binding experiments with protein H. Like N-CAM, radiolabeled fibronectin showed affinity for protein H immobilized on PVDF membranes and Sepharose (not shown). Protein H-expressing *S.pyogenes* binds IgG efficiently (Åkesson *et al.*, 1990, 1994). *S.pyogenes* also produces a cysteine proteinase (Elliott, 1945) which is connected with essential functions in these bacteria (Björck *et al.*, 1989). When incubated together with the activated cysteine proteinase at concentrations >2 μg/ml 10% bacterial suspension, all IgG-binding activity is removed from the bacterial surface (A.Berge and L.Björck, submitted). As demonstrated in Figure 5, streptococci of the AP1 strain pretreated with the proteinase also lost their fibronectin-binding activity indicating that protein H also at the bacterial surface has affinity for fibronectin.

Discussion

Proteins A, G and H are bacterial surface proteins interacting with the Cγ2–Cγ3 interface region of IgG (Stone *et al.*, 1989; Frick *et al.*, 1992). Therefore, the initial observation that protein H, but not proteins A and G, bound to N-CAM indicated that perhaps regions other than the Ig-like domains of N-CAM were responsible for the interaction. Nevertheless it was surprising to find that the FNIII domains of N-CAM appeared to mediate the interaction with protein H. Pathogenic bacteria of many different species express surface proteins with affinity for fibronectin (reviewed by Westerlund and Korhonen, 1993). In cases where the interaction has been mapped, the NH_2-terminal 29 kDa part of fibronectin has mostly been claimed to be the binding region; there is no previous report of FNIII domains participating in the interaction with bacterial surface proteins.

The three-dimensional structure of FNIII domains has been determined (Leahy *et al.*, 1992; Dickinson *et al.*, 1994; Huber *et al.*, 1994) and in relation to this study it is interesting to note that the folding topology was found to be similar to that of Ig constant domains, except for the sheet switching of one of the β strands (Leahy *et al.*, 1992). Although three-dimensionally related it should be emphasized that the pattern of conserved hydrophobic residues is distinctly different between FNIII and Ig constant domains (Leahy *et al.*, 1992). Our observation that protein H has different binding sites for Ig and FNIII domains is therefore not in conflict with previously published structural data. Some FNIII domains in extracellular matrix proteins contain the RGD sequence which mediates cell adhesion by binding to integrins (Pierschbacher and Ruoslahti, 1984). However, the interaction between protein H and FNIII domains appears independent of RGD, as a large molar excess of this peptide could not block the binding of N-CAM to protein H. Moreover, one of the cytotactin/tenascin fragments (FN3) used in this study is devoid of RGD, but still inhibited the N-CAM–protein H interaction similarly to fragment FN1 which contains the RGD sequence.

Protein H belongs to the M protein family (see Fischetti, 1989; Kehoe, 1994), a large family of streptococcal surface proteins showing considerable homology in their COOH-terminal regions, whereas the NH_2-terminal parts contain unique sequences. None of these proteins have previously been shown to interact with fibronectin. However, a fibronectin-binding protein, called protein F, was recently identified and found to be an important adhesin in strains of *S.pyogenes* (Hanski and Caparon, 1992). Among eight strains analysed, only the strain used in this study (AP1) was devoid of the gene encoding protein F. The AP1 strain was also reported not to bind fibronectin (Hanski *et al.*, 1992) whereas in the present study these bacteria were found to bind fibronectin through protein H. This discrepancy could be explained by different binding assays. For instance Hanski *et al.* (1992) used lower bacterial concentrations. Protein F contains two regions interacting with fibronectin (Sela *et al.*, 1993). The region binding to the 29 kDa part of fibronectin consists of four repeats of 37 amino acid residues each. However, on the NH_2-terminal side of these repeats there is an additional region (43 amino acids long) called the upstream fibronectin-binding domain (UFBD). Also this part of protein F interacts with fibronectin but the binding area in fibronectin has not been defined. Interestingly, a sequence alignment of UFBD (introducing one gap of two residues) and the FNIII-binding A domain (residues 16–60) of protein H reveals 21% identity and 47% homology suggesting that perhaps also protein F has affinity for FNIII domains. As mentioned, protein H-expressing AP1 bacteria do not carry the gene encoding protein F. If the ability to bind fibronectin is critical to *S.pyogenes*, the AP1 strain may compensate for the absence of protein F by interacting with FNIII domains through protein H. It has been

estimated that as many as 2% of all animal proteins contain FNIII domains (Bork and Doolittle, 1992) including many extracellular proteins like fibronectin and cytotactin/tenascin, some intracellular proteins, and the extracellular domains of various membrane-receptor proteins. It remains to be established to what extent protein H is capable of interacting with other FNIII-like domains, but the present study suggests that protein H-expressing bacteria could establish multiple protein–protein interactions with cell surfaces and the matrix of the infected host through FNIII-like domains.

Host–parasite relationships represent a delicate balance between multiple molecular events, and pathogenicity and virulence are highly polygenic properties. The emergence of two closely located but separate binding sites for Ig and FNIII domains, two of the most abundant structural motifs in eukaryotic proteins, in a bacterial surface protein, further underlines the complexity of the molecular interactions creating the relationship between an infecting microorganism and its host. For the prevention, diagnosis and treatment of infectious diseases, a better understanding of such host–parasite interactions is crucial. However, we believe that the focusing on widespread protein folds in the evolution of the protein-binding regions of protein H is interesting also from a more general biological point of view.

Materials and methods

Bacterial strain

The group A streptococcal strain 40/58 of serotype M1, designated AP1, was from the WHO Collaborating Centre for Reference and Research on Streptococci, Prague, Czech Republic.

Proteins and peptides

Chicken embryonic and adult N-CAM were prepared as described earlier (Hoffman *et al.*, 1982) as were recombinant protein H and protein H fragments (Åkesson *et al.*, 1990; Frick *et al.*, 1994). Protein G comprising two IgG Fc binding domains (CDC) was purchased from Pharmacia and protein A, also comprising two IgG Fc binding domains (ED), was kindly provided by Dr J.Sjöquist, Uppsala University, Uppsala, Sweden. An *E.coli*-produced protein L fragment containing four Ig light chain binding domains was purified as described (Kastern *et al.*, 1992). Polyclonal IgG was isolated from human plasma by ion-exchange chromatography and gel filtration. ICAM-1 was kindly provided by Dr T.Springer, Center for Blood Research, Harvard Medical School, Boston, USA. Human plasma fibronectin and an Arg-Gly-Asp (RGD) peptide were from Sigma Chemical Co., St Louis, MO, and human fibronectin α-chymotryptic fragment 120 kDa (cell attachment) from Chemicon, Temecula, CA. Cytotactin/tenascin fusion proteins FN1 and FN3 were prepared as previously described (Prieto *et al.*, 1992).

Labeling of proteins

N-CAM, ICAM-1 and protein H were labeled with ^{125}I using the Bolton and Hunter (1973) reagent (Amersham, UK). Human IgG, protein G and human fibronectin were labeled with ^{125}I using the chloramine T method (Greenwood *et al.*, 1963). The ^{125}I was from Nordion Int. Co. (Canada). Specific activities were 5–10 mCi/mg protein.

Analysis of proteins applied to polyvinylidene difluoride (PVDF) membranes

Proteins were applied to PVDF membranes (Immobilon, Millipore, Bedford, MA) using a Milliblot-D system (Millipore). Membranes were blocked at room temperature for 1 h in VBS (10 mM veronal, 0.15 M NaCl pH 7.4) containing 0.25% Tween-20 and 0.25% gelatin or 2% skim milk. After incubation at room temperature for 3 h with radiolabeled protein (2×10^5 c.p.m./ml corresponding to ~10–30 ng protein/ml) in VBS containing 0.1% gelatin or 2% skim milk, the membranes were washed four times with 1.0 M NaCl, 10 mM EDTA, pH 7.7, 0.25% Tween-20 and 0.25% gelatin or 2% skim milk. The filters were air-dried and autoradiographed at −70°C using Kodak X-Omat AR films and Kodak X-Omat regular intensifying screens.

Coupling of protein H to Sepharose

Protein H (1.5 mg) was dialysed against coupling buffer (0.1 M $NaHCO_3$ pH 8.3, 0.5 M NaCl) and mixed with 3.5 ml CnBr-activated Sepharose 4B (Pharmacia) prewashed with 1 mM HCl followed by coupling buffer. The mixture was rotated overnight at 4°C. Excess ligand was removed by washing with coupling buffer and remaining active groups were blocked with 0.2 M glycin, pH 8.0, at room temperature for 2 h. The Sepharose was then rinsed with coupling buffer and wash buffer (0.1 M $CH_3COOH-CH_3COONa$ pH 4.0, 0.5 M NaCl). After a final wash with PBSA (0.12 M NaCl, 0.03 M phosphate, pH 7.4 + 0.05% NaN_3) the Sepharose was resuspended in this buffer.

Affinity chromatography

^{125}I-labeled N-CAM was run on protein H–Sepharose and protein G–Sepharose (Pharmacia) columns. The columns were extensively rinsed with PBSAT (PBSA + 0.05% Tween-20). Bound material was eluted with 3 M KSCN and the radioactivity in the fractions was measured in a gammacounter. Fractions were also analysed by SDS–PAGE.

Electrophoresis

SDS–PAGE was performed using the buffer system described by Laemmli (1970). The acrylamide content was 8% in the separation gels with a crosslinking of 2.7%. Samples were boiled for 3 min in an equal volume of sample buffer containing 2% SDS and 5% 2-mercaptoethanol. Gels were fixed with a mixture of 7% acetic acid and 10% ethanol, dried and autoradiographed.

Binding assays and determination of affinity constants

^{125}I-labeled proteins were bound to streptococcal cells as described (Björck and Kronvall, 1984). Competitive binding assays and calculations of affinity constants were also performed as reported (Åkerström and Björck, 1986, 1989) with the exception that N-CAM was diluted in PBS containing 0.5% NP-40.

Digestion of protein H at the bacterial surface

AP1 bacteria (10^{10} in 1 ml PBS) were incubated with 10 μg of activated streptococcal cysteine proteinase for 3 h at 37°C. The enzyme was inactivated with iodoacetic acid added to 6 mM and cells were washed and used in binding experiments with ^{125}I-labeled IgG and fibronectin.

Acknowledgements

We are indebted to Dr Ulf Sjöbring for helpful suggestions. This work was supported by grants from the Swedish Medical Research Council (projects 7480 and 10434), King Gustaf V's 80-years foundation, the Medical Faculty, Lund University, the Foundations of Kock and Österlund, High Tech Receptor AB, the Swedish Research Council for Engineering Sciences (project 123) and USPHS Grant HD-09635 to GME.

References

Åkerström,B. and Björck,L. (1986) *J. Biol. Chem.*, **261**, 10240–10247.
Åkerström,B. and Björck,L. (1989) *J. Biol. Chem.*, **264**, 19740–19746.
Åkerström,B., Lindahl,G., Björck,L. and Lindqvist,A. (1992) *J. Immunol.*, **148**, 3238–3243.
Åkesson,P., Cooney,J., Kishimoto,F. and Björck,L. (1990) *Mol. Immunol.*, **27**, 523–531.
Åkesson,P., Schmidt,K.-H., Cooney,J. and Björck,L. (1994) *Biochem. J.*, **300**, 877–886.
Björck,L. and Kronvall,G. (1984) *J. Immunol.*, **133**, 969–974.
Björck,L., Åkesson,P., Bohus,M., Trojnar,J., Abrahamson,M., Olafsson,I. and Grubb,A. (1989) *Nature*, **337**, 385–386.
Bolton,A.E. and Hunter,W.M. (1973) *Biochem. J.*, **133**, 529–539.
Bork,P. and Doolittle,R.F. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 8990–8994.
Boyle,M.D.P., ed. (1990) *Bacterial Immunoglobulin Binding Proteins*. Academic Press, San Diego.
Cremer,H. *et al.*(1994) *Nature*, **367**, 455–459.
Cunningham,B.A., Hemperly,J.J., Murray,B.A., Prediger,E.A., Brackenbury,R. and Edelman,G.M. (1987) *Science*, **236**, 799–806.

Dickinson,C.D., Veerapandian,B., Dai,X.-P., Hamlin,R.C., Xuong,N.-h., Ruoslahti,E. and Ely,K.R. (1994) *J. Mol. Biol.*, **236**, 1079–1092.
Dustin,M.L., Rothlein,R., Bhan,A.K, Dinarello,C.A. and Springer,T.A. (1986) *J. Immunol.*, **137**, 245–254.
Edelman,G.M. and Crossin,K.L. (1991) *Annu. Rev. Biochem.*, **60**, 155–190.
Elliott,S.D. (1945) *J. Exp. Med.*, **81**, 573–592.
Fischetti,V.A. (1989) *Clin. Microbiol. Rev.*, **2**, 285–314.
Forsgren,A. and Sjöquist,J. (1966) *J. Immunol.*, **97**, 822–827.
Frick,I.-M., Wikström,M., Forsén,S., Drakenberg,T., Gomi,H., Sjöbring,U. and Björck,L. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 8532–8536.
Frick,I.-M. *et al.* (1994) *Mol. Microbiol.*, **12**, 143–151.
Gomi,H., Hozumi,T., Hattori,S., Tagawa,C., Kishimoto,F. and Björck,L. (1990) *J. Immunol.*, **144**, 4046–4052.
Greenwood,F.C., Hunter,W.M. and Glover,J.S. (1963) *Biochem. J.*, **89**, 114–123.
Hanski,E. and Caparon,M.G. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 6172–6176.
Hanski,E., Horwitz,P.A. and Caparon,M.G. (1992) *Infect. Immun.*, **60**, 5119–5125.
Heath,D.G. and Cleary,P.P. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 4741–4745.
Hemperly,J.J., Edelman,G.M. and Cunningham,B.A. (1986a) *Proc. Natl Acad. Sci. USA*, **83**, 9822–9826.
Hemperly,J.J., Murray,B.A., Edelman,G.M. and Cunningham,B.A. (1986b) *Proc. Natl Acad. Sci. USA*, **83**, 3037–3041.
Hoffman,S. *et al.* (1982) *J. Biol. Chem.*, **257**, 7720–7729.
Huber,A.H., Eureka Wang,Y.-m., Bieber,A.J. and Bjorkman,P.J. (1994) *Neuron*, **12**, 717–731.
Hynes,R.O. (1990) *Fibronectins.* Springer Verlag, New York, pp. 113–175.
Kastern,W., Sjöbring,U. and Björck,L. (1992) *J. Biol. Chem.*, **267**, 12820–12825.
Kehoe,M.A. (1994) *New Comp. Biochem.*, **27**, 217–261.
Kronvall,G. (1973) *J. Immunol.*, **111**, 1401–1406.
Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
Leahy,D.J., Hendrickson,W.A., Aukhil,I. and Erickson,H.P. (1992) *Science*, **258**, 987–991.
Pierschbacher,M.D. and Ruoslahti,E. (1984) *Nature*, **309**, 30–33.
Prieto,A.L., Andersson-Fisone,C. and Crossin,K.L. (1992) *J. Cell Biol.*, **119**, 663–678.
Raeder,R., Otten,R.A., Chamberlin,L. and Boyle,M.D.P. (1992) *J. Clin. Microbiol.*, **30**, 3074–3081.
Reis,K.J., Ayoub,E.M. and Boyle,M.D.P. (1984) *J. Immunol.*, **132**, 3091–3097.
Retnoningrum,D.S., Podbielski,A. and Cleary,P.P. (1993) *J. Immunol.*, **150**, 2332–2340.
Rothbard,J.B., Brackenbury,R., Cunningham,B.A. and Edelman,G.M. (1982) *J. Biol. Chem.*, **257**, 11064–11069.
Sela,S., Aviv,A., Tovi,A., Burstein,J., Caparon,M.G. and Hanski,E. (1993) *Mol. Microbiol.*, **10**, 1049–1055.
Staunton,D.E., Marlin,S.D., Stratowa,C., Dustin,M.L. and Springer,T.A. (1988) *Cell*, **52**, 925–933.
Stenberg,L., O'Toole,P. and Lindahl,G. (1992) *Mol. Microbiol.*, **6**, 1185–1194.
Stone,G.C., Sjöbring,U., Björck,L., Sjöquist,J., Barber,C.V. and Nardella,F.A. (1989) *J. Immunol.*, **143**, 565–570.
Westerlund,B. and Korhonen,T.K. (1993) *Mol. Microbiol.*, **9**, 687–694.

Received on November 14, 1994; revised on January 19, 1995