Communication

An IgA-binding Peptide Derived from a Streptococcal Surface Protein*

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Surface proteins that bind to the Fc part of human IgA are expressed by many strains of Streptococcus pyogenes, a major human pathogen. Studies of these proteins have been complicated by their size and by their ability to bind human plasma proteins other than IgA. Here, we describe a synthetic 50-residue peptide, derived from streptococcal protein Sir22, that binds human IgA but not any of the other plasma proteins known to bind to Sir22. The peptide binds serum IgA and secretory IgA and binds IgA of both subclasses. Evidence is presented that the peptide folds correctly both in solution and when it is immobilized and that it readily reassociates after denaturation. Together, these data indicate that the peptide corresponds to a protein domain that binds IgA with high specificity. This is the first report of an IgA-binding domain that retains its properties in isolated form.

An important property of human IgA is the ability of the Fc part to interact with different receptors. Binding to one of these receptors, CD89, is believed to be essential for the effector functions of serum IgA (1, 2), whereas binding to the poly-Ig receptor on mucosal epithelial cells is required for secretion of dimeric IgA (3).

The Fc part of human IgA also binds to surface proteins expressed by some pathogenic streptococci (4, 5). These proteins are of interest for analysis of pathogenetic mechanisms in bacterial infections and are also of potential value as model systems and analytical tools for studies of IgA. The most extensively studied of these bacterial IgA-binding proteins are those expressed by Streptococcus pyogenes (group A streptococcus), a major human pathogen. IgA-binding proteins expressed by this bacterium are members of the M protein family (6–8), a family of coiled-coil proteins that are important for virulence. However, characterization of several of the IgA-binding proteins of S. pyogenes has shown that they not only bind IgA but also bind other human plasma proteins (9–11). This property is of interest with regard to pathogenetic mechanisms but has complicated studies of the IgA binding property of the proteins.

For structural analysis, it would also be desirable to have access to an IgA-binding molecule of smaller size.

In this study, we explored the possibility of studying the IgA-binding region of an S. pyogenes protein in isolated form. We show that a 50-residue synthetic peptide, derived from streptococcal protein Sir22,1 binds IgA but not any of the other plasma proteins that bind to Sir22. The properties of this peptide indicate that it corresponds to a protein domain that binds IgA with high specificity.

EXPERIMENTAL PROCEDURES

Synthetic Peptide and Purified Proteins—The 50-residue synthetic peptide (see Fig. 1A) was purchased from the Department of Clinical Chemistry (Malmö General Hospital, Sweden). It corresponds to amino acid residues 35–83 of protein Sir22 (10) and includes a C-terminal cysteine residue not present in Sir22. The peptide was ≥95% pure. Recombinant Sir22 was purified as described (10). Purified human C4BPa was the kind gift of Dr. B. Dahlbäck (Malmö General Hospital).

Immunoglobulins and Human Serum—All immunoglobulins were of human origin. Polyclonal serum IgA was from Cappel-Organon Teknika (Turnhout, Belgium). S-IgA was purified from colostrum (12). Monoclonal IgA proteins were isolated from serum of patients with IgA multiple myeloma (13). Three of the monoclonal IgA proteins and all monoclonal IgG proteins were kindly provided by Dr. F. Skvaril (Bern, Switzerland). Monoclonal IgM and IgD were from The Binding Site (Birmingham, United Kingdom), and IgE was the gift of Dr. I. Olsson (Lund University). Polyclonal IgG was from Kabi (Stockholm, Sweden). Fab and Fc fragments of an IgA1 protein were purified after cleavage with IgA protease from Haemophilus influenzae (12). Serum from an IgA-deficient individual was kindly provided by Dr. A. Sjöholm (Lund University).

Binding Assays and Competitive Inhibition—Binding of radiolabeled human proteins was analyzed after immobilization of peptide or Sir22 in microtiter wells. The wells were coated overnight at 4 °C with 50 μg of a solution of peptide or Sir22 (100 μg/ml in PBS). After washing and blocking with PBSAT (PBS with 0.02% sodium azide and 0.05% Tween 20), radiolabeled ligand (~25,000 cpm) was added to each well, and the plates were incubated for 2 h at room temperature. After washing with PBSAT, the radioactivity associated with each well was determined. Nonspecific binding (<0.7%) was determined for wells coated only with PBSAT. The IgGs used were polyclonal.

For inhibition tests, wells of microtiter plates were coated with the peptide (5 μg/ml for tests with serum IgA and 50 μg/ml for tests with S-IgA). After blocking, radiolabeled serum IgA or S-IgA (~25,000 cpm) was added together with inhibitor as indicated. The final concentration of radiolabeled IgA was ~0.3 nM. Binding was analyzed as described above. Nonspecific binding was <0.25%.

Other Methods—Western blot and dot-blot analysis on polyvinylidene difluoride membranes were performed as described (10). Protein concentrations were determined with the MicroBCA kit from Pierce using BSA as a reference. Radiolabeling of peptides and proteins with 125I was performed with the chloramine-T method.

RESULTS AND DISCUSSION

Design of a Synthetic Peptide Including the IgA-binding Region of Protein Sir22—Previous studies of the S. pyogenes protein Arp4 demonstrated that the IgA-binding region of this protein is localized in a 29-residue region in the N-terminal part of the molecule (8, 13). An attempt was made to demon-

1 In an alternative nomenclature (14), Sir22 is designated Emm22.
2 The abbreviations used are: C4BP, C4b-binding protein; S-IgA, secretory IgA; PBS, phosphate-buffered saline; BSA, bovine serum albumin.
Microtiter wells coated with Sir22 (A) peptide. and a C-terminal cysteine not present in Sir22. The 29-residue IgA-binding region, (8), 10 additional residues on either side, duces 35–83 of the processed form of Sir22 and includes the predicted actual shared binding sites. The synthetic peptide corresponds to residues 35–83 of the processed form of Sir22 and includes the predicted IgA-binding region (8), 10 additional residues on either side, and a C-terminal cysteine not present in Sir22. B, specificity of binding. Microtiter wells coated with Sir22 (left) or peptide (right) were analyzed for ability to bind radiolabeled human proteins. C, competitive inhibition. Microtiter wells were coated with the peptide. Binding of radiola beled serum IgA (left) or S-IgA (right) was inhibited with unlabeled protein or peptide, as indicated. Inhibition by BSA was tested at one concentration. In the experiments in B and C, each value is the average of duplicate samples. All experiments were performed twice with similar results.

strate IgA binding for a 33-residue synthetic peptide including this region from Arp4, but no binding was observed (data not shown). A longer peptide was therefore tested as described below. This new peptide was derived from the Sir22 protein, which is closely related to Arp4 (10) and is expressed by a bacterial strain that is suitable for analysis of pathogenetic mechanisms (14).

Protein Sir22 is a dimeric coiled-coil molecule that binds at least three different human plasma proteins, IgA, IgG, and C4BP (10, 11, 15) (Fig. 1A). The regions in Sir22 that bind these ligands have been partially identified. The 29-residue IgA-binding region (defined by homology with Arp4) overlaps with an upstream region that includes a C4BP-binding site (16). Because IgA and C4BP do not compete for binding to Sir22 (11), these ligands may bind to separate domains. The IgA-binding region also overlaps with a downstream region including an IgG-binding site (10, 17). The bindings of IgA and IgG to Sir22 are mutually exclusive, indicating shared or contiguous binding sites (10). In addition to these ligands, serum albumin may bind to the central C repeat region of Sir22 (18).

The design of an IgA-binding peptide was based on the hypothesis that C4BP, IgA, and IgG bind to separate domains in Sir22 and that the inhibition of IgA binding by IgG (10) was due to steric hindrance not shared binding sites. The synthetic 50-residue peptide covers the predicted IgA-binding region of Sir22 and also includes 10 residues on either side, added to enhance the probability for correct folding. Because the Sir22 protein must form a coiled-coil dimer to bind IgA (15, 19), a C-terminal cysteine residue was included to ensure that dimerization could occur. However, it also seemed possible that the peptide would be able to form a coiled-coil by itself (20).

The Peptide Specifically Binds IgA—Wells of microtiter plates were coated with the peptide or with Sir22 and analyzed for the ability to bind radiolabeled ligands (Fig. 1B). As expected, immobilized Sir22 bound all four ligands. For unknown reasons, the binding of IgG was lower than for serum IgA in this test, although Sir22 has similar affinity for these two ligands (10). The immobilized peptide bound serum IgA and S-IgA, but not C4BP or IgG, indicating that it specifically binds IgA. The binding of serum IgA and S-IgA to the immobilized peptide could be completely inhibited by unlabeled IgA but not by BSA, confirming that the binding of IgA was specific and showing that the binding was not due to the radiolabeling (Fig. 1C). Moreover, binding of serum IgA or S-IgA to immobilized peptide could be inhibited by free peptide, indicating that the ability to bind IgA was not due to a conformational change in the peptide upon immobilization (Fig. 1C). Finally, the binding of serum IgA and S-IgA to immobilized peptide could be inhibited by a purified monoclonal IgA1 protein, showing that the binding of IgA was independent of the antigen-binding site (data not shown).

The binding properties of the peptide were further studied by Western blot analysis under nonreducing conditions (Fig. 2A). Radiolabeled peptide, used as the probe, bound to serum IgA but not to IgG. Thus, the peptide in solution retains its binding ability after radiolabeling. Striking evidence for the specificity of the peptide came from analysis of whole normal serum and serum from an IgA-deficient individual. The peptide lacked reactivity with any of the many proteins present in IgA-deficient serum, but bound to IgA present in normal serum. Sim-
The Peptide Binds IgA-Fc and Binds IgA of Both Subclasses—As expected, the peptide binds to the Fc part of IgA (Fig. 3A). To analyze whether the peptide binds IgA of both subclasses, a dot-blot analysis was performed with purified monoclonal proteins (Fig. 3B). The analysis also included analysis of monoclonal IgG, IgM, IgD, and IgE proteins, and the binding properties of the peptide were compared with those of the parental Sir22 molecule. In agreement with previous results (10), Sir22 was found to bind most of the IgA and IgG proteins. The peptide only bound IgA proteins, and binding was observed for 8 of 9 IgA1 proteins and for 10 of 11 IgA2 proteins. However, this semi-quantitative analysis indicated that the affinity varied considerably for different IgA proteins. Possible reasons for this variation are differences in glycosylation and loss of binding ability during purification of monoclonal proteins. The ability of the peptide to bind different IgA proteins was similar to that of protein Sir22, supporting the hypothesis that the peptide corresponds to an IgA-binding domain in Sir22.

Western Blot Analysis and Effect of Heating—Western blot analysis of the peptide under nonreducing conditions showed that it bound radiolabeled IgA (Fig. 4A). This analysis included an initial step, in which the peptide was boiled in SDS-containing buffer, a step that most likely caused denaturation. Thus, these results suggest that at least some peptide molecules reaturated during the analysis. The peptide, probably a dimer, moved more slowly than expected, a common property in streptococcal surface proteins (10). Under reducing conditions, when the peptide migrated as a monomer, it also bound IgA after blotting. In this regard, the peptide behaved like protein Sir22, which also binds IgA in Western blot analysis, after having migrated as a monomer (10). Because Sir22 must form a coiled-coil dimer to bind IgA (15, 19), it seems likely that dimerization of Sir22 can occur on the blotting membrane, allowing binding of IgA. Similarly, the ability of the peptide to bind IgA in Western blot analysis during reducing conditions might have been due to formation of coiled-coil dimers on the blotting membrane. Thus, the results of this analysis cannot be taken as evidence that a monomeric form of the peptide binds IgA. Structural analysis of the peptide will now be of considerable interest and will show whether it has a coiled-coil structure, as suggested by the available data (15, 16, 19). Interestingly, the C-terminal part of the peptide includes a sequence (LEEEKKNLEKK) that is similar to a consensus “trigger” sequence implicated in the formation of coiled-coils (21).

The ability of the peptide to bind IgA after heating was further analyzed in an inhibition test (Fig. 4B). In this test, the ability of radiolabeled IgA to bind to immobilized peptide was inhibited by free peptide. Boiling of the peptide for 5 min, which most likely caused denaturation, had no effect on the ability of the peptide to subsequently inhibit binding, implying that most peptide molecules refolded into the native form after denaturation. Taken together, the data in Fig. 4 indicate that the peptide is a very stable molecule. The radiolabeled form of the peptide was also stable, because it could be kept frozen for 4 months without losing the ability to specifically bind IgA (data not shown).

Concluding Remarks—The properties of the peptide studied here indicate that it corresponds to an IgA-binding domain that binds human IgA-Fc with high specificity. To our knowledge, this represents the first example of an IgA-binding domain that retains its binding properties in isolated form. Importantly, the peptide binds human IgA of both subclasses and binds both serum IgA and S-IgA, suggesting that it may become a useful tool for studies of human IgA. With regard to applications, it is of interest that the peptide showed specificity for IgA both when it was immobilized and when it was present in soluble form. Previous studies of Sir22 and other IgA-binding streptococcal proteins suggest that the peptide may have lower affinity for S-IgA than for serum IgA, due to the presence of secretory component in S-IgA (10, 22), but the peptide could readily detect S-IgA in Western blot analysis, implying that it may become valuable also for studies of this form of IgA.

A bacterial IgA-Fc-binding protein unrelated to Sir22 has been described in group B streptococcus (5, 23, 24), and a fusion protein including a 73-residue sequence from this protein was found to bind IgA (25). Direct comparison of this 73-residue sequence with the peptide described here did not disclose any similarity. The group B streptococcus protein has little or no ability to bind S-IgA, which may limit its use as an immunological tool (26), but structural comparisons of the different IgA-binding regions would be of interest.

The size and stability of the peptide described here suggest that it may be amenable to structural analysis and could become a useful model for studies of human IgA receptors. An interesting parallel has already been identified, because human CD89 was found to bind to a site on IgA-Fc analogous to the site on IgG-Fc that is recognized by staphylococcal protein A (2), stressing the value of bacterial proteins as model systems. Further characterization of the peptide will also be of interest for analysis of pathogenetic mechanisms in S. pyogenes infections.

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