Identification of the transferrin- and lactoferrin-binding proteins in *Haemophilus influenzae*

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**Summary.** An affinity procedure with purified, biotinylated human transferrin and streptavidin-agarose was used to identify the transferrin-binding proteins in strains of *Haemophilus influenzae*. Proteins of 58 and 98 Kda were isolated from total membranes prepared from iron-deficient but not iron-sufficient *H. influenzae* KC548 cells. The 58-Kda protein was capable of binding human transferrin after sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) and electroblotting. Isolation of transferrin-binding proteins from type-b and non-typable *H. influenzae* strains demonstrated some variability in the size of the higher mol. wt protein (94–106 Kda) and in ease of elution of the smaller protein from the affinity resin. Use of purified, biotinylated human lactoferrin in the affinity isolation procedure with membranes from a strain expressing lactoferrin-binding activity resulted in isolation of proteins of 105 and 106 Kda distinct from the transferrin-binding proteins.

**Introduction**

Bacterial pathogens have to deal with an environment containing low levels of free iron in the human host (Weinberg, 1978). The iron-binding protein lactoferrin is present on mucosal surfaces to sequester iron (Masson *et al.*, 1966) and in the systemic circulation transferrin is largely responsible for reducing the level of available iron (Weinberg, 1978). It has been suggested that the ability to compete with lactoferrin and transferrin for iron is essential for the pathogenesis of a number of bacterial infections (Bullen *et al.*, 1974; Kochan *et al.*, 1977; Miles and Khimji, 1975; Williams and Warner, 1980). *Haemophilus influenzae* has been shown to utilise transferrin-bound iron for growth and the demonstration of transferrin-iron utilisation in almost all invasive strains but not in most non-typable, avirulent isolates is consistent with an important role in pathogenesis (Herrington and Sparling, 1985). In addition to being capable of utilising iron from the readily available protein transferrin, *H. influenzae* can obtain iron from haem and haemoglobin (Stull, 1987). Although these sources of iron would not be expected to be present in significant quantities in the normal host, they could possibly be released during infection. In this context, it is interesting that *H. influenzae* has the capacity to obtain haem from the serum protein haemopexin and from haemoglobin complexed with haptoglobin (Stull, 1987). Haemopexin and haptoglobin are responsible for binding free haem and haemoglobin released in serum (Muller-Eberhard, 1970; Eaton *et al.*, 1982).

Recently, we have demonstrated that a specific transferrin-binding activity was present in all *H. influenzae* strains tested but not in other species in the family Pasteurellaceae (Schryvers, 1988). The specificity of binding of transferrin by *H. influenzae* was essentially identical to that observed for *Neisseria meningitidis* (Schryvers and Morris, 1988a). Similarly, a lactoferrin-binding activity detected in one strain of *H. influenzae* (Schryvers, 1988) had essentially the same specificity as that observed in *N. meningitidis* (Schryvers and Morris, 1988b). In this paper we report on the identification of proteins in *H. influenzae* that bind specifically to human transferrin and lactoferrin.

**Materials and methods**

**Bacterial strains**

A collection of type-b *H. influenzae* isolates from clinical infections was obtained from Dr E. Hansen, Dallas, Texas. These included strains OA104, DL41, DL67, DL90 and NA103. In addition, several well characterised non-typable isolates, strains BF100, BF103...
and TN100, were obtained from Dr Hansen. *H. influenzae* strain KC548 was obtained from Dr W. Albritton, Saskatoon, Saskatchewan. This strain was isolated from a routine eye swab and although it had not been serotyped originally, one-dimensional protein-profile analysis suggested that it was related to serotype-d strains (unpublished observations, W. Albritton).

**Growth of bacteria**

*H. influenzae* strains were grown routinely on chocolate-agar plates supplemented with CVA enrichment (Gibco Laboratories, Grand Island, NY) in an atmosphere containing CO₂ 5% v/v. Liquid cultures were grown on supplemented brain-heart infusion (BHI) broth (Herrington and Sparling, 1985) and were inoculated with a suspension of cells harvested from fresh chocolate-agar plates. Supplemented BHI broth contained NAD (Sigma Chemical Co., St Louis, MO) 2 μg/ml and protoporphyrin IX (Sigma) 2 μg/ml. Iron-deficient medium was prepared by the addition of the chelator ethylenediamine di-orthophenylacetic acid (EDDA, Sigma) to a final concentration of 100 μM, unless otherwise indicated. The iron deficiency was reversed by the addition of ferric chloride to 120 μM, unless otherwise indicated.

**Chemicals**

Human transferrin, human lactoferrin, pre-stained mol. wt standards and diaminobenzidine hydrochloride were obtained from Sigma. Biotin-NHS was from Calbiochem, San Diego, CA. Horseradish peroxidase (HRP)-conjugated streptavidin and HRP-conjugated human transferrin were obtained from Jackson Immunoresearch Labs, Avondale, PA. Streptavidin-agarose was from Bethesda Research Laboratories, Bethesda, MD. The anti-human transferrin monoclonal antibody (from HT1/13.6.3 hybridoma line) was obtained from Cedarlane Laboratories Ltd, Hornby, ON. Anti-human lactoferrin HRP-conjugated rabbit hyperimmune serum was obtained from United States Biochemical Corp., Colombus, OH. The acrylamide gel exclusion column was from Beckman Instruments, Fullerton, CA.

**Preparation and biotinylation of human transferrin and lactoferrin**

Human lactoferrin and transferrin were saturated with iron by dissolving in a 0.1 M sodium citrate, 0.1 M Na₂CO₃, pH 8-6 buffer and adding a 50-fold molar excess of FeCl₃ dissolved in the same buffer. The reagents were removed by two sequential passages through an acrylamide gel exclusion column equilibrated with 20 mM Tris-HCl, pH 7.0. Protein preparations were concentrated by ultrafiltration with an Amicon Centriflo membrane cone. Preparations of iron-saturated human transferrin or lactoferrin were equilibrated with 50 mM Tris-HCl, pH 7.5 buffer by cycles of gel filtration and ultrafiltration and diluted to 1 mg/ml. Biotin-NHS 250 μg dissolved in 16 μl of dimethylformamide was added per ml of the protein solution and the mixture was incubated with gentle agitation at 4°C for 2 h. The reaction was stopped by the addition of 100 μl of glycine (10 mg/ml) to each ml and the mixture was incubated for a further 2 h with agitation at 4°C. The samples were dialysed against three changes of 50 mM Tris-HCl, pH 8-0, 100 mM NaCl. one change of 50 mM Tris-HCl, pH 7-5 and concentrated by ultrafiltration with an Amicon Centriflo membrane cone.

**Preparation of membranes**

Cells were harvested and washed in 50 mM Tris-HCl, pH 7.5 buffer and resuspended to a concentration of 0.2 g of cells/ml in buffer containing phenylmethylsulphonyl fluoride 50 μg/ml. After cell lysis by passing twice through a French pressure cell at 16 000 p.s.i., cell debris was removed by centrifugation at 8000 g for 15 min. Crude total membranes were collected by centrifugation at 140 000 g for 1 h and resuspended in the above buffer.

**Affinity isolation of binding proteins**

Affinity isolation of transferrin- and lactoferrin-binding proteins was achieved by the method shown schematically in fig. 1. Biotinylated human lactoferrin 14 μg or biotinylated human transferrin 23 μg was added per ml of total membranes (5 mg of protein/ml) suspended in 50 mM Tris-HCl, 100 mM NaCl, pH 8-0 buffer and the mixture was incubated with gentle agitation for 60 min at 37°C. The membranes were pelleted by centrifugation at 16 000 g for 10 min, the supernate was discarded and the pellet was resuspended in the original volume of buffer. The membranes were solubilised by addition of EDTA to 10 mM and Sarkosyl NLS97 to 2% w/v. Streptavidin-agarose (100 μl of a 1 in 2 dilution) was added to the solubilised membranes after centrifugation at 16 000 g for 10 min to remove insoluble material. After incubation at 22°C for 60 min the mixture was centrifuged at 750 g for 3 min and the supernate was removed. In reverse order of addition experiments, the biotinylated protein was added directly to the streptavidin-agarose resin and, after incubation for 1 h, membranes solubilised as above (without prior exposure to biotinylated ligand) were added to the resin-biotinylated ligand mixture. After incubation at 22°C for 1 h, the mixture was centrifuged as above and the supernate was removed. The affinity resin pellet was subjected to one of several different washing regimens in which buffers of different compositions were added to the pellet and incubated for 15 min at 22°C, the mixture was centrifuged as above and the supernate was removed. The standard (high-salt) washing procedure consisted of the following washing steps: (i) twice with a 50 mM Tris-HCl, 1 mM NaCl, pH 8-0 buffer containing 10 mM EDTA and Sarkosyl 0.5 w/v; (ii) once with the above buffer without EDTA and detergent; and
indicated concentration was included in the high-salt buffer during the first three washes. After the final washing step the pellet was resuspended in 200 μl of sample buffer (0.2M Tris-HCl, pH 6.8, SDS 2% w/v, glycerol 30% w/v, bromophenol blue 0.1% w/v) without reducing reagent and heated at 100°C for 5 min to elute bound proteins. After boiling, the sample was quickly cooled on ice for 1 min and then centrifuged at 750 g for 3 min and the supernate immediately transferred to a separate tube to which β-mercaptoethanol was added to 1-4M.

**Electrophoresis and electroblotting**

For analysis of the affinity-isolated preparations, 40-μl volumes of the samples prepared as described in the previous section were applied to a 7-10% acrylamide gradient SDS-PAGE gel with the buffer system of Laemmli (1970) and electrophoresed at 9 mA/gel overnight. For analysis of protein composition of the samples, the SDS-PAGE gel was silver-stained according to the method of Oakley et al. (1980) with the following minor modifications. The gel was first fixed overnight with a solution of isopropanol 25% v/v and acetic acid 7% v/v. After removal of the developing solution, the development was stopped with a solution of acetic acid 0.35% v/v for 1 h, and then washed with water. In experiments involving Western-blot analysis, samples on parallel gels, or on sections of the same gel, were electroblotted on to nitrocellulose paper for 2 h at 60 V in a BioRad Transblot apparatus with a 20 mM Tris-HCl, 192 mM glycine, pH 8.3 buffer containing methanol 20% v/v. The nitrocellulose paper was then incubated at 37°C for 45 min in blocking solution (Schryvers and Morris, 1988a), washed with TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.5) and then exposed to blocking solution containing the indicated reagents for 1 h at 37°C. After the final incubation step the paper was washed once with TBS, followed by two washes with 100 mM, pH 7-8 sodium phosphate buffer. The paper was then exposed to a substrate mixture in the above buffer containing diaminobenzidine hydrochloride 2 mg/ml, 20 mM imidazole, Ni(II) SO₄ 0.02% w/v, and H₂O₂ 0.03% v/v. After incubation at room temperature in the dark for 15 min, the substrate mixture was removed and the paper was rinsed three times with distilled water.

**Determination of protein concentration**

Protein was estimated by the method of Lowry et al. (1951) with bovine serum albumin as standard.

**Results**

**Isolation of transferrin-binding proteins from H. influenzae strain KC548**

Strain KC548 was selected for identification of binding proteins because this strain possessed both
transferrin- and lactoferrin-binding activities (Schryvers, 1988). Preliminary fractionation studies demonstrated that the binding activities were associated with the outer-membrane fraction but that considerable activity was lost after addition of detergent for the selective detergent extraction procedure used for outer-membrane preparation (data not shown). These results prompted us to develop an affinity isolation method, using biotinylated transferrin (or lactoferrin) and streptavidin-agarose, which is schematically illustrated in fig. 1. This procedure was selected in preference to conventional affinity chromatography because it provided the opportunity to bind ligand to receptor in intact membranes before detergent solubilisation. The rationale for this step was that the ligand bound to the receptor was presumed to stabilise the receptor and reduce the loss of binding activity which was observed when receptor was first solubilised with detergents. The importance of this step is illustrated in fig. 2, which demonstrates that solubilisation of membrane before binding to the column-bound human transferrin resulted in no binding proteins being isolated (lane C).

Fig. 2 illustrates that proteins of 58 and 98 Kda were isolated from total membranes prepared from iron-deficient KC548 cells when biotinylated human transferrin was used as binding ligand (lane E). These proteins were absent when membranes from iron-sufficient cells were used (lane A) which correlates with the low level of receptor activity detected in iron-sufficient cells (Schryvers, 1988). When biotinylated human transferrin was omitted from the procedure (lane B) or when biotinylated human lactoferrin was used instead of transferrin (fig. 5, lane E) these proteins were not detected, indicating that specific binding to transferrin was involved. As noted above, these proteins were also absent when membranes were solubilised before binding to the ligand (lane C). Transferrin-binding activity was detected in the 58-Kda protein (lane E4, arrow) but not the 98-Kda protein after SDS-PAGE and electroblotting with HRP-conjugated human transferrin.

The 80-Kda protein present in the purified transferrin-receptor preparation (lane E, asterix) was identified as biotinylated human transferrin because it co-migrated with control biotinylated human transferrin added directly to the SDS-PAGE gel (lane D) and biotinylated human transferrin. The mol. wts (Kda) of marker proteins are shown on the left.

**Fig. 2.** Identification of the transferrin-binding proteins in *H. influenzae* strain KC548. Membranes from iron-sufficient cells or iron-deficient cells were used in the affinity isolation procedure and resulting samples were electrophoresed as described in the Methods section. Lanes A–E, silver-stained portion of the gel; C2–E2, electroblotted section exposed to 1 in 500 dilution of the anti-transferrin monoclonal antibody, washed and exposed to HRP-conjugated goat anti-mouse IgG 1 μg/ml; C3–E3, electroblotted section exposed to HRP-strepavidin 200 ng/ml; C4–E4, electroblotted section exposed to HRP-human transferrin 420 ng/ml. Lane A, iron-sufficient membranes, biotinylated human transferrin (bhTf); lane B, iron-deficient membranes, no bhTf; lane C, iron-deficient membranes, bhTf, reverse order procedure; lane D, 300 ng of bhTf control; lane E, iron-deficient membranes, bhTf. *, bhTf in lane E. →, 58-Kda protein binding human transferrin. The mol. wts (Kda) of marker proteins are shown on the left.
transferrin eluted from the column (lane C). Fig. 2 demonstrates that biotinylated human transferrin present in the control lanes (C and D) reacted with an anti-human transferrin monoclonal antibody (lanes C² and D²) and HRP-conjugated streptavidin (lanes C³ and D³). The 80-Kda band present in the receptor preparations also reacted with these reagents but this is not demonstrated in fig. 2 (lanes E² and E³) because of the relatively small amount of this protein present in this experiment. It is salient to note that the 58- and 98-Kda proteins did not react with the anti-transferrin monoclonal antibody (lane E²) nor with HRP-streptavidin (lane E³) indicating that they are not merely derivatives of the biotinylated human transferrin. This is also evidenced by the lack of the 58- and 98-Kda proteins in samples in which solubilised membranes were added to biotinylated transferrin pre-bound to streptavidin-agarose (lane C).

The results in fig. 2 demonstrating that the 58-Kda protein but not the 98-Kda protein is capable of binding to human transferrin after SDS-PAGE and electroblotting might suggest that it is the 58-Kda protein that is responsible for binding of human transferrin in intact membranes. An experiment in which the proteins bound to the affinity resin were washed with various buffers was performed to see if the 98-Kda protein could be removed with harsher washing conditions. The results in fig. 3 show that washing the affinity resin with increasing concentrations of guanidine hydrochloride (lanes D–G) did not result in preferential release of the 98-Kda protein indicating that either it directly binds to transferrin or it is very tightly

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**Fig. 3.** Effect of washing conditions on affinity isolation of proteins with biotinylated human transferrin. Membranes isolated from iron-deficient *H. influenzae* strain KC548 cells were subjected to the affinity isolation procedure with biotinylated human transferrin as the binding ligand followed by different washing procedures. Proteins were eluted in sample buffer, subjected to SDS-PAGE and silver stained as in fig. 2. Lane A, low-salt wash; B, low-pH wash; C, high-salt wash; D–H, high-salt wash containing: D, 250 mM guanidine hydrochloride (GHCI); E, 500 mM GHCI; F, 1 mM GHCI; G, 2 mM GHCl; → indicates position of biotinylated human transferrin.
associated with the 58-Kda protein. The fact that the 98-Kda protein is detected in lane F (1 M guanidine hydrochloride wash) without detectable association with the 58-Kda protein suggests that the 98-Kda protein binds directly to transferrin. Samples obtained from the affinity resin after milder washing conditions (lanes A and B) contain an additional protein of 67 Kda. This band was not observed in control samples (iron-sufficient membranes or no biotinylated transferrin added, data not shown) washed under similar conditions, indicating that it is associated with the binding of human transferrin and probably is binding to the resin via the 58- or 98-Kda transferrin-binding proteins.

**Isolation of transferrin-binding proteins from different H. influenzae strains**

Earlier studies had demonstrated that the ability to use transferrin-iron as a sole source of iron for growth was present in all type-b clinical isolates of *H. influenzae* but was relatively uncommon in nontypable strains tested (Herrington and Sparling, 1985). A more recent study showed that all type-b and nontypable strains tested were capable of using human transferrin to obtain iron for growth (Pidcock et al., 1988) which is consistent with our observation that transferrin-binding activity was present in all type-b clinical isolates and in all nontypable strains tested (Schryvers, 1988). Affinity isolation of transferrin-binding proteins was performed with several of these strains to determine whether there were differences in the transferrin-binding proteins in type-b and non-typable strains (fig. 4). As with strain KC548, a high mol. wt protein, ranging from 94 to 106 Kda, was found in all the type-b clinical isolates (lanes A-F) and nontypable isolates tested (lanes G-I). There were no systematic differences evident between receptors from type-b and non-typable strains other than a reduced intensity of the high mol. wt band in samples from the non-typable isolates, perhaps reflecting quantitative differences of receptor present in the membrane. The results in fig. 4 show that an additional protein of c. 80 Kda was observed in several of the type-b clinical isolates (lanes E and F). When milder washing conditions were used (1 M NaCl versus 250 mM guanidine hydrochloride) a protein in this mol. wt range was observed in all of the type-b isolates tested (data not shown). However, since these proteins migrated in the same region as biotinylated human transferrin, a number of different gels run under different conditions (varying run time and acrylamide concentration) were necessary to resolve these proteins from the biotinylated transferrin. The 78–86-Kda proteins were observed to bind HRP-transferrin after SDS-PAGE and electroblotting in several, but not all, of the type-b strains (OA104 and DL67) but the reaction was much weaker than that observed with the 58-Kda protein in strain KC548 (data not shown). A minor band in the mol. wt range 58–87 Kda was also detected in the non-typable isolates when milder washing conditions were used.

**Identification of the lactoferrin-binding protein in H. influenzae strain KC548**

In a previous study (Schryvers, 1988) lactoferrin-binding activity was detected in only one strain of *H. influenzae*, strain KC548. An affinity isolation experiment was performed with this strain and with biotinylated human lactoferrin in an attempt to identify the lactoferrin-binding protein(s). Fig. 5 illustrates that proteins of 105 and 106 Kda were isolated from total membranes prepared from iron-deficient KC548 cells when biotinylated human lactoferrin was used as binding ligand (lane E). When membranes from iron-sufficient cells, which contain little lactoferrin-binding activity (Schryvers, 1988), were used, these proteins were not isolated (lane A). When biotinylated human lactoferrin was omitted from the procedure (lane B), or when biotinylated human transferrin was used instead of biotinylated human lactoferrin (lane F), the 105- and 106-Kda proteins were not detected, indicating that specific binding to lactoferrin was involved. As observed with the transferrin-binding proteins, solubilisation of the membranes before ligand binding resulted in little or no protein being isolated (lane C).

To exclude the possibility that the 105- and 106-Kda proteins were simply derivatives of the biotinylated ligand, the purified receptor preparation was analysed with HRP-streptavidin (lane E2) and an anti-lactoferrin hyperimmune serum (lane E3) after SDS-PAGE and electroblotting. No reactivity with either of these reagents was observed under conditions in which reactivity with control biotinylated human lactoferrin (lanes D2 and D3) was evident. The lack of detectable 105- or 106-Kda proteins in the sample in which solubilised membranes were added to biotinylated lactoferrin pre-bound to streptavidin-agarose (lane C) also suggests that these proteins are not derivatives of the binding ligand.

**Discussion**

Although it has been shown that *H. influenzae* can use human transferrin-bound iron as a sole
Fig. 4. Identification of the transferrin-binding proteins in different *H. influenzae* isolates. The transferrin-binding protein(s) were isolated from iron-deficient cells by the affinity isolation procedure described in the Methods section. The affinity resin was washed with a high-salt wash containing 250 mM guanidine hydrochloride before elution of proteins in sample buffer. Electrophoresis and silver staining were performed as described in fig. 2. Lane A, strain OA104 (type b); B, DL41 (type b); C, DL67 (type b); D, DL90 (type b); E, DL63 (type b); F, NA103 (type b); G, BF100 (non-typable); H, BF103 (non-typable); I, TN100 (non-typable).

source of iron for growth (Herrington and Sparling, 1985; Pidcock et al., 1988) the mechanism of iron acquisition from transferrin and its importance in pathogenesis are still unknown. Recently we have used a solid-phase binding assay to detect transferrin-binding activity in isolates of *H. influenzae* (Schryvers, 1988) and demonstrated that the binding specificity was similar to that observed in *N. meningitidis* (Schryvers and Morris, 1988a). The fact that *N. meningitidis* binds only the human form of transferrin, can use only human transferrin to support the growth of iron-deficient cells in vitro (Schryvers and Morris, 1988a) and can use only human transferrin to support infection in mice (data not shown), indicates that the binding activity detected is a functional receptor activity involved in transferrin-iron acquisition. The striking similarities between the specificity of binding of human transferrin to *N. meningitidis* and *H. influenzae* suggests that this binding activity probably also indicates a functional transferrin receptor in *H. influenzae*. Therefore, we attempted to capitalise on the specific transferrin binding as a means of isolating and identifying the binding protein(s) postulated to be involved in specific iron acquisition from human transferrin.
Two proteins of 98 and 58 Kda were isolated from *H. influenzae* strain KC548 (fig. 2) by an affinity isolation method with biotinylated human transferrin and streptavidin-agarose. The importance of binding biotinylated human transferrin to intact membranes in the first step of this procedure was demonstrated. These results do not allow us to discriminate between the possibilities that: (i) the detergent was directly interfering with the binding reaction by binding to sites of interaction of either or both receptor and ligand; or (ii) the presence of detergent destabilised the conformation of the receptor (complex). However, our observation that there was a significant loss in binding activity when outer membranes were prepared by selective detergent extraction with either Sarkosyl NL30 or Triton X-100, which was not restored by successive washes with buffer alone (data not shown), suggests that the latter consideration may be more important.

Several lines of evidence indicate that the proteins isolated by our affinity immobilisation procedure are the *H. influenzae* proteins involved in binding to human transferrin. Firstly, these proteins were isolated from membranes prepared from iron-deficient cells but not from iron-sufficient cells, indicating that these proteins are iron-regulated in the same fashion as transferrin-binding activity (Schryvers, 1988). Secondly, the proteins were isolated when biotinylated human transferrin was used in the affinity procedure but not when it was absent or replaced by human lactoferrin. The lack of reactivity of these proteins with HRP-streptavidin, a monoclonal antibody to human transferrin or concanavalin A (data not shown) indicates that these proteins were not merely derivatives of the ligand used in the affinity procedure. The ability of the 58-Kda protein to bind HRP-transferrin after SDS-PAGE and electroblotting, albeit weakly, indicates that at least this protein is involved in binding to human transferrin. The observation that the 98-Kda protein remained bound to the column after the 58-Kda protein had been eluted suggests that the 98-Kda protein may also directly bind to human transferrin. The lack of transferrin binding by the 98-Kda protein after SDS-PAGE and electroblotting does not exclude this possibility and may merely reflect a reduced capacity to restore the protein conformation necessary for binding of ligand. Since the binding of human transferrin to *H. influenzae* would be anticipated to involve not only simple binding but also removal of iron from
transferrin, the involvement of more than one protein in the binding reaction is not unreasonable. In this context, it is interesting to speculate that the additional 67-Kda protein remaining bound to the affinity resin after milder washing conditions may be associated with the putative 58- and 98-Kda binding proteins in an iron acquisition complex. More direct evidence for the involvement of these proteins in iron acquisition from human transferrin awaits production of specific deletion mutants.

Analysis of transferrin-binding proteins isolated from serotype b and non-typable strains (fig. 4) indicated that a high mol. wt protein of 94–106 Kda was present in all strains tested. The fact that these proteins shared the property of being retained on the affinity resin after washing with 250 mM guanidine hydrochloride suggests that they may represent functionally similar proteins in the different strains. A lower mol. wt protein of 78–90 Kda was observed in samples from all type-b strains when milder washing conditions were used (data not shown). In samples from some of the type-b strains we were able to detect transferrin-binding activity by the lower mol. wt. protein after SDS-PAGE and electroblotting but the reaction was very weak (data not shown). In fig. 4, these bands were evident only in several of the type-b strains (lanes E and F), indicating that they were susceptible to elution by 250 mM guanidine hydrochloride in many of the strains. Even when using samples prepared with milder washing conditions, the interpretation was complicated by the fact that biotinylated human transferrin migrated in the same region. This made it necessary to run gels with different acrylamide concentrations (including gradient gels) for longer periods of electrophoresis in order to resolve the lower mol. wt binding protein from the biotinylated human transferrin in a number of the strains. This type of analysis will be much simpler once we have prepared hyperimmune serum or monoclonal antibody against these proteins.

The detection of lactoferrin-binding activity in H. influenzae strain KC548 (Schryvers, 1988) convinced us to also attempt to identify the lactoferrin-binding protein(s) from this strain. Using biotinylated human lactoferrin in the affinity immobilisation procedure we isolated 105- and 106-Kda proteins from iron-deficient cells of strain KC548. The lack of these bands in samples prepared from iron-sufficient cells and in samples prepared without biotinylated human lactoferrin indicates that there are iron-regulated proteins specifically binding to human lactoferrin. As observed with the transferrin-binding proteins, prior solubilisation of the membrane resulted in little or no binding protein being isolated. The mol. wts of the lactoferrin-binding proteins in H. influenzae strain KC548 (105 and 106 Kda) are nearly identical to the binding protein isolated from N. meningitidis strains (105 Kda, Schryvers and Morris, 1988b). The similarity in size and in binding specificity (Schryvers, 1988) of the lactoferrin receptor in this strain of H. influenzae with the lactoferrin receptor in meningococcal strains suggests a structural and functional conservation. Further studies are needed to determine if this apparent conservation is also reflected in a conservation of antigenic epitopes that might be useful as immunotherapeutic targets.

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REFERENCES


